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Ameliorative effects of microbial fertiliser on yield and quality parameters of curly lettuce and cucumber with fertiliser saving

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ABSTRACT

This research aimed to evaluate the potential effects of microbial fertilizer on the growth, yield and quality parameters and fertiliser savings of lettuce and cucumber. The study had control (C), microbial fertilisation (MF), chemical fertilisation (CF), chemical fertilisation + microbial fertilisation (CF + MF), 50% chemical fertilisation + microbial fertilisation (50% CF + MF) and dipping into microbial fertiliser + chemical fertilisation (D + CF) treatments. The highest head length, root collar diameter, soluble solids and leaf number of lettuce were obtained in D+CF. Maximum chlorophyll contents were obtained in CF, CF + MF and D + CF, all of which were higher than the C application. D + CF and CF + MF resulted in the highest total and marketable yields. In cucumber cultivation, although the highest fruit length was obtained in CF, CF + MF, 50% CF + MF and D + CF were only slightly less than CF. The highest fruit diameters were in CF + MF and 50% CF + MF. The chlorophyll contents were significantly more in 50% CF + MF and D + CF than in other applications. The highest first- and second-class yields were in 50% CF + MF and were significantly higher than those in C and CF. Average fruit weight was also higher in all MF applications. It counclud that the alone MF application is not important, so MF has to be used with organic or chemical fertilisers in lettuce and cucumber cultivation.

Keywords: bacteria, cucumber, lettuce, microbial fertiliser

INTRODUCTION

Lettuce is one of the annual leafy cool climate vegetables that stands out in the national and international vegetable market (Aćamović-Đoković et al., 2011; Colonna et al., 2016). Lettuce is consumed mainly as a salad and processed vegetable, and it has great importance in human nutrition due to its vitamin, mineral and antioxidant contents. The advantage of eating raw lettuce is that it contains more nutrients than thermally processed lettuce (Aćamović-Đoković et al., 2011). Cucumber, one of the most popular vegetables all over the world, is commonly monocultured under greenhouse conditions (Zhao et al., 2020). It has several phytonutrients with antioxidant, anti-inflammatory and anti-cancer properties. As it is difficult to maintain plant growth and soil fertility along with higher yields in protected cultivation of cucumber (Singh and Sirohi, 2002), excess chemical fertilisers and pesticides are often used to obtain higher yields (Choudhari and More, 2002; Qiu et al., 2012).

Cocktail mixtures consisting of lactic acid bacteria, phototrophic bacteria and yeast, known as effective microorganisms (Goessler and Kuehenelt, 2002), are used in crop cultivation, and they are physiologically compatible with one another and can coexist in the environment working in a symbiotic relationship.

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There is evidence in the literature that these beneficial microorganisms can improve the quality of soil, plant growth and yield (Kengo and Hui-lian, 2000). Moreover, yeasts produce hormones and enzymes that are known to promote plant cell and root division. Different microorganisms in the cocktail mixtures complement each other and are in a mutually beneficial relationship with the roots of plants in the soil ecosystem. Thus, plants grow exceptionally well in soils inhabited and dominated by effective microorganisms (Pei-Feng et al., 2014).

Plant growth-promoting bacteria, such as rhizobacteria (PGPR), are commonly used as biofertilisers in several crops, including vegetables, grown under both field and greenhouse conditions (Gravel et al., 2007). Such organisms has potential to enhance the availability of nutrients to the host plant by lowering ethylene levels in plants or by increasing the production of plant growth regulators such as indole-3-acetic acid (IAA). Since such physiological mechanisms also alter the morphology of the roots, it facilitates better root growth and thus increases nutrient uptake (Vessey, 2003; Antoun and Prévost, 2005; Richardson et al., 2009). It is also known that these microorganisms show enhanced defence against broad biotic and abiotic stress factors (Pieterse et al., 2014; Prasanna et al., 2014; Triveni et al., 2015).

Beneficial microorganisms not only suppresses soil-borne pathogens but also increases the decomposition of organic materials and, consequently, the availability of mineral nutrients and important organic compounds to plants (Singh et al., 2003). Improvement in soil fertility has a significant positive effect on plant growth, flowering, fruit development and ripening in crops (Lévai et al., 2006).

They can be used in traditional, organic and integrated farming systems to reduce the consumption of fertilisers and pesticides and minimise their negative effects on the ecosystem (Molla et al., 2012). Microorganisms are responsible for activating nutrients (macro- and microelements) and supporting plant growth (Colla et al., 2010). Leafy vegetable production is largely based on the presence of NO_3, which is the most important source of N (Colla et al., 2011). The availability of excess N may reduce leaf quality as it causes nitrate accumulation in the above-ground parts of vegetables with a short life cycle such as lettuce (Awaad et al., 2016). Some root bacteria that stimulate growth in plants are *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Paenibacillus* and *Pantoea* (Chen et al., 1996; Fálico et al., 2000; Luz, 2000; Pal et al., 2000; Wall, 2000). Research studies have focused especially on *Pseudomonas* and *Bacillus* genera (Compant et al., 2005), and it has been found that many species of *Pseudomonas* stimulate rapid growth by affecting seed germination, growth and development of roots (De Salamone et al., 2006). *Bacillus megaterium* helps dissolve P and K in the soil (Wu et al., 2012; Keshavarz Zarjani et al., 2013; Sindhu et al., 2016; Zhao et al., 2019) and promotes plant growth (Zou et al., 2010; Zhou et al., 2016; Korir et al., 2017). In a study, biofertilisers enriched with inorganic fertilisers played a significant role in the growth and yield of tomato and reduced the inorganic fertiliser cost by 50% (Haque et al., 2012). As an alternative approach, NPK fertilisers were reduced by 25% by microbial inoculation in French bean cultivation (Chauhan and Bagyaraj, 2015). Conventional fertilisation treatment with *B. megaterium* significantly increased cucumber yield by 11.8%–15.2%, and when P and K were reduced from conventional fertilisation, the yield and quality of cucumber did not decrease (Zhao et al., 2021). Microorganisms significantly increased the weight of red lettuce and contributed the highest vitamin C concentration (Stojanović et al., 2020).

This research aimed to determine the effects of a microbial fertiliser comprising a cocktail of beneficial microorganisms on plant growth, yield and some quality criteria in lettuce and cucumber cultivation under greenhouse conditions.

