Article

Evaluation on the effect of Difulai microbial fertilizer in Weibei Loess Plateau Area, China

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Abstract

Microbial fertilizers are considered an alternative for chemical fertilizers to improve soil fertility and crop production in sustainable farming. This study aimed to evaluate the effects of microbial fertilizer application in Weibei arid area, China. The study was conducted in an apple orchard and a tomato greenhouse between February and November in 2015. Microbial fertilizer application in the apple orchard significantly increased the longitudinal diameter, fruit weight, leaf thickness, and leaf weight by 3.03%, 6.97%, 8.93%, and 3.39%, respectively. In tomato, the use of microbial fertilizer significantly increased the flower number; plant height; and the transverse diameter, sugar content, and hardness of fruit by 7.93%, 14.24%, 6.84%, 4.03%, and 7.92%, respectively. In general, the Difulai microbial fertilizer had noticeable promoting effects on the apple and greenhouse tomato yield and quality. In addition, this study proposed a new method to characterize the apple yield by using fruit weight, and established a model to predict the apple yield by using leaf weight during the growth period.

Keywords microbial fertilizer; Loess Plateau; quality; yield; field experiment.

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1 Introduction

Chemical fertilizers are inorganic compounds added to the soil to sustain crops, and have played a significant role in promoting agricultural production and ensuring national food security. Over the years; however, the persistent and excessive use of chemical fertilizer has caused serious problems in the soil and environment (Li and Zhang, 2002; Tang, 2014); therefore, their usage needs to be controlled. Microbial fertilizers contain beneficial microbial flora, active enzymes, organic matter, and trace elements, which can improve soil properties and crop yield as well as quality. Derived from nature itself (Bhardwaj et al., 2014), microbial fertilizers are more environmentally friendly and do not have evident side effects like chemical fertilizers.

The studies and applications of microbial fertilizer in China have been ongoing since the 1950s. At present, it has been widely used in China, in food crops as well as economic and ornamental plants such as fruit trees, vegetables, and flowers (Wu and Lin, 2002; Meng et al., 2008). However, as microbial fertilizer is a living material, certain environmental conditions are needed to ensure its optimal functioning. These conditions include soil types and climatic conditions (Yuan et al., 2009; Xu et al., 2016); particularly, the lack of water can have an adverse effect on the effectiveness of microbial fertilizer.

The Loess Plateau region is an important fruit and vegetable production base in China. A rational use of microbial fertilizer in this region could be of great significance for the improvement of the quality, yield, and commercial grade of these highly productive cash crops. However, drought in this area limits the usage of microbial fertilizer. Therefore, it is extremely important to investigate the effect of microbial fertilizer under drought conditions in this region.

2 Materials and Methods

2.1 Materials

The microbial fertilizer, Difulai microbial fertilizer (Difulai), provided by Beijing Difulai Biotechnology Co. Ltd., China, is a pure, natural, and highly concentrated microbial fertilizer. There are two formulas, one for fruits and another for vegetables, which were used in this study in an apple orchard with Red Fuji apples and a tomato greenhouse, respectively.

2.2 Experimental areas

The row and plant spacing in the apple orchard was 4 m × 5 m. The majority of apple trees were 15 years old. The orchard soil showed medium fertility and lack of water and was mainly fertilized with chemical fertilizer prior to the experiment.

Row and plant spacing of the tomato plants was 30 cm × 50 cm. The greenhouse soil showed medium fertility, was irrigable, and was fertilized mainly with chemical fertilizer prior to the experiment.

2.3 Experimental design

The application of chemical fertilizer in conjunction with microbial fertilizer is more effective than chemical fertilizer alone (Liu et al., 2007; Meng et al., 2008; Li et al., 2015); therefore, the apple test group was fertilized with Difulai as well as chemical fertilizer. The test was conducted from February to November 2015, in four orchards in different counties (Luochuan, Fuxian, Huangling, and Baishui) in the Shaanxi Province. The test area was rectangular with a flat contour and uniform soil fertility. Each orchard was divided into two test plots for the test group and control group, respectively. The area of each plot was 667 m^2. The experimental design of the apple test is shown in Table 1.

