

Full Length Research Paper

Evaluation of the impact of various tillage systems on rhizobiyal parameters

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Accepted 15 July, 2020

The effects of different soil tillage methods on rhizobial nodulation, biomass yield (root, shoot and nodule) and nitrogen content of soybean as a second crop have been investigated. The experiment was conducted as a field trial in Cukurova Region, Turkey in 2008. Six different tillage methods have been studied in this research. These methods were conventional tillage with residue (CTR), conventional tillage with burnt residue (CTBR), reduced tillage with heavy disking (RTHD), reduced tillage with rotary tiller (RTR), no- tillage with heavy disking (NTHD) and no tillage with direct seeding (NTDS). At the stage of inflorescence, nodulation was investigated in the root, shoot, and nodule samplings by determining the weights of the samples and analyzing them for nitrogen contents. The results have been statically analyzed by using MSTAT-C package programme. According to the results of the study, under the conventional methods in which deep tillage operations are applied to soil, it has been found out that nodule formation and plant nitrogen contents have been negatively affected. The differences among the applications were found to be statistically significant. In general, the best nodulation has been obtained with NTDS and RTHD plots. Moreover, the effects of other tillage methods on some parameters related with rhizobiyal N₂-fixation have caused to considerable decrease on the parameters mentined above.

Key words: N₂-fixation, soil tillage methods, microbial activity.

INTRODUCTION

Soil tillage methods have complex effects on physical, chemical and biological properties of soil. Because of the changing physical and chemical properties of soil by soil tillage methods, the biological properties of soil may also change. Actually these changes are indirect results of tillage. Changed physical and chemical soil properties by soil tillage methods effect the parameters directly related with soil microbial activities such as organic matter, soil humidity, temperature and ventilation as well as the

degrees of interaction between soil mineral and organic matter. As a result of these effects, significant differences can be observed in the population of microbial activities in soil (Wardle, 1995; Lavelle, 2000; Kladvko, 2001; Sagar et al., 2001).

Microbiological properties of soil are affected from any soil problem or productivity and they are accepted as indicator (Sagar et al., 2001; Jinbo et al., 2007). Soil microorganisms are important elements for ecosystem that have active roles on properly functioning of nutrition circulation mechanism. Disconnection or variation at any links of many reaction chains affecting nutrition circulation mechanism can also cause changes the plant-microorganism interactions. Plant and microorganism interactions in rhizosfer region are very important for plant growth. In the rhizosfer region, rhizobial and mycohorizal activities occur as reciprocal and compulsory interactions (symbiosis) of plant-microorganism (Altieri, 2000; Garcia and Altieri, 2005). One of the important

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Abbreviations: CTR, Conventional tillage with residue; CTBR, conventional tillage with burnt residue; RTHD, reduced tillage with heavy disking; RTR, reduced tillage with rotary tiller; NTHD, no- tillage with heavy disking; NTDS, no tillage with direct seeding; CRB, complete randomized block.

activities related to soil qualities is beneficial micro-organism activities. The most important of these activities is a root nodule bacterium which provides biological N₂-fixation (Ferreira et al., 2000).

Microorganisms, that are important parts of the nature, are considerably affected by the environmental conditions. These organisms which rapidly reproduce and function in proper environmental conditions, also struggle to continue their functions under poor conditions (Dogan et al., 2007). Although N₂ exists in the atmosphere with the rate of 78%, it is not in the useful form for plants and animals. Lots of creatures can't sustain their life activities in the lack of N₂, despite its abundance in the atmosphere. Atmospheric N₂ has to be converted to available form (NH₄⁺, NO₃⁻) for plants. These conversions are carried out mostly by bacteria. Symbiotically N₂-fixation rhizobiums and leguminosae plants are very important for annual nitrogen fixation balance and these bacteria add 75 tonne nitrogen in a year all over the world (Lindermann and Glower, 2003; Dogan et al., 2007). In agricultural systems, mean symbiotic nitrogen fixation is 150 – 200 kg/ha and it may reach the value of 300 kg/ha when grafting is done suitable rhizobium strain (Gok et al., 1995; Lindermann and Glower, 2003).

As a result of symbiotic N₂-fixation, legumes supply nitrogen to the soil not only with their nodules, but also by decomposition of their roots and shoots. Nitrogen might have formed by mixing the separated dead nodule tissues into the soil. This situation can be accelerated by cutting of the plant's shoots (Werner, 1987; Goormachting et al., 2004).