MATERIALS AND METHODS

Experimental site and materials

In this study, experiments were conducted in the Research and Application Area of Akdeniz University (36°39′ north, 30°39′ east) under greenhouse conditions. During the research period, the minimum, average and maximum temperature values with humidity were recorded in a data logger in the greenhouse (Figure 1). Curly lettuce (*Lactuca sativa* var. *crispa* cv. 'Campania') and cucumber (*Cucumis sativus* cv. 'PTK-40') seedlings were used as plant materials. A microbial fertiliser, consisting of a cocktail of microorganisms in a liquid form including *Lactobacillus* strains (*Lactobacillus lactis*, *Lactobacillus diacetylactis*, *Lactobacillus cremoris*, *Lactobacillus acidophilus*), *Rhodopseudomonas palustris*, *Bacillus subtilis* bacteria and a yeast (*Kluyveromyces marxianus*), was investigated in this study.

The physical and chemical properties of the greenhouse soil in which the research was conducted were analysed according to methods used by Kaçar (1995) and Kaçar and Kovanci (1982) in soil samples taken from a depth of 0-30 cm. The greenhouse soil had 39.5% lime and 1.71% organic matter, and its pH was 8.1% and salinity was 0.013%. The total nitrogen content was detected as 0.1%. P_2O_5, K_2O, extractable CaO and extractable MgO concentrations of the soil were analysed as 186.5 kg · ha^{-1}, 442.0 kg · ha^{-1} and 10,399.0 kg · ha^{-1}, respectively. Available Fe, Mn and Zn from microelements were determined as 3.75 mg · kg^{-1}, 5.52 mg · kg^{-1} and 2.49 mg · kg^{-1}, respectively.

Experimental design

In this research, six different applications including the control were investigated with three repetitions.

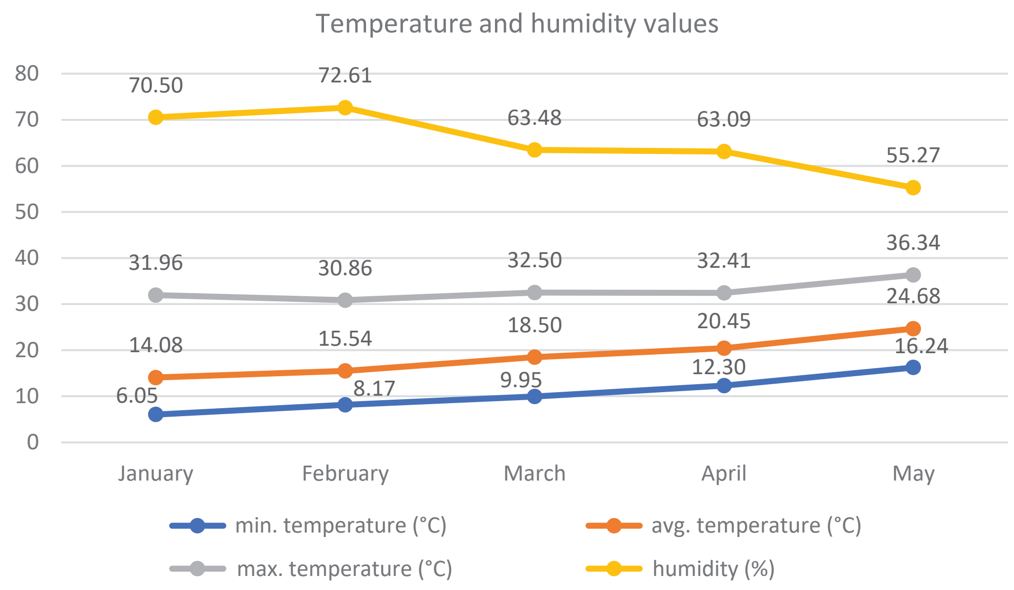

Figure 1. Temperature and humidity values recorded in the greenhouse during the research periods.

The plot size was 2.88 m^2 in curly lettuce cultivation, 16 plants were included in each plot and the seedlings were planted on January 18 at 40-cm intervals with 50-cm intra-row distances. In cucumber cultivation, the plot size was planned as 2.52 m^2, and 14 seedlings were included in each plot. Seedlings were planted on February 8, with 90 cm between wide rows, 50 cm between narrow rows and 60 cm inline according to the double-row planting distances. The research was carried out during the growing periods from January to June. Before cultivation, organic fertilisation is routinely applied to soils to improve soil properties and to improve microbial activity. For this purpose, 2,500 kg · ha^{-1} of Biofarm commercial organic fertiliser was applied to all plots, except the control plot, to accelerate bacterial colonisation before planting.

Studied applications

Different applications in the study are presented together with their corresponding abbreviations in Table 1.

The following is information on how to conduct the applications in the research:

- **Control**: No application was made before and after the seedling plantation.
- **Microbial Fertilisation (MF) Alone**: After planting the seedling, MF was applied in the irrigation water at 15-day intervals.
- **Chemical Fertilisation (CF):** CF was applied as 65 kg · ha^{-1} N, 25 kg · ha^{-1} P_2O_5 and 60 kg · ha^{-1} K_2O at the recommended dose according to Aybak (2002) after planting the seedling.
- **CF + MF**: MF was applied at 15-day intervals in addition to the recommended dose of a chemical fertiliser in 65 kg · ha^{-1} N, 25 kg · ha^{-1} P_2O_5 and 60 kg · ha^{-1} K_2O, according to Aybak (2002) after planting the seedling.
- **50% CF + MF**: MF was applied at 15-day intervals in addition to the half dose of the recommended chemical fertiliser according to Aybak (2002) after planting the seedling.
- **Dipping + CF:** After the seedlings were planted by dipping into a microbial fertiliser solution prepared with non-chlorinated water, CF was applied as 65 kg · ha^{-1} N, 25 kg · ha^{-1} P_2O_5 and 60 kg · ha^{-1} K_2O alone at the recommended dose according to Aybak (2002).

Table 1. Applications involved in the study.

