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Effect of manure and NPK to increase soil bacterial population of *Azotobacter* and *Azospirillus* in chili (*Capsicum annum*) cultivation

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Abstract. *Mujiyati, Supriyadi. 2009. Effect of manure and NPK to increase soil bacterial population of* Azotobacter *and* Azospirillum *in chili (*Capsicum annum*) cultivation. Nusantara Bioscience 1: 59-64.* The objectives of this research were to find out the increasing number of two bacterial populations, *Azotobacter* and *Azospirillum,* due to the use of manure fertilizer. The experiment was conducted using group randomly designed with two treatments. The plant populations were treated (i) without fertilizer as the control, (ii) with manure fertilizer, and (iii) with NPK fertilizer. Data was experimentally collected by planting chili in several plots treated by manure, with three replications. The field experiment was conducted in Gathak Village, Karangnongko Sub-district, Klaten District, Central Java. The data collected consist of the total population of *Azotobacter* and *Azospirillum*, nitrogen content in soil and the chili yield. The primary data of research were analyzed using ANOVA test and followed by LSD test, with the degree of significance by 95 %. The results showed that the manure fertilizer can increase the population of bacteria as many as 0.02% (*Azotobacter*) and 0.46% (*Azospirillum*) when they were compared to the control one. So that it can increase the soil fertility when they were used in long time. Therefore increasing the nutrient availability in the soil was occurred. Application of manure fertilizer could increase the total nitrogen content in the soil and it is very useful for the fertilizing of plants.

Keywords: chili, manure fertilizer, *Azotobacter*, *Azospirillum*, nitrogen, fixation.

Abstrak. *Mujiyati, Supriyadi. 2009. Pengaruh pupuk kandang dan NPK terhadap populasi bakteri* Azotobacter *dan* Azospirillum *dalam tanah pada budidaya cabai (*Capsicum annum*). Nusantara Bioscience 1: 59-64.* Tujuan penelitian ini adalah mengetahui peningkatan populasi bakteri *Azotobacter* dan *Azospirillum* akibat pemberian pupuk kandang. Percobaan menggunakan rancangan acak kelompok dengan perlakuan: (i) tanpa pupuk sebagai kontrol, (ii) dengan pupuk kandang, (iii) dengan pupuk NPK. Data dikumpulkan secara eksperimen dengan menanam cabai pada beberapa petak percobaan dengan perlakuan penggunaan pupuk sebanyak tiga kali ulangan. Data yang dikumpulkan terdiri atas jumlah populasi *Azotobacter* dan *Azospirillum*, kandungan nitrogen dalam tanah dan hasil cabai. Percobaan lapangan dilakukan di Desa Gathak, Kecamatan Karangnongko, Kabupaten Klaten, Jawa Tengah. Data primer dari hasil penelitian di analisis dengan uji ANAVA dan dilanjutkan dengan uji BNT dengan tingkat kepercayaan 95%. Hasil penelitian menunjukkan bahwa menggunakan pupuk kandang dapat meningkatkan populasi bakteri *Azotobacter* (0,02%) dan *Azospirillum* (0,46%) apabila dibandingkan kontrol, sehingga dapat meningkatkan kesuburan tanah dalam waktu yang cukup lama yaitu dengan meningkatkan ketersediaan hara dalam tanah. Kandungan nitrogen total tanah setelah diberi pupuk kandang juga meningkat dan sangat bermanfaat untuk bahan penyusun tubuh tumbuhan.

Kata kunci: cabai, pupuk pupuk kandang, *Azotobacter, Azospirillum*, nitrogen, fiksasi.

INTRODUCTION

Soil is an important environmental factor, because it has a close reciprocal relationship with the plants growing on it and the soil microbes that exist in it. Soil generally contains many nutrients needed by plants. Nevertheless, nutrient content in agricultural land is slowly reduced because it is absorbed by plants to meet its growth needs (Sutejo et al. 1991). The continuous intake of nutrients by plants from the soil will result in poor nutrients soil, which will result in the degradation of soil fertility, the plant growth, and its productivity will be disturbed (Syekhfani 2003). The addition of nutrients is needed to cope with this circumstance, namely through fertilization.