The tomato test was conducted in three greenhouses in Yangling County in the Shaanxi Province, from February to August 2015. The test group was fertilized with only Difulai. Each greenhouse was divided into two test plots for the test group and control group, respectively; the area of each plot was 333 m^2. The experimental design of the tomato test is shown in Table 2. The control groups in both experiments were fertilized with chemical fertilizer as in the previous seasons.

Table 1 Experimental design of the apple group.

Group	Treatment
Control	Base fertilizer: chemical fertilizer, applied at 0.22 kg/m2, (urea: DAP: potassium sulfate = 1:1:1) Top dressing: chemical fertilizer, applied at 0.13kg/m2, on June 20 (urea: DAP: potassium sulfate = 1:1:1)
Test	Base fertilizer: chemical fertilizer, applied at 0.22 kg/m2, (urea: DAP: potassium sulfate = 1:1:1) Top dressing: Difulai microbial fertilizer, applied at 0.6 ml/m2, on April 18 and June 20.

Base fertilizer used on March 5. DAP: diammonium phosphate

Table 2 Experimental design of the tomato group.

Group	Treatment
Control	Chemical fertilizer: N-P2O5-K2O:0.024-0.012-0.15 kg/m2, applied three times: on February 4, February 26, and April 5
Test	Difulai microbial fertilizer, 0.6ml/m2, applied as above

2.4 Determination of related indices

In the apple test, six apple trees were selected randomly from each plot, and an apple was picked randomly from each side of the tree. A total of 24 apples were picked. Their main indices, such as transverse diameter, longitudinal diameter, fruit weight, sugar content, and hardness were measured (Dong et al., 2011; Xu et al., 2011; Bai, 2012). Fifty leaves were selected randomly in each plot from the middle part of annual branches in the central crown of the apple trees; the thickness and weight of these leaves were measured.

At the flowering stage of the tomato plants, three rows of plants were randomly selected and marked from each test plot. Ten tomato plants were randomly selected from each row. The plants from top to bottom were divided into four layers; the bottom one was the first layer, and the top one was the fourth layer. The number of flowers in each layer was then counted. In the midway through fruit maturation, the plant height and the stem diameter (the measuring point in the middle of each layer) of all the selected tomato plants were measured by the tape and vernier caliper. At the same time, we also measured transverse diameter, longitudinal diameter, sugar content, and hardness of three tomato fruits in the second layer of each selected tomato plant by vernier caliper, saccharimeter and hardness tester.

2.5 Data analysis

The data were analyzed using Microsoft Excel 2007 Office and IBM Statistics SPSS 20.

3 Results

3.1 Fertilizer test in apple orchard

The yield is determined by the amount of fruit and the weight of a single fruit, and the amount of fruit is mainly determined by the process of blossom and fruit thinning which was standardized in the test, thus the yield and the fruit weight were expected to show a positive correlation. In this test, the fruit weight was used to characterize the yield. The results of variance analysis of the single apple weight are shown in Table 3.

Table 3 Variance analysis of the single-apple weight.

Variation source	DF	SS	MS	F	
Between groups	1	598.93	598.93	25.58**	$F_{0.05}$ (1, 6) = 5.99
Within groups	6	140.47	23.41		$F_{0.01}$ (1, 6) = 13.75
Total	7	739.40			

As indicated in Table 3, the variation between the control and test groups was significantly higher than that within groups; thus, the difference of soil fertility between the control and test groups could be ignored. The variation was generated mainly by the different treatments.

The results of the apple test are shown in Table 4.

Table 4 The results of the apple test.