Application	Abbreviations
Control	C
Microbial fertilisation alone	MF
Chemical fertilisation	CF
Chemical fertilisation + microbial fertilisation	CF + MF
50% Chemical fertilisation + microbial fertilisation	50% CF + MF
Dipping into microbial fertilisation + chemical fertilisation	D + CF

Microbial fertiliser application before and after planting seedlings

Before planting the lettuce and cucumber seedlings, a solution was prepared by mixing 1.5 L of the microbial fertiliser with 100 L of unchlorinated water. This solution was sprayed on the planting holes of seedlings, except for CF and control (C) applications. In addition to this, lettuce and cucumber seedlings (except C and CF) were dipped into a solution prepared by adding 750 mL of the microbial fertiliser to 100 L of unchlorinated

Figure 2. Lettuce before harvesting (6-1: D + CF) and chlorophyll measurement in cucumber leaves. D, dipping; CF, chemical fertilisation.

water for 10 min before being planted into the soil. After planting the seedlings, the first irrigation was carried out straight away. After planting the seedlings, microbial fertiliser was applied to MF alone, CF + MF and 50% CF + MF at 15-day intervals, at a rate of 750 mL per 100 L. Microbial fertiliser application was continued till 1 week before the harvesting of lettuce and cucumber. Lettuce before harvesting and cucumber plants with chlorophyll measurement are shown in Figure 2.

The criteria examined in the research

Within the scope of the research, head height (cm), root collar diameter (mm), number of leaves per plant, total chlorophyll content, total and marketable yield (kg · ha^{-1}), average head weight (g · plant^{-1}), leaf colour (L, C, H°), total soluble solid (%) and pH criteria in lettuce juice were investigated. In the cucumber experiment, fruit height (cm); fruit stalk length (cm); fruit stalk thickness (mm); fruit diameter (mm); total chlorophyll content in leaves; colour values in fruits (L, C, H°); peeled and unpeeled hardness (kg · cm^{-2}); total soluble solid; pH values in the cucumber juice (%); earliness, first- and second-class yield (kg · ha^{-1}); average fruit weight (g · fruit^{-1}); and total yield (kg · ha^{-1}) criteria were evaluated (Polat et al., 2009; Sahin et al., 2015; Olawuyi and Lee, 2019).

The total chlorophyll content was determined by using a SPAD 502 (Konica Minolta Sensing, Inc., Osaka, Japan) chlorophyll meter. The colour values of L, *a and *b for both the lettuce and cucumber plants were measured by using a Minolta CR400 (Konica Minolta Sensing, Inc., Osaka, Japan) colour chromameter. The measured *a and *b values were used to calculate the C (Chroma) and H° (Hue) angle values by using the following formulas (Siomas et al., 2002; Madeira et al., 2003):

C: $\sqrt{(a^2 + b^2)}$

H (°): $\tan^{-1}$ (b/a)

Statistical analysis

The research was carried out in three repetitions according to the randomised plot design. The findings were evaluated with the SAS (version 9.00; SAS Institute Inc, Cary, NC, USA) statistical package program.

RESULTS AND DISCUSSION

Head height, root collar diameter, number of leaves and chlorophyll contents in curly lettuce

As seen in Table 2, the highest head length was obtained from D+CF with 20.44 cm, while the second highest head length value was 19.03 cm in CF. There were not many differences between other applications, where chemical fertilisers were used in combination with the microbial fertiliser. The highest value in terms of root collar diameter was measured in D + CF and CF with 20.22 mm and 20.05 mm, respectively. These were followed by 18.89 mm and 18.82 mm for 50% CF + MF and CF + MF applications, respectively. The highest number of leaves was counted in D + CF as 62.57 leaves/plant, followed by CF and 50% CF + MF applications with 58.00 and 57.00 leaves/plant, respectively ($p < 0.05$). The lowest head height, root collar diameter and leaf values were determined in C and MF applications.

The highest SPAD values in terms of chlorophyll content were measured as 26.33, 25.98 and 25.70 in CF, CF + MF and D + CF applications, respectively. These applications were in the same group, and no significant differences were considered to exist between them. The application following the highest value group was 50% CF + MF with 25.20. As shown in Table 2, the lowest chlorophyll contents were found in C and MF alone (MF) applications, which were applied only at 15-day intervals from the seedling planting. When the criteria given in Table 2 are evaluated, the D + CF application yielded best results. Ucok et al. (2019) reported that in curly lettuce, the head length varied between 18.27 cm and 23.10 cm and the root collar diameter in the range

Table 2. Effects of different applications on head height, root collar diameter, number of leaves and total chlorophyll contents in curly lettuce.

Application	Head height (cm)	Root collar diameter (mm)	Leaves (per plant)	Chlorophyll contents
C	16.95 cd*	18.17 b	52.00 c	23.85 b
MF	16.71 d	17.86 b	53.57 bc	21.87 c
CF	19.03 b	20.05 a	58.00 ab	26.33 a
CF + MF	18.05 bc	18.82 ab	56.10 bc	25.98 a
50% CF + MF	18.03 bc	18.89 ab	57.00 abc	25.20 ab
D + CF	20.44 a	20.22 a	62.57 a	25.70 a
$LSD_{5\%}$	1.1165	1.8548	5.9853	1.4752

*The difference between values not shown with the same letter are significant at a $p < 0.05$ level.
LSD, Least Significant Difference; C, control; CF, chemical fertilisation; MF, microbial fertilisation; D, dipping.

of 20.25–25.38 mm, and these values increased with organic fertilisers. On the contrary, Sonmez et al. (2017) showed that the head length in curly lettuce varied between 13.3 cm and 21.5 cm according to the use of organic matter in autumn and spring periods, and the number of leaves increased compared to that in the control. Rabiei et al. (2020) found that plant height was not significant in coriander plants inoculated with *Azospirillum brasilense* and *Azotobacter chroococcum*. When Miskoska-Milevska et al. (2018) applied Slavol (including *A. chroococcum*, *Azotobacter vinelandii*, *Derxia* sp., *B. megaterium*, *Bacillus licheniformis* and *B. subtilis* microorganisms) on cauliflower by foliar spraying and drip irrigation system, leaf length and width increased.

In our study, the chlorophyll content was found to be higher in the trials combined with chemical fertilisers, while Cocetta et al. (2021) found that *Paenibacillus pasadenensis* strain R16, *Pseudomonas syringae* strain 260-02 and *Bacillus amyloliquefaciens* strain CC2 bacteria were not effective on the total chlorophyll content in romaine lettuce. According to Moncada et al. (2021), the use of *B. subtilis* did not affect plant growth criteria such as leaf area, stem diameter, plant height and leaf colour in basil. Sahandi et al. (2019) found that when phosphate-solubilising *Pseudomonas putida* and *Pantoea agglomerans* bacteria were applied together with 50 kg $\cdot$ ha^{-1} of phosphorus, the number of leaves, plant height and total chlorophyll content of mint increased due to a synergistic effect of these microorganisms. Han and Lee (2005) reported that the total chlorophyll content of lettuce increased with the application of *Serratia* spp. and *Rhizobium* spp. in different saline soil conditions. Ozbay et al. (2010) revealed that *Trichoderma harzianum* increased plant height by 7%, chlorophyll content by 10% in arugula and by 4% and 7% in cress, and there was no significant difference in the number of leaves in both plants. Yıldırım et al. (2011) used some PGPR applications under salt stress conditions in lettuce cultivation. *Kocuria erythromyxa* increased the head length (30%) and yielded the highest number of leaves compared to the control. *B. subtilis* and *Staphylococcus kloosii* bacteria provided the best results in chlorophyll measurements. In addition, they determined that *S. kloosii* and *Bacillus sphaericus* bacteria increased all of these growth parameters.