The types of fertilizer that can be given to soil for nutrients additions are organic fertilizers and inorganic fertilizers. According to Winston (1999), the use of inorganic fertilizers continuously and excessively, without using organic fertilizers as a balancer may cause the soil becoming barren and decrease productivity. Therefore, organic fertilizer needs to be added to increase nutrient content, both the macro and microelements. Manure can improve the physical, chemical and biological properties of soil through its role as a source of food microbes in the soil (Sugito et al. 1995) and increase the microbes' varieties and populations so that the activity of microbes in the soil increases (Sarief 1989).

Soil plays important role in the mineral cycle consisting mainly of the nitrogen, phosphorus, sulfur and carbon cycles. The bacteria that play roles in the nitrogen cycle are *Azotobacter* and *Azospirillum*. The bacteria are non-symbiotic which are capable of binding free N_2.

Azotobacter, for example, is the bacteria that live in rhizosphere areas which are heterotrophic. These bacteria have a function as a binder of free N_2 which influence physical and chemical properties of soil to increase soil fertility. The population of nitrifying bacteria in the soil affects the concentration ratio of nitrogen in the soil, so that the microbial population is an indicator of soil fertility levels (Allen and Allen 1981). The use of manure can improve soil fertility for a long period of time.

This study aims to: (i) determine the effect of manure and NPK in increasing the bacteria population of *Azotobacter* and *Azospirillum* in the rhizosphere area of chili planting, (ii) determine the effect of manure and NPK on soil chemical improvement in the rhizosphere area of chili planting, (iii) determine the effect of manure and NPK on component of chili yield.

MATERIALS AND METHODS

The field research was conducted in the Gathak Village, Karangnongko Subdistrict, Klaten District, Central Java. Identification of types and populations of free-nitrogen banding bacteria was conducted in Laboratory of Science, Faculty of Soil Agriculture, Sebelas Maret University, Surakarta. The study was conducted in September 2006-March 2007. Seed material used was a fantastic variety of large hybrid chili (*Capsicum annuum*), where the characteristics are found in Kepmentan (2006).

This type of research is an experiment by using a randomized complete block design (RCBD). The independent treatments variables are the fertilizer namely: the type of fertilizers (manure and NPK fertilizer). The dependent variables consist of: (i) The population of *Azotobacter* and *Azospirillum*, (ii) the character of soil chemistry (total nitrogen, Cation Exchange Capacity (CEC), organic carbon, organic matter), and (iii) The yield of chili. The layout of experimental design is shown in Figure 1.

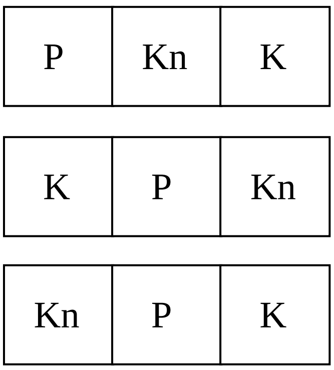


Figure 1. Plan of chili planting experiment with two fertilizer treatments (Gomez and Gomez 1983). Note: K: control (no NPK fertilizer/manure). P: given with a dose of NPK fertilizer application of 200 mL/plant. Kn: given with manure, the dosage of 2 kg/plant.

This study used experimental methods for data collection. To know the bacteria *Azotobacter* and *Azospirillum*, the land which is associated with the nitrogen cycle, soil content and the content of organic manure and NPK fertilizer was observed in the laboratory. The sample is part of the population taken for examination. The sample population of bacteria was taken from 3 blocks of land used in the experiment, using the drawing to take 5 numbers randomly from plant number one to plant number twenty. Of the 5 points in each treatment, the samples were mixed and immediately brought to the Laboratory of Soil, Faculty of Agriculture, Sebelas Maret University, Surakarta for microbial testing. Calculation of free nitrogen-banding bacteria was conducted using "Plate Count" method. The *Azotobacter* and *Azospirillum* observation method used the method of Rao (1982). To calculate total N concentration used the total nitrogen method of Kjeldahl macro way.

The primary data as the results of experiments were being analyzed with ANOVA test followed by LSD test with 95% confidence level.