Treatment		Transverse diameter (mm)	Longitudinal diameter (mm)	T-diameter /L-diameter	Fruit weight (g)	Sugar content (%)	Hardness (kg/cm2)	Leaf thickness (mm)	Leaf weight (g)
Control	1	83.37	70.99	1.17	244.20	14.96	7.08	0.171	0.353
	2	82.86	71.07	1.17	247.22	14.78	6.91	0.160	0.357
	3	83.72	72.83	1.15	251.00	14.28	6.73	0.172	0.355
	4	85.03	71.67	1.19	251.11	15.08	6.77	0.170	0.357
Test	1	86.83	74.42	1.17	269.18	14.73	6.86	0.180	0.377
	2	85.01	72.77	1.17	262.43	15.03	6.82	0.184	0.368
	3	84.70	75.44	1.12	272.07	14.21	6.72	0.183	0.369
	4	85.36	72.60	1.18	259.07	14.89	7.01	0.183	0.364
Mean	Control	83.75a	71.64b	1.17a	248.38b	14.78a	6.87a	0.168b	0.356b
	Test	85.48a	73.81a	1.16a	265.69a	14.72a	6.85a	0.183a	0.370a
Increased by (%)		—	3.03	—	6.97	—	—	8.93	3.93

For each response variable, values (means of four replicates) not sharing a common letter differ significantly ($p < 0.05$) from each other (t-test).

As indicated in Table 4, there is a significant difference in transverse diameter, sugar content, and hardness between the two groups, but the differences in leaf thickness, fruit weight, leaf weight, and fruit longitudinal diameter were significant. Their values in the test group increased by 8.93%, 6.97%, 3.93%, and 3.03%, respectively. Increased leaf thickness and weight indicated the enhanced vigor of the apple trees and the potential to improve yield, the significantly increased fruit weight reflected the increased yield. The hardness is mainly associated with the annual mean temperature and daily temperature range, whereas the sugar content is mainly related to air temperature, daily temperature range, precipitation, sunlight intensity, and relative humidity (Yu and Pu, 1991; Gao et al., 2009; Duan et al., 2014). The differences in these two indices were not significant between the two groups.

The eight indices in Table 4 were defined as X1-X8 respectively, and then Pearson correlation analysis was performed for each pair of indices; the results are shown in Table 5.

Table 5 Pearson correlation values in the apple test.

	X1	X2	X3	X4	X5	X6	X7	X8
X1	1.000	0.609	0.154	0.746*	0.126	-0.171	0.705	0.832*
X2	0.609	1.000	-0.684	0.937**	-0.640	-0.548	0.711*	0.794*
X3	0.154	-0.684	1.000	-0.474	0.894**	0.481	-0.271	-0.215
X4	0.746*	0.937**	-0.474	1.000	-0.365	-0.427	0.803*	0.929**
X5	0.126	-0.640	0.894**	-0.365	1.000	0.544	-0.116	-0.129
X6	-0.171	-0.548	0.481	-0.427	0.544	1.000	-0.138	-0.235
X7	0.705	0.711*	-0.271	0.803*	-0.116	-0.138	1.000	0.713
X8	0.832*	0.794*	-0.215	0.929**	-0.129	-0.235	0.713*	1.000

Level of significance: * $p < 0.05$, ** $p < 0.01$. X1-X6 stand for the apple fruit transverse diameter (mm), longitudinal diameter (mm), t-diameter /l-diameter, fruit weight (g), sugar content (%) and hardness (kg/cm^2). X7 and X8 stand for the apple leaf thickness (mm) and weight (g).

As shown in Table 5, the Pearson correlation values for X4 and X2, X4 and X8, and X3 and X5 are 0.937, 0.929, and 0.894, respectively, reaching the extremely significant level. The value for X7 and X4 is 0.803, reaching the significant level. Their unary linear regression equations are shown below:

$X4 = -190.821 + 6.158 \cdot X2$; $r2 = 0.879$, $F = 43.542$, $p = 0.0006 < 0.01$**
$X4 = -155.802 + 1138.862 \cdot X8$; $r2 = 0.863$, $F = 37.807$, $p = 0.0008 < 0.01$**
$X5 = -1.347 + 13.813 \cdot X3$; $r2 = 0.798$, $F = 23.773$, $p = 0.0028 < 0.01$**
$X4 = 87.095 + 969.0081 \cdot X7$; $r2 = 0.645$, $F = 10.899$, $p = 0.0164 < 0.05$*

Based on the stepwise regression equation between X4 and other indices, two linear regression models are obtained:

$X4 = -223.388 + 3.553 \cdot X2 + 612.539 \cdot X8$; $r2 = 0.971$, $F = 84.417$, $p = 0.0001 < 0.01$**

The above results show that there is a significant linear relationship between fruit weight and longitudinal diameter, leaf thickness, and leaf weight. Thus, the rate of change of these three indices can give an indication of the fruit weight, and thus the yield. The apple yield can be compared between different treatments during the growth period based on the regression equation between X4 and X8. Sugar content can be predicted from the transverse diameter/longitudinal diameter ratio based on the regression equation between X5 and X3. This method can avoid the mechanical damage to apple samples during the determination of sugar content.