Table 3. Effects of different applications on the total soluble solid and pH in curly lettuce.

Application	Soluble solid (%)	pH
C	3.80 ab*	5.83 a
MF	4.00 a	5.76 cd
CF	3.63 b	5.78 bc
CF + MF	3.83 ab	5.80 ab
50% CF + MF	4.10 a	5.74 d
D + CF	4.10 a	5.80 ab
$LSD_{5\%}$	0.3138	0.0358

*The difference between values not shown with the same letter are significant at a $p < 0.05$ level.
LSD, Least Significant Difference; C, control; CF, chemical fertilisation; MF, microbial fertilisation; D, dipping.

Soluble solid and pH of lettuce juices

The highest soluble solids called Brix were detected in 50% CF + MF (4.10%), D + CF (4.10%) and MF (4.0%) applications, and the difference between them was not significant (Table 3).

The lowest soluble solid (3.63%) was obtained from the CF experiment performed at the recommended dose. When evaluated in terms of soluble solid, it was observed that the microbial fertiliser applications increased the total soluble solid in lettuce juice. When the acidity of the juice obtained from lettuce was measured, the highest value, that is, the most alkaline application, was determined in C (5.83). Polat et al. (2004) reported that the total soluble dry matter of iceberg lettuce juice was 3.86%–4.06% and 4.40%–4.60% in cos lettuce. They also reported that the pH values varied between 5.99 and 6.06 in iceberg lettuce and 5.89 and 5.94 in cos lettuce. The soluble solids and pH values obtained from our research are similar to these normal values. Ozbay et al. (2010) found that the application of *T. harzianum* bacteria increased the total soluble solid content by 13% in arugula and 11% in cress.

Colour values in lettuce leaves

While applications were found to be statistically ($p < 0.05$) effective on L, C and H° colour values,

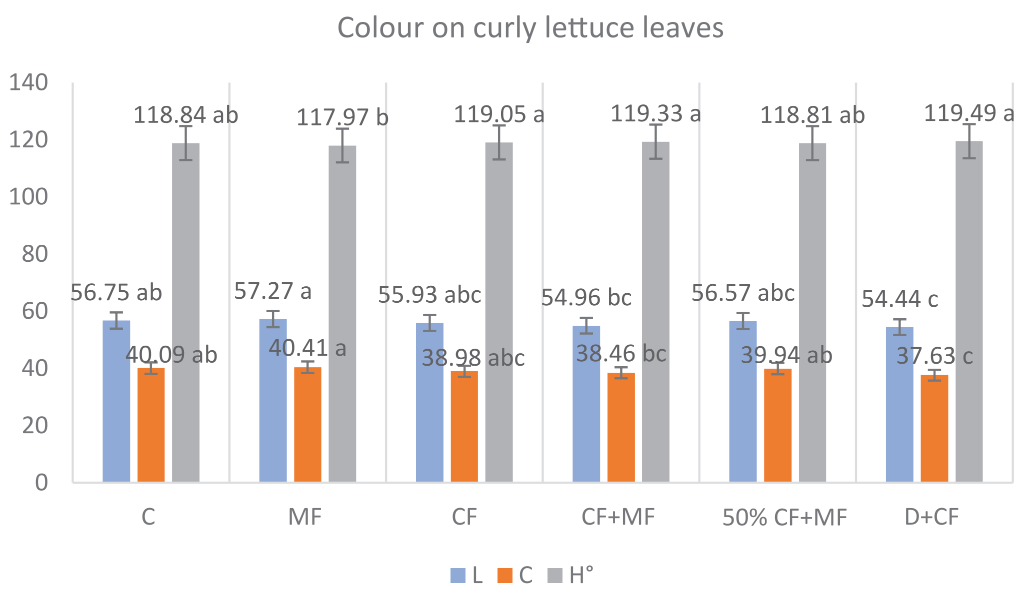

Figure 3. Effects of different applications on L, chroma (C) and hue (H°) angle values in curly lettuce leaves. The difference between values not shown with the same letter are significant at a $p < 0.05$ level. C, control; CF, chemical fertilisation; MF, microbial fertilisation; D, dipping.

the highest L and C values were obtained at the MF application. The lowest value was measured in the D + CF application (Figure 3).

Very light-coloured leaves are among the undesirable features in lettuce as the L value indicates the lightness of the leaf colour and will turn white as the value approaches 100. In this case, the leaves of the plants in which D + CF and CF + MF applications are applied have the desired properties in terms of L colour value. Since the chroma (C) value expresses the saturation of the leaf colour, the best applications in this respect are similarly D + CF and CF + MF applications. When the hue (H°) angle value is zero (0), it shows red colour, and when it is 270°, it means blue colour. In this respect, when evaluated, darkest green leaves were obtained in the D + CF, CF + MF and CF applications. Ucok et al. (2019) pointed out that the colour values of curly lettuce (L: 56.23–59.43; C: 37.19–38.82; H: 115.56–117.61) varied according to different organic fertilisers, and the values increased with some organic inputs. On the contrary, Sonmez et al. (2017) found that there was no change in colour values when different organic fertilisers were used in lettuce production. Our study also confirms that colour values, in other words total chlorophyll contents, vary with different organic inputs. This is in compliance with the studies of Uçok et al. (2019) and Cocetta et al. (2021).

Yield values in lettuce

Microbial applications, as indicated in Table 4, had a significant effect on the total yield, marketable yield, average head weight and average marketable head weight ($p < 0.05$). Accordingly, the highest total yield was obtained from D + CF with 30,486.1 kg · ha^{-1}. This was followed by CF + MF with 29,513.9 kg · ha^{-1} and CF with 29,265.0 kg · ha^{-1}. In terms of marketable yield, the highest values were obtained from the same applications in the same order as in the case of total yield. In other words, the highest marketable yield value was found in D + CF with 28,750.0 kg · ha^{-1}, and this was followed by CF + MF with 27,297.4 kg · ha^{-1} and CF applications with 26,981.5 · kg ha^{-1}. The lowest values in terms of total and marketable yields were obtained from C and MF applications. When the yield values obtained were examined, it could be seen that when the microbial fertiliser was added to the plant together with chemical fertiliser applications, it increased the yield values compared to that of only chemical fertiliser application.