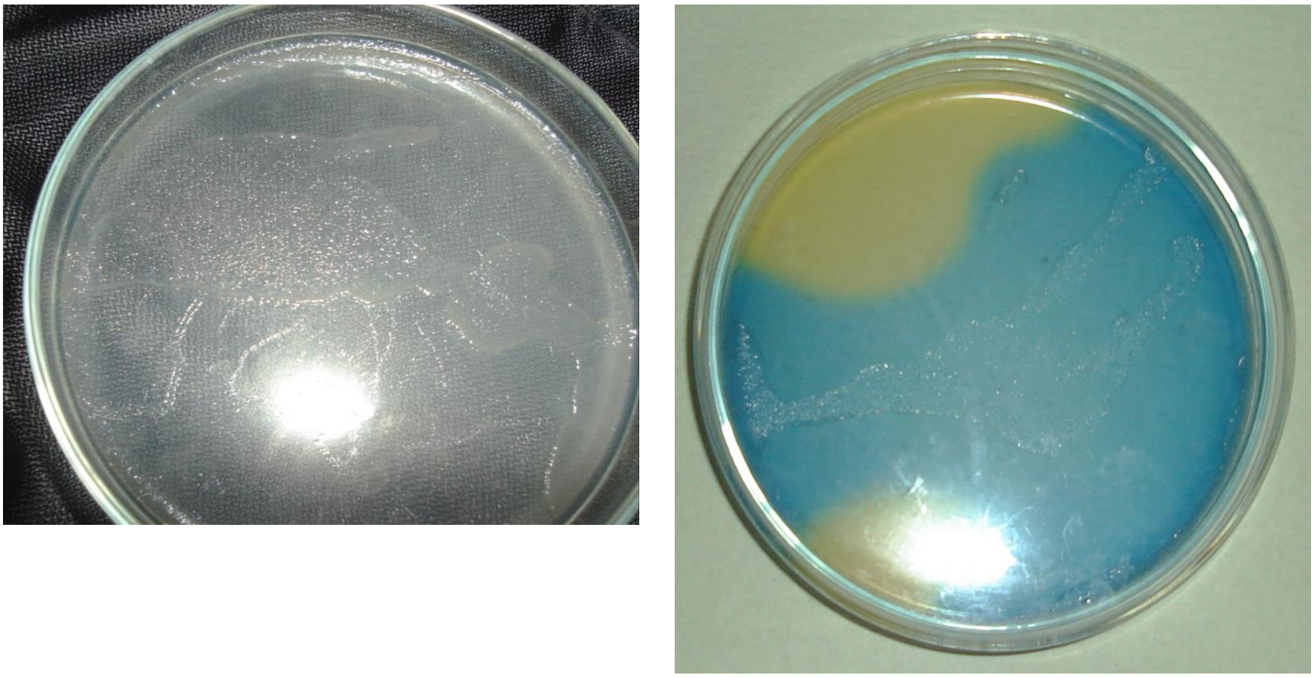
RESULTS AND DISCUSSION

Population of *Azotobacter* and *Azospirillum*

Population of Azotobacter

From the observation of *Azotobacter* population, with different fertilizer treatments, *Azotobacter* population, without any treatment reached the mean of 4333.33 cfu/mg, with manure treatment reached the mean of 4466.66 cfu/mg, whereas *Azotobacter* population, with NPK treatment, reached the mean of 6666.67 cfu/mg (Table 1). From these observations, it can be seen that the giving of NPK may increase the population of *Azotobacter* to the highest value when compared to the manure treatment and control. Manure also increases the *Azotobacter* population, when compared to the controls.

From the analysis of variance, it can be known that there was effect of treatment on the population of *Azotobacter*. The giving of manure did not significantly affect *Azotobacter* population, while the provision of artificial fertilizer significantly increased *Azotobacter*. *Azotobacter* population, with greater manure treatment compared with the control and NPK fertilizer, was 6666.67 (cfu/mg) (b). The population of *Azotobacter* in the control and NPK treatments, respectively, was 4333.33 (cfu/mg) (a) and 4466.66 (cfu/mg) (a) (Table 1). *Azotobacter* population got higher value when compared with manure was understandable due to the granting of NPK fertilizer that can decrease soil pH to 6.31 compared to soil pH with manure treatment which was 7.08 and with no treatment (control) which was 7.08. *Azotobacter* bacteria are commonly found in neutral or acid soil (Rao 1994). Nitrogen banding bacteria, when added with a special substrate, the number of bacteria will increase and gradually decrease if that additional substrate increasingly ran out. Other factors that influence bacterial populations in soil are pH, farming practices, fertilizer and pesticide used and also the addition of organic material.