Table 6 Total number of flowers per tomato plant.

Treatment		1st layer	2nd layer	3rd layer	4th layer	Total
Control	1	4.7	5.0	5.0	5.1	19.8
	2	6.2	6.0	5.2	8.3	25.7
	3	5.4	5.5	5.4	6.4	22.7
Test	1	4.9	5.6	5.7	5.7	21.9
	2	7.5	6.1	6.0	7.8	27.4
	3	5.9	5.8	5.5	6.9	24.1
Mean	Control	5.4a	5.5a	5.2a	6.6a	22.7b
	Test	6.1a	5.8a	5.7a	6.8a	24.5a
Increased by (%)		—	—	—	—	7.93

For each response variable, values (means of three replicates) not sharing a common letter differ significantly ($p < 0.05$) from each other (t-test).

3.2 Fertilizer test in tomato greenhouse

The average flower number of tomato plants is shown in Table 6. The results of the t-test showed that the difference in the flower number of the same layer between two groups was not significant, but the differences in total flower number of the plants were significant. The total flower number in the test group is 7.93% higher than that of the control group. From the results of the t-test in Table 7, one can see significant differences in plant height and fruit hardness, transverse diameter, and sugar content between the two groups. The values of these indices in the test group increased by 14.24%, 7.92%, 6.84%, and 4.03%, respectively. Plant diameter and fruit longitudinal diameter did not show significant differences. Because the fruit transverse diameter was larger in the test group and the longitudinal diameter did not significantly differ between the two groups, the fruit size was larger than that in the control group. At the same time, the tomato plants in the test group had more flowers; thus, yield was higher in the test group than in the control group. These results are consistent with the findings of previous studies which showed that tomato fertilized with organic fertilizer had higher fruit sugar content than that fertilized with chemical fertilizer (Chu and Ye, 1987; Wang et al., 2013).

Table 7 The results of the tomato test.

Treatment		Plant		Fruit				
		Height (cm)	**Diameter (mm)**	**Transverse diameter (mm)**	**Longitudinal diameter (mm)**	**T- diameter /L- diameter**	**Sugar content (%)**	**Hardness (kg/cm2)**
Control	1	171.0	10.99	76.33	61.29	1.24	4.76	3.09
	2	162.0	14.53	64.25	49.04	1.31	4.72	2.94
	3	164.0	12.43	72.47	57.06	1.27	4.67	3.06
Test	1	195.0	11.76	81.36	62.73	1.30	4.90	3.31
	2	184.0	14.98	67.62	52.49	1.29	4.93	3.27
	3	189.0	14.26	78.65	60.97	1.29	4.89	3.23
Mean	Control	165.7b	12.65a	71.02b	55.80a	1.27a	4.72b	3.03b
	Test	189.3a	13.67a	75.88a	58.73a	1.29a	4.91a	3.27a
Increased by (%)		14.24	—	6.84	—	—	4.03	7.92

For each response variable, values (means of three replicates) not sharing a common letter differ significantly ($p < 0.05$) from each other (t test).

The seven indices in Table 7 were defined as X1-X7 respectively, and then Pearson correlation analysis was performed for each pair of indices. The results are shown in Table 8.

Table 8 Pearson correlation values in the tomato test.