The highest average head weight based on total yield was determined in D + CF (548.75 g · plant^{-1}), followed by CF + MF (531.25 g · plant^{-1}) and CF (526.77 g · plant^{-1}) applications. The highest average head weight in terms of marketable yield was determined in D + CF (517.50 g · plant^{-1}), and it was followed by CF + MF (491.36 g · plant^{-1}) and CF (485.67 g · plant^{-1}) applications. These three applications constitute a higher group in terms of yield. The lowest yield values in terms of average head weights were determined in C and F applications.

Some researchers reported that the effect of biofertilisers on yield varied between 35% and 65%, and this performance depends on the biological interaction between fauna, microflora and the host plant in the rhizosphere (Berg, 2009). Some researchers also reported that the use of nitrogen, phosphorus and potassium (NPK) can be reduced with the use of microbial fertilisers. For example, Chauhan and

Table 4. Effects of different applications on total yield, marketable yield, total average head weight and marketable average head weight in curly lettuce.

Application	Total yield (kg · ha^{-1})	Marketable yield (kg · ha^{-1})	Total average weight (g · plant^{-1})	Mark. average weight (g · plant^{-1})
C	20,127.3 d*	18,978.0 d	362.29 d	341.60 d
MF	20,329.9 d	19,053.2 d	365.94 d	342.96 d
CF	29,265.0 b	26,981.5 b	526.77 b	485.67 b
CF + MF	29,513.9 ab	27,297.4 b	531.25 ab	491.36 b
50% CF + MF	25,665.5 c	23,607.6 c	461.98 c	424.94 c
D + CF	30,486.1 a	28,750.0 a	548.75 a	517.50 a
$LSD_{5\%}$	999.12	1,044.9	17.983	18.809

*Difference between values not shown with the same letter are significant at a $p < 0.05$ level.
LSD, Least Significant Difference; C, control; CF, chemical fertilisation; MF, microbial fertilisation; D, dipping.

Table 5. Effects of different applications on the fruit length, fruit stalk length, fruit stalk thickness and fruit diameter in cucumber.

Application	Fruit length (cm)	Fruit stalk length (cm)	Fruit stalk thickness (mm)	Fruit diameter (mm)
C	11.18 b*	1.80	3.57	23.76 d
MF	11.54 ab	2.01	3.28	24.84 c
CF	12.18 a	2.03	3.52	25.20 bc
CF + MF	11.55 ab	1.71	3.52	26.44 a
50% CF + MF	11.79 ab	1.82	3.59	26.19 a
D + CF	12.03 ab	1.82	3.58	25.82 ab
$LSD_{5\%}$	0.928	NS	NS	0.691

*Difference between values not shown with the same letter are significant at a $p < 0.05$ level.
NS, not significant.
LSD, Least Significant Difference; C, control; CF, chemical fertilisation; MF, microbial fertilisation; D, dipping.

Bagyaraj (2015) reported that NPK fertilisers could be reduced by 25% through microbial inoculation in French beans. However, reducing the recommended nitrogen, phosphorus and potassium amounts by 50% in our lettuce experiment reduced the total yield and marketable yield by 12.3% and 12.5%, respectively. On the contrary, our results also showed that dipping + full chemical fertiliser (D + CF) increased the marketable yield by 6.6%. Considering this, dipping into a beneficial microorganism cocktail before planting could possibly, to some extent, compensate the yield loss due to 50% less chemical fertiliser use, and hence, D + MF + 50% CF could be the ideal solution for greenhouse farmers. Vejan et al. (2016) reported that the use of PGPR in all products increased the yield by between 7% and 33%. Stojanovic et al. (2020) investigated two different commercial liquid microbial fertilisers containing effective microorganisms (*Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus lactis*, *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*, *Saccharomyces cerevisiae*, *Streptomyces albus*, *Streptomyces griseus*, *Aspergillus oryzae*, *B. subtilis*, *B. megaterium* and *A. chroococcum*) and *Trichoderma* spp. and their combinations on different lettuce varieties in different seasons and found that the varieties, periods and fertilisers were effective on the fresh weight of lettuce. In the autumn period, the highest fresh weight was obtained in one variety by using a combination of effective microorganisms and *Trichoderma* spp.. By using effective microorganisms, the highest yield was achieved in the same variety in winter. In another lettuce study (Tošić et al., 2016), higher values were obtained in fresh weight and in early head formation with bioactive microbial fertiliser (containing *B. subtilis, Azotobacter* spp., *Penicillium oxalicum* and *Fusarium* spp.) Yıldırım et al. (2011) used some PGPR applications in lettuce cultivation under salt stress conditions and found that the head weight increased by 40% compared to the control.

Fruit properties of cucumber

It was determined that the applications had significant effects on the fruit length and fruit diameter of cucumber. There were no significant differences between fruit stalk length and fruit stalk thickness values (Table 5).

The highest fruit length was obtained from CF with 12.18 cm. The lowest value is seen in C, which does not apply anything. There were no significant differences between other treatments and were statistically in the same group. The highest fruit diameter was found in the same group with 26.44 mm and 26.19 mm in CF + MF and 50% CF + MF applications, respectively. The lowest fruit diameter was measured at 23.76 mm in C application. Dash et al. (2018) used farm manure (10 t · ha^{-1} and 20 t · ha^{-1}), vermicompost (2 t · ha^{-1} and 4 t · ha^{-1}) and recommended NPK fertilisers alone and by adding

Table 6. Effects of different applications on total soluble solid, pH and hardness.