A B

Figure 2. Colony of bacteria: A. *Azotobacter* spp. (transparent), B. *Azospirillum* spp. (original color is blue).

Azotobacter is a heterotrophic nitrogen fixation bacterium that lives freely and many are found in acid to neutral soil. Fertilization with NPK can increase *Azotobacter*, but if it is done with inorganic fertilizers continuously, it will reduce the level of soil fertility, because the element of K is one nutrient that easily washed, so the soil will lack K element which can reduce soil fertility. *Azotobacter* has a beneficial effect on the rate of seed development, plant growth, standing crop, and vegetative growth. So with the increase in *Azotobacter*, the yields of crops will increase (Rao 1994).

Populations of Azospirillum

From the observation of *Azospirillum* populations, with different fertilizer treatments, the population of *Azospirillum*, without any treatment reached the mean of 52666.66 cfu/mg, with manure treatment reached the mean of 221666.66 cfu/mg, while the population of *Azospirillum*, with NPK treatment, reached the mean of 52833.33 cfu/mg (Table 1). From the observation, it is known that the provision of manure would increase the population of *Azospirillum*, the highest number when compared to the NPK fertilizer treatment and control. When compared to controls, NPK fertilizer would also increase the population of *Azospirillum*.

From the analysis of variance, it can be known that there was effect of treatment on the population of *Azospirillum*. The giving of manure increased the population of *Azospirillum*, whereas NPK fertilizer did not affect the population of *Azospirillum*. The number of *Azospirillum* populations was greater with manure treatment than with the control and NPK fertilizer treatment namely 221,666.66 (cfu/mg) (b). While *Azospirillum* populations in the control and the NPK fertilizer treatments, respectively, were 52666.66 (cfu/mg) (a) and 52833.33 (cfu/mg) (a) (Table 1).

Azospirillum is bacteria that has associative symbiotic to mention any fixation nitrogen in plants. *Azospirillum* bacteria require low oxygen conditions, and can grow rapidly in environments containing ammonium without fixating nitrogen. *Azospirillum* is bacteria that can encourage the growth of various plant species, where this advantageous ability is because of its ability to produce phytohormone, including gibberellins (Okon and Labandera-González 1994; Cassán et al. 2001).

Manure application can increase the content of ammonium in the soil to obtain a high *Azospirillum* population compared with NPK fertilizer treatment and no treatment. This proves that manure can increase the population of bacteria in the soil. It is expected to increase soil fertility in a long time. With soil fertility, the nutrient availability for plants growing on it is enough, so it can increase crop yields.

Table 1. The result of the mean number of *Azotobacter* and *Azospirillum* treated with two fertilizers (cfu/mg).

Treatment (cfu/mg)	Control	Manure	NPK
Azotobacter	4333.33 (a)	4466.66 (a)	6666.67 (b)
Azospirillum	52666.66(a)	221666.66 (b)	52833.33 (a)

Note: Number followed by the same letter on the same line showed no significant differences in LSD with 95% confidence level.

The chemical content of soil in the relationship of manure and NPK

From soil analysis, the results obtained were the difference of total nitrogen content of various fertilizer treatments. The treatment of NPK fertilizer produced the highest nitrogen content of 0.41%. The treatment of manure produced the nitrogen content of 0.36%. While no treatment (control) produced the nitrogen content of 0.225% (Table 2). The highest total nitrogen content by providing the NPK fertilizer can be understood as it provides direct supply of nitrogen.

Table 2. Results of analysis of soils at location of study, i.e. Gathak Village, Karangnongko Subdistrict, Klaten District.

Treatment	Mean			
	N total (%)	CEC (Me%)	Organic C (%)	Organic matter (%)
Control	0.225	19.795	1.575	2.71
Manure	0.36	24.11	2.47	4.26
NPK	0.41	21.625	2.43	4.18

Note: N total = total nitrogen. CEC = Cation Exchange Capacity.