	X1	**X2**	**X3**	**X4**	**X5**	**X6**	**X7**
X1	1.000	0.024	0.656	0.561	0.267	0.921**	0.952**
X2	0.024	1.000	-0.627	-0.710	0.661	0.299	-0.008
X3	0.656	-0.627	1.000	0.983**	-0.278	0.349	0.600
X4	0.561	-0.710	0.983**	1.000	-0.447	0.263	0.536
X5	0.267	0.661	-0.278	-0.447	1.000	0.328	0.123
X6	0.921**	0.299	0.349	0.263	0.328	1.000	0.897*
X7	0.952**	-0.008	0.600	0.536	0.123	0.897*	1.000

Level of significance: * $p < 0.05$, ** $p < 0.01$. X1 and X2 stand for the tomato plant height (cm) and diameter (mm) X3-X7 stand for the tomato fruit Transverse diameter (mm), Longitudinal diameter (mm), T- diameter /L- diameter, Sugar content (%) and Hardness (kg/cm^2).

As indicated in Table 7, the Pearson correlation values for X1 and X6, and X1 and X7 are 0.921 and 0.952, respectively, reaching the extremely significant level. The value for X7 and X6 is 0.893, reaching the significant level. Their unary linear regression equation is shown as below:

$X6 = 3.519 + 0.007 \cdot X1$; $r2 = 0.849$, $F = 22.413$, $p = 0.0091 < 0.01$**
$X7 = 1.393 + 0.010 \cdot X1$; $r2 = 0.907$, $F = 38.849$, $p = 0.0034 < 0.01$**
$X6 = 2.663 + 0.682 \cdot X7$; $r2 = 0.805$, $F = 16.465$, $p = 0.0154 < 0.05$*

These results show that plant height has a significant linear relationship with sugar content and hardness, and so does hardness with sugar content. Thus, sugar content can be predicted based on hardness and plant height, and hardness can be predicted based on plant height.

4 Discussion and Conclusions

In the apple test, which used chemical fertilizer as base fertilizer in both groups, and Difulai as top dressing in the test group, the yield (indicated by apple weight) was increased. The increase in leaf thickness and weight indicated that the Difulai microbial fertilizer could promote nutrient assimilation and enhance vigor in apple trees. Owing to the low soil organic matter content and the lack of organic fertilizer in most orchards, the rate of fertilizer utilization is very low (Zhao, 2007; Wang et al., 2007). In this case, it is not feasible to improve the yield and quality of the apples by continuing to use excessive amounts of fertilizer. It is necessary to improve the soil structure and increase apple trees' absorption and utilization of nutrients. The effect of Difulai in this test is evident; therefore, it can replace chemical fertilizer as top dressing, and thereby reduce the harm of excessive use of chemical fertilizer in the top-dressing process.

In the tomato test, using Difulai only in the test group, the yield increased. Moreover, the fruit sugar content and hardness and plant weight increased. the fruit quality and tree vigor were enhanced compared to the control group. Chemical fertilizer, which is commonly used these days in tomato greenhouses, can be replaced with Difulai microbial fertilizer. The noticeable effect of Difulai may be related to the accessible irrigation facilities in the greenhouse which lead to improved ambient conditions for microbial fertilizer to take effect.

In addition, this study proposes a new method to characterize apple yield by using the weight of a single apple. This method is especially suitable for comparing apple yield among different treatments. Linear relationships can be seen between apple fruit weight and leaf weight, fruit diameter and leaf thickness, as well as sugar content and the transverse diameter/longitudinal diameter ratio. The apple yield can also be predicted during the growth period on the basis of the linear regression model of fruit weight and leaf weight. Linear relationships were also observed between tomato plant height, fruit sugar content, and fruit hardness, and between fruit sugar content and hardness.

In conclusion, the application of Difulai microbial fertilizer showed a endorsing effect on the yield and quality of apples and greenhouse tomatoes in the Weibei Loess Plateau, China. Satisfactory effects can be achieved in fruit-producing areas by using chemical fertilizer as base fertilizer and Difulai as top dressing, and by using only Difulai in greenhouses or areas where irrigation facilities are accessible and soils are rich. Thus, it is feasible to promote the application of microbial fertilizers in this arid area, and reduce the excessive use of and reliance on chemical fertilizers, which will lead to an improvement of the quality and yield of food crops, and development of sustainable agriculture.

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