Application	Soluble solid (%)	pH	Unpeeled hardness (kg · cm^{-2})	Peeled hardness (kg · cm^{-2})
C	3.37 b*	5.50	1.98 b	1.35
MF	3.40 b	5.53	2.16 a	1.41
CF	3.34 b	5.50	2.21 a	1.40
CF + MF	3.46 ab	5.50	2.24 a	1.42
50% CF + MF	3.42 b	5.50	2.12 ab	1.36
D + CF	3.57 a	5.50	1.97 b	1.33
$LSD_{5\%}$	0.133	NS	0.1536	NS

*Difference between values not shown with the same letter are significant at a $p < 0.05$ level.
NS, not significant.
LSD, Least Significant Difference; C, control; CF, chemical fertilisation; MF, microbial fertilisation; D, dipping.

a biofertiliser (4 kg · ha^{-1} *Azotobacter* + 4 kg · ha^{-1} phosphorus-solubilising bacteria) at different ratios in a 3-year study. The highest fruit length was obtained in the application of NPK at the recommended dose. In terms of fruit diameter, fruits with the highest values were obtained in vermicompost + biofertiliser, and then the second highest fruit diameter was obtained in the half of the recommended NPK dose + farm manure + vermicompost + biofertiliser application. We have to point out that the two highest values in the fruit diameter were very close to each other.

Fruit hardness of cucumber, total soluble solid and pH values in juices

It was determined that microbial fertiliser applications caused significant differences in total soluble solid and unpeeled hardness. However, differences in terms of pH measured in fruit juices and peeled hardness in fruits were not significant (Table 6).

D+CF application increased the total soluble solid content of cucumber (3.57%) higher than the others. The second highest content (3.46%) was measured in the CF+MF combination. All other applications were in the same group, and the differences between them were not significant. The highest values in the hardness measurements of cucumber fruits were found in CF + MF, CF and MF applications with 2.24 kg · cm^{-2}, 2.21 kg · cm^{-2} and 2.16 kg · cm^{-2}, respectively. When Table 6 is evaluated, chemical fertiliser application after dipping into a diluted MF solution and microbial fertiliser applications at 15-day intervals after chemical fertiliser application may have a positive effect on the taste and aroma of cucumbers.

Kafi et al. (2021) studied the effect of *P. putida* and the microbial fertiliser Barvar (containing *A. vinelandii*, *P. agglomerans*, *P. putida*, *Bacillus circulans*, *B. megaterium*) on cucumber and pointed out that the sugar ratio in the cucumber fruit increased with the reduction of chemical fertiliser, and the highest value was obtained in the Barvar application alone. In contrast to our results, Murtic et al. (2018) found that microbial fertiliser application did not increase the amount of soluble dry matter and titratable acidity in dry and regular irrigation conditions. Göktekin and Ünlü (2016) studied commercial microbial fertilisers called Crop-Set (*L. acidophilus*) and Bionem (*Pseudomonas fluorescens*) under negative and positive control (conventional) applications by adding different organic materials to tomato: the highest fruit hardness was obtained in the plots, where Crop-Set was used alone without the addition of any organic materials.

Table 7. Effects of different applications on L, C and H° colour values of fruits and on chlorophyll of cucumber leaves.

Application	L	C	H°	Chlorophyll contents
C	35.92	21.61	123.25	43.98 d*
MF	36.32	20.79	123.42	53.34 bc
CF	36.32	21.35	123.49	54.13 b
CF + MF	36.57	21.10	123.47	49.33 c
50% CF + MF	37.50	22.86	122.95	60.84 a
D + CF	35.62	20.66	123.58	60.83 a
$LSD_{5\%}$	N.S.	N.S.	N.S.	4.1579

*Difference between values not shown with the same letter are significant at a $p < 0.05$ level.
NS, not significant.
LSD, Least Significant Difference; C, control; CF, chemical fertilisation; MF, microbial fertilisation; D, dipping.

Colour values of cucumber fruits and chlorophyll content in cucumber leaves

It was determined that microbial fertiliser, C and CF applications did not make a significant difference in L, C and H° colour values determined in cucumber fruits. However, the applications created significant differences in the chlorophyll content of the leaves (Table 7).

Accordingly, the highest chlorophyll content was observed in the applications of 50% CF + MF and D + CF, with values of 60.84 and 60.83, respectively. From these results, it can be derived that 50% less chemical fertiliser plus microbial fertiliser application can be sufficient for the chlorophyll content in cucumber and that plants with sufficient chlorophyll will have a positive impact on the plant as a whole, and it will be positively reflected on the yield and quality of the produce. A microbial fertiliser containing plant growth hormones, nitrogen-fixing and phosphorus-solubilising bacteria was studied by Murtic et al. (2018) in cherry tomato under drought and regular irrigation conditions and was found to increase the amount of chlorophyll in regular irrigation conditions by increasing the amount of this microbial fertiliser. However, there was no change in the chlorophyll b content. Simranjit et al. (2019) also reported that different microbial inoculants increased the amount of chlorophyll in cucumber plants grown under greenhouse conditions compared to untreated cucumber.

Yield in cucumber

The differences due to average fruit weights were not statistically significant. First- and second-class average fruit weights varied between 49.20 and 52.22 g · fruit^{-1}, and 22.83 and 24.77 g · fruit^{-1}, respectively. However, there were significant differences between first- and second-class yield values ($p < 0.05$). The effects of the applications on the first- and second-class yield (kg · ha^{-1}) are given in Figure 4.

Both highest first-class and second-class yield values were obtained from 50% CF + MF application, where 50% less chemical fertiliser was applied with a microbial fertiliser. Accordingly, the highest first-class yield value was 73,304 kg · ha^{-1}, and the highest second-class yield value was 3,701 kg · ha^{-1}. Consequently, the highest value in terms of total yield was again achieved in the 50% CF + MF application. This was followed by CF, CF + MF and D + CF applications, respectively, with 67,360.1 kg · ha^{-1}, 65,457.8 kg · ha^{-1} and 63,346.9 kg · ha^{-1}, which were all in the same group in first-class yield. In the second-class yield, the second highest value was determined in CF + MF application with 2,947.5 kg · ha^{-1}. The lowest first- and second-class yields were identified in C with 46,796.0 kg · ha^{-1} and 1,724.3 kg · ha^{-1}, respectively. Because of the data obtained, it was seen that the microbial fertiliser was effective on the productivity of cucumbers. When comparing 50% CF + MF with CF at the recommended dose, 50% CF + MF increased first- and second-class yields by 8.11% and 30.33%, respectively. In addition, the total yield increased by 9.18%. With this application in cucumber, the highest yield was achieved and 50% fewer NPK fertilisers were used. The fact that the 50% CF + MF application provides the highest efficiency is of great importance in terms of saving expensive chemical fertiliser and reducing the negative effects of chemical fertiliser applications on soil fertility. It can also be noted that the use of microbial fertilisers has a positive effect on soil fertility in the long term.