In soil analysis, the results were the differences of CEC come from various treatments fertilizer. Manure treatment gave the highest number of 24.11%. Treatment with NPK fertilizer produced CEC of 21.625%. And no treatment (control) resulted in CEC of 19.795% (Table 2). The exchange of cations or the nature of alkali exchange in the soil determines the soil fertility and plant nutrition. The exchangeable cations are hydrogen, calcium, magnesium, potassium, sodium, ammonium, manganese, zinc, copper and aluminum. These ions have different energy content that determines its binding with the solid soil material. The energy will also determine the ease of ions to conduct mutual exchanges of ions in the soil. The nature of alkali exchange in the soil allows those ions to be bound and ready as plant nutrition, and also prevent it from being dissolved (Camberato 2001; Rashidi and Seilsepour 2008)

Ions present in the soil. They are positive known as cation (K^+, Mg^{++}, Ca^{++}, Fe^{+++}, Mn^{++}, Zn^{++} and Cu^{++}) and negative known as anions (NO_3^-, $H_2PO_4^-$, SO_4^-, Cl^-, $HB_4O_7^-$ and $HmoO_4^-$). In the soil, ions always flow due to the absorption of ions by plant roots and the release of ions from the soil. Transfer of ions into the roots include ion exchange between roots and soil solution. For example, H^+ ion is released into soil solution and the root replaces it with cation. Similarly, the anion is absorbed by the roots with ion exchange of OH^- and HCO_3^- (Rao 1994). CEC with the highest manure application is very influential with the provision of nutrients in the soil for a long time.

From the soil analysis, the result is different organic carbons derived from different fertilizer treatments. The highest number of organic carbon from manure treatment was 2.47%. Treatment with NPK fertilizer produced organic carbon as high as 2.43%, whereas, without treatment (control), it produced organic carbon as high as 1.575% (Table 2). The organic carbon with the highest manure application is very influential with carbon in the soil nutrient supply. From the soil analysis, the result is different soil organic matters derived from different fertilizer treatments. Manure treatment is highest with 4.26%. Treatment with NPK fertilizer produced 4.18% of soil organic matter. While no treatment (control) produced soil organic matter 2.71% (Table 2).

Soil organic matter is a potential source of nitrogen, phosphorus, and sulfur for plant growth. Microbiology is the decomposing agent for organic materials and it is capable of releasing the nutrient banding from organic materials into a form that can be utilized by plants. Increasing soil organic matter due to the manure application can manage moisture and aeration, soil structure stabilizer, increase the CEC, as a source of nutrients for plants and as a source of energy for the micro body activity (Suryantini 2002).

The effect of manure and NPK on the chili yields

From the observation of chili production with different fertilizer treatments, the average production of chili without any treatment is 0.850 kg/plant, with manure treatment is 0.973 kg/plant, while the average production of chili with NPK treatment is 1.060 kg/plant (Table 3).

Table 3. The yield of hybrid chili (kg/plant)

Treatment	Production (kg/plant)	
	Mean	Notation 5%
Control	0.850	a
Manure	0.973	b
NPK	1.060	c

From the results of observation, it can be seen that the NPK fertilizer treatment increases the production of chili higher than manure treatment and control. Manure treatment also increases the production of chili, but it is not as high as the NPK fertilizer treatment. From the results of data analysis, it can be stated that the provision of artificial fertilizers is very effective on the production of chili, namely increasing the production of chili up to 21% when it is compared with controls, but when it is compared with the manure treatment, the effect on production of chili peppers is up to 8.7% (Table 4).

Table 4. Test results of two fertilizer treatments on production of chili (kg/plant)

Treatment	Mean			Notation 5%
Control	0.850			a
Manure	0.973	0.123		b
NPK	1.060	0.210	0.087	c

The treatment of NPK fertilizer will supply nitrogen, phosphorus, and potassium directly into the ground. Of the three elements, nitrogen has a prominent role in increasing the production of chili. The higher the nitrogen fertilization is the higher the content of chlorophyll on leaf, it is because nitrogen is an important component of chlorophyll which gives the green color on leaves, which is required in the process of photosynthesis. When the process of photosynthesis increases, it also increases the production of chili (Supriyadi 2002).

Although the production of chili is really increased with NPK fertilizer treatment, the provision of inorganic fertilizers continuously can lead to deterioration of soil productivity either chemically, physically and biologically (Adiningsih and Rochayati 1988). On the other hand, the manure treatment can increase the number of *Azospirillum* population and contribute microorganisms to the soil (Roeslan 2004). *Azospirillum* bacteria make the use of nitrogen fertilizer more efficient. In addition, *Azotobacter* and *Azospirillum* also have the ability to produce growth hormone which is useful for root growth, so it will increase the plant growth (Gunarto 2000).