Similarly, Zhao et al. (2021) studied the effects of chemical fertiliser + *B. megaterium* (BM) on cucumber for 2 years and obtained the highest cucumber yield in the chemical fertiliser + *B. megaterium* application compared to CF application alone. The application of chemical fertiliser + BM increased the yield by 15.2% in the first year and 11.8% in the second year in comparison to chemical fertiliser application alone. In the same study and in the same application (chemical fertiliser + BM), when P (10%–20%) and K (5%–10%) minerals were reduced, the yield did not decrease either. Dash et al. (2018) used farm manure (10 t · ha^{-1} and 20 t · ha^{-1}), vermicompost (2 t · ha^{-1} and 4 t · ha^{-1}) and NPK fertilisers (at the recommended rate and half of the recommended rate) alone and by adding a biofertiliser (4 kg · ha^{-1} *Azotobacter* +4 kg · ha^{-1} phosphorus-solubilising bacteria) in a 3-year study. The application of farm manure and vermicompost was at a rate of 10 t and 2 t, respectively, when a biofertiliser was added. Otherwise, they were used twice as much as these doses (20 t · ha^{-1} and 4 t · ha^{-1}) when they were applied alone

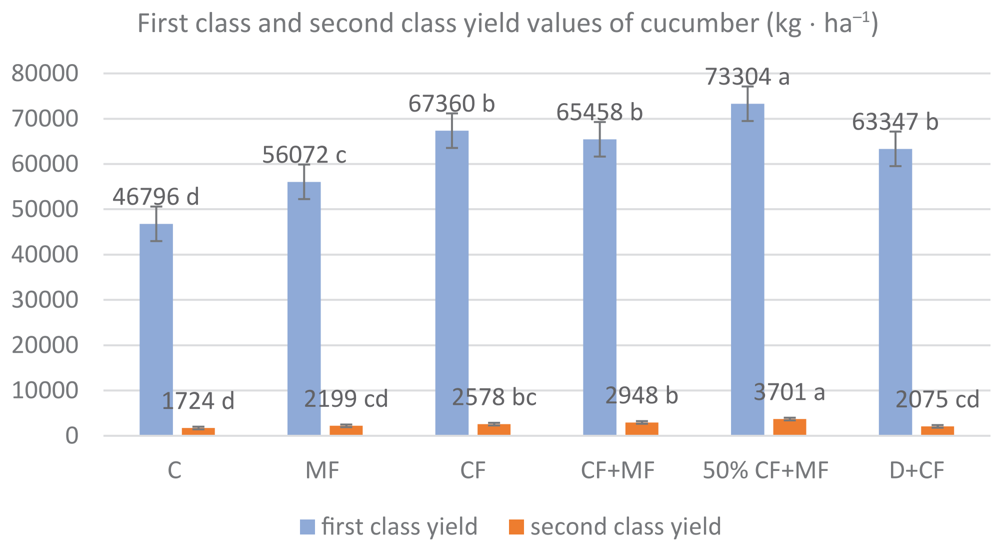

Figure 4. Histogram showing the effects of different applications on the first- and second-class yields (kg · ha^{-1}) of cucumber. The difference between values not shown with the same letter are significant at a $p < 0.05$ level. C, control; CF, chemical fertilisation; MF, microbial fertilisation; D, dipping.

without a biofertiliser. As a result of the research, a higher yield was obtained in the half the recommended NPK + farm manure + vermicompost + biofertiliser and half the recommended NPK + farm manure + biofertiliser applications than in NPK fertilisation alone at the recommended dose. The highest average fruit weight was found in half of the recommended NPK + farm manure + vermicompost + biofertiliser application.

Kafi et al. (2021) compared *P. putida* and Barvar (containing *A. vinelandii*, *P. agglomerans*, *P. putida*, *B. circulans* and *B. megaterium* bacteria) biofertilisers with a control, alone and with a 30% reduction of the recommended dose of NPK applications in cucumber in autumn and spring. It was pointed out that while there was no difference between reduced NPK and biofertilisers in the spring period, the yield in the biofertiliser applications increased slightly compared to that of the control in the autumn period, but there was not much difference between different biofertiliser applications. Rajeela et al. (2017) obtained the highest tomato yield in the recommended dose of chemical fertiliser application and in the *P. putida* + coconut leaf vermicompost (CLV) application compared to that of control and of *B. megaterium* + CLV applications. Haque et al. (2012) compared the performance of various biofertilisers enriched with *T. harzianum*, required and 50% reduced doses of NPK and control applications, and the average fruit weight was determined to be the highest in the 50% N + 50% BioF/suspension and 50% N + 50% BioF/pellets applications. The highest yield per plant was obtained in the 50% N + 50% BioF/suspension application. When we compare the tomato yield obtained in the 50% N + 50% BioF/compost, 50% N + 50% BioF/pellets and 50% N + 50% BioF/suspension applications, we get 28%, 36% and 46% more yield, respectively, compared to that of the required dose of NPK.

Earliness in cucumber

Earliness was determined by comparing the first female flower formations and the number of flowerings at the stage when 50% of the plants flowered in cucumber cultivation with microbial fertiliser (Figure 5). The highest number of female flowers in the first flowering was obtained from the CF + MF experiment. D + CF and alone CF treatments had an equal flower number. There was minimal and equal flower formation in C, MF alone and 50% CF + MF treatments. When 50% of the plants flowered, the highest number of female flowers was seen in CF + MF, and the least number of flowers was seen in C.

When the earliness was examined in terms of the first harvest and the effectiveness of MF, the highest yield was found in D + CF and 50% CF + MF with 25.27% and 21.83%, respectively, compared to CF. These results show that the use of microbial fertilisers is effective on earliness, and the effectiveness of microbial fertilisers increases gradually in the days after seedling planting. As a result of the research, the highest efficiency was found in the 50% CF + MF experiment, with 8.8% more value than the CF application. The reason for the 8.8% increase in efficiency with 50% fertiliser savings can be explained by the observation made in the next criterion.

Observation on cucumber roots

The roots in Figure 6 are the roots of cucumbers grown in CF (3-1), D + CF (6-1), 50% CF + MF (5-1) and CF + MF (4-1) experiments, respectively. During this

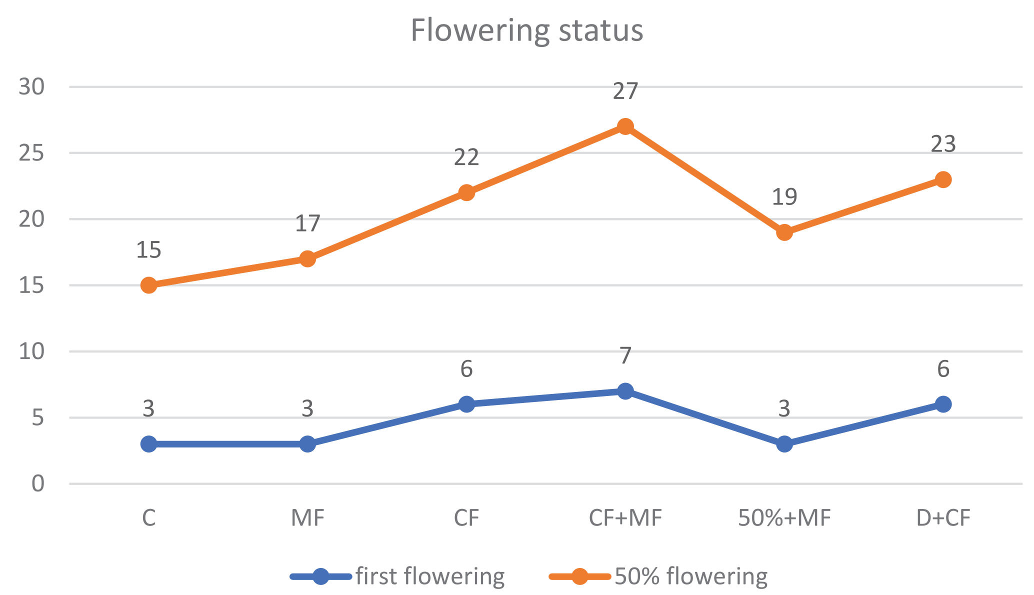

Figure 5. First female flower formation in cucumber plants and number of female flowers when 50% of plants flower. C, control; CF, chemical fertilisation; MF, microbial fertilisation; D, dipping.

Figure 6. Effect of different applications on the development of cucumber roots.

research, no observation and measurement planning were made regarding the root since yield and quality criteria were considered. While the plants were removed manually from the field after the vegetation period, it was noticed that it was more difficult to remove the plant roots from the soil in 50% CF + MF application. Thereupon, the roots of the uprooted plants were examined, and as a result, it was observed that the roots of the plants belonging to 50% CF + MF application spread deeper and to the sides. The same application indicates that the earliness characteristics and yield values mentioned earlier are also related to root development.

With regard to the effect of microbial fertilisers on root formation, some research results in the literature support our findings. For example, Ejaz et al. (2020) used PGPR (containing *Azospirillum* Er-20 and *Agrobacterium tumefaciens*) in pea with CF consisting of nitrogen and phosphorus and found that PGPRs applied together with full (100%) and reduced CF increased root length. Zdravkovska et al. (2016) employed a microbial fertiliser containing various azotobacter groups, nitrifying microorganisms and phosphorus-solubilising microorganisms, and a separate commercial microbial fertiliser added with the same microorganisms and iron mineral on carrot and found that microbial fertilisers increased root length and width compared to the control. According to Pushpa et al. (2021), microbial fertiliser applications increased the root length in cowpea compared to that in the control, and the longest root length was observed in the combination of *Rhizobium* + vermicompost + arbuscular mycorrhizal fungi. These researchers stated that *Rhizobium*, vermicompost and arbuscular mycorrhizal fungi provide the necessary nutrients for plant growth and that such microbial products colonise plant roots and induce plant growth mechanism. Gupta et al. (2021) studied various combinations of *B. licheniformis* and *P. fluorescens* bacteria together with the commercial product Kelpak seaweed on onion and determined a positive effect of *P. fluorescens* on the root length of onion.

CONCLUSION

In this study, the effects of a microbial fertiliser consisting of a mixture of *Lactobacillus* strains, a phototrophic bacterium and a yeast on yield and quality of lettuce and cucumber were investigated. In our study, which was carried out under greenhouse conditions, head length, root collar diameter and chlorophyll amounts that enable plants to produce more carbohydrates, as well as the number of leaves, which are the criteria that determine the morphological quality of lettuce, reached the best results in D + CF application. When compared to the CF application using NPK at the recommended dose, neither significant difference between them was obtained nor the best results were obtained with D + CF. In terms of total water-soluble dry matter determined in lettuce juice, the best practices were D + CF, 50% CF + MF and MF applications. Similarly, in terms of leaf colour values (L, C, H), which is one of the morphological quality criteria, the best results were obtained in the microbial fertiliser applications, D + CF and MF alone. The highest total and marketable yield and average head weights were determined in the D + CF application.

There were significant differences between treatments in the cucumber trials. Microbial applications combined with CF produces best results in terms of the morphological features of cucumber fruits examined in our study. While the highest water-soluble dry matter was in D + CF, the SPAD values measured in leaves were highest in D + CF and 50% CF + MF. The absence of significant differences between some criteria indicates that there are no negative effects of microbial applications. In terms of yield in cucumber, the highest value was obtained in the 50% CF + MF application. In this application, 50% less fertiliser was used than in CF containing NPK at the recommended dose, and 8.11% higher yield in first-class yield and 9.18% higher yield in the total yield were achieved than that of the recommended dose of NPK. In other words, the more economic gain was obtained with less fertiliser in cucumber production.

As a result, it was concluded that the use of microbial fertilisers is effective in both lettuce and cucumber production. In addition, such natural products with biological origins should not be evaluated by the numerical growth performance alone in a short time. The use of microbial fertilisers in the long term is important to improve the soil structure and microbiological properties in soil. Considering cucumber cultivation is carried out for a much longer time than lettuce cultivation since root and plant characteristics of cucumber are different from lettuce, the amount of input fertilisers and other input used in the production are also much more than those for the lettuce cultivation. Therefore, fertiliser savings are of much greater importance in cucumber cultivation.

Future Work

Various organic wastes that are fermented by microbial solutions consisting of lactic acid bacteria, phototrophic bacteria and yeast, namely Bokashi, attract attention in agricultural applications. Bokashi should also be incorporated into studies investigating the effect of liquid microbial solutions by either irrigation or spraying or both. It would also be interesting to note that "dipping the seedlings" or "immersing seeds" into microbial solutions should be combined with other microbial applications. This combined treatment could give enhanced results and the least chemical fertiliser input in many agricultural applications.

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AUTHOR CONTRIBUTIONS

D.H. – experiment design, conducting research and writing the manuscript. Y.H.K. – English editing of the manuscript. K.A. – conducting the research under greenhouse and laboratory conditions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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