The highest number of bacteria is obtained with the treatment of manure. Manure is one of crop cultivation components which are environmentally friendly and plays a role in improving soil fertility, soil structure, both physically, chemically or biologically (Sarwanto et al. 1997; Sudiarto et al. 2002). Manure has a role in increasing the physical fertility of soil because it can reduce plasticity, increase the aggregate pore space, water availability, viscidity and soil aeration (Janariah and Sulichantini 2004). While in chemical fertility, it plays a role of binding or absorbing larger ions and also increasing cations. In biological fertility, manure has a role in forming tissues that form the body of microorganisms and as the source of energy for soil microorganisms (Sukur 2005). Manure is also able to increase the efficiency of fertilizer usage (Adiningsih and Rochayati 1988).

Discussion

Nitrogen content in soil

After the elements of hydrogen, carbon, and oxygen, Nitrogen is the element that plant needs in large quantities as the building substance for its body. Nitrogen content is generally low and dynamic so its formation and its availability in adequate number need to be measured (Julianto 2004).

The observation results show that the total nitrogen content in soil when compared between the total nitrogen content in soil treated with total nitrogen content in control, are known to greater nitrogen content of the treatment, which is 0.36 (36%) with manure treatment and 0.46 (46%) with NPK fertilizer treatment. The total nitrogen content in soil with the treatment of manure shows the result of 0.36 (36%) while in soil with treatment of NPK shows the result of 0.46 (46%), if it is compared to the total nitrogen content in control. Those two nitrogen contents are categorized into moderate (Jacob 2001). This proves that the addition of NPK fertilizer can increase the nitrogen content in soil. The increasing of nitrogen in the soil will affect the soil in providing the nutrients needed by plants. The nitrogen content in soil with the NPK fertilizer treatment had higher levels when it is compared with the total nitrogen content in soil with manure treatment. This is understandable because by giving NPK fertilizer, the nitrogen will be available directly in a short time.

The content of nitrogen in the soil with manure treatment is smaller than that of NPK fertilizer. This occurred because the appliance of manure into the soil will produce CH_4. Methane gas is released into the atmosphere in the form of NO_2, which is the result of nitrification and denitrification processes (Mulyadi et al. 2002). So, if the total nitrogen of soil with NPK treatment is calculated, it will give a higher number than of soil with manure treatment.

Cations Exchange Capacity

CEC is very important to be understood because it provides the criteria for soil fertility. The higher the CEC is, the higher the level of soil fertility in the long term (Sukur; 2005). With manure treatment, the obtained CEC results are higher than the one with NPK fertilizer treatment. With manure treatment, CEC reaches the value of 24.4% including a high criterion, while without treatment (control) the CEC value is 19.79%, which is moderate, and with NPK fertilizer treatment the CEC value is 21.63%, which is moderate too (Jacob 2001) (Table 2). If CEC in the soil is high, it indicates that the soil has a high negative charge so the soil can absorb more potassium; henceforth it will be released again when the potassium concentration in solution decreases (Subiksa et al. 2004).

K fertilization increases the saturation of potassium on the sorption complex, so that the potassium's ability to bind and to support the potassium change in the solution decreased. Potassium is a nutrient that is easily washed; as a consequence, land will often lack this element. The treatment of manure can increase the CEC with the result that it will improve long-term soil fertility because the nutrient in soil is available for long-term crop. The relation with population of microbes to the treatment of manure is that it can increase the population of bacteria.

CONCLUSION

Manure increases *Azotobacter* population by 29% and the population of bacteria *Azospirillum* by 68%, while the NPK fertilizer increases *Azotobacter* population by 43% and *Azospirillum* population by 16% in rhizosphere areas of chili planting. Manure treatment increases the total nitrogen by 36%, the Cations Exchange Capacity by 24.36%, the organic carbon by 2.45% and the organic material by 4.25%, while the NPK fertilizer increases the total nitrogen by 41%, the CEC by 21.63%, the total nitrogen by 41%, the organic carbon by 2.43% and the organic materials by 4.21% in the rhizosphere area of chili planting. Manure treatment increases chili yields by 34% while NPK fertilizer increases chili yield by 37%.

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