Soybean plant that is an important food supply for human beings and animals introduced to our country in the 1930s and was cultivated only in the Black Sea Region for many years. With the secondary crop project, conducted in the last 20 years, soybean plant has been cultivated in the irrigated Egean and Mediterranean Region soils but today it has been mostly cultivated in Çukurova Region. 80-85% of Turkey's soybean production is came from Adana and Osmaniye, but in recent years it is reported that soybean production has been decreased (FAO, 2005; Arıoğlu, 2007). In this study, the effects of six different tillage systems on rhizobiyal parameters such as nodulation, plant biomass weight and plant nitrogen content of soybean plant were investigated.

MATERIALS AND METHODS

Study field

This study has been carried out in the Arık soil series that is located at Çukurova University Agricultural Faculty's application farm. According to the soil taxonomy classification system, the soil varieties are at Vertisol ordo, Haploxerert big group and Typic Haploxerert sub group (Soil Survey Staff, 1999). Arık series soils are plain and nearly plain 0+1% sloping and deep profiled located on very old alluvial subsoil. They have thick A horizon with A-C

horizon arrangement. The color of soil profile is dark brown or brown. In dry summer months, there are wide-deep cracks that have shiny surfaces and make 60° of angle from vertical and horizontal surfaces in the upper part of the soil. The whole soil profile has clay texture and calcareous, and there is a little calcium wash-outd (Gulez and Senol, 2002).

Climate conditions

Adana, which is in the Mediterranean climate zone, has a warm and rainy winter, dry and hot summer climate. According to 30 years of mean climate data, annual mean temperature is 19.1°C in Adana. Annual total evaporation rate is 1536 mm and total raining rate is 670 mm and 75% of raining occurs in winter and spring. The average climate data for the last three years (2006-2009) including the data for 2008 when the experiment was conducted is given in Table 1.

Experimental design

A Complete Randomized Block (CRB) design was implemented within the field with subplots of different tillage methods. There were six different soil tillage methods and each soil tillage method was replicated three times yielding 18 subplots. Each plot was 12 m in width and 40 m in length covering an area of 480 m².

Soil tillage methods

Six different soil tillage methods have been applied in this study. These methods were conventional tillage with residue (CTR), conventional tillage with burnt residue (CTBR), reduced tillage with heavy disking (RTHD), reduced tillage with rotary tiller (RTR), no-tillage with heavy disking (NTHD) and no tillage with direct seeding (NTDS). These methods were replicated three times. As secondary crop, soybean was planted to the plots after winter wheat. The details of the tillage operations applied for winter wheat and soybean are given in Table 2.

Cultivation, maintenance and harvest processes

Soil tillage applications, mentioned in previous section and wheat cultivation have been performed in 17.11.2007 for the 2007- 2008 production periods. In the years of 2007-2008, 161 kg N ha⁻¹ nitrogen and 40 kg P₂O₅ ha⁻¹ phosphours have been applied as pre-plant and top-dress fertilization. No-irrigation has been applied in the wheat growing season and herbicide has been applied to kill the weeds and wild oats before spiking. Wheat was harvested 25. 05.2008. The next application was to cultivate soybean by using six different soil tillage methods in 30.05.2008 as a secondary crop according to the objectives of the research.

Arisoy variety has been used as a secondary soybean. Before the cultivation Bradyjaponicum bacteria inoculation (400 g/100 kg seed) has been applied to the soybean seeds. Soybean seeds, inoculated with bacteria, were planted in 70 cm of row width and 6 cm of on-row spacing by direct seeding machine. While cultivating soybean, 0.4 kg N da⁻¹ nitrogen (encouragement N) and 0.4 kg P₂O₅ da⁻¹ phosphorus have been applied according to soil test results. After germination, the plots were irrigated 8 times with the 12-13 day- intervals by sprinkler irrigation method. Since climate conditions of the study area was appropriate for development of deseases and pests, herbicide application was made three times for successful weed and pest management. By analyzing root and leaf samples, nodule development was investigated, biomass yield and nitrogen contents were also determined at the blooming stage.

Table 1. 2006-2009 years of climate data that belong to centre of Adana (Anonymous, 2009).

Climate parameters	Months												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average
Max. Temp. (°C)	11.1	12.3	16.5	19.5	24.5	27.3	29.7	30.7	26.9	23.4	17.3	12.7	21
Min. Temp. (°C)	6.8	8.0	10.2	14.2	21.1	25.5	27.9	26.6	24.4	19.6	13.2	8.8	17.2
Average Temp. (°C)	9.1	10.1	13.4	17.4	22.3	26.3	28.8	29	26.1	21.6	15.4	10.7	19.2
Average Mosit. (%)	63.9	71.6	70.8	67.8	68.6	71.8	75.7	76.4	68.2	66.3	67.2	64.6	69.6
Total evaporation (mm)	53.5	50.4	88.4	147.8	183.7	238	253.2	235.6	178	126.1	73	52.7	1674.7
Ave. Total rains (mm)	62.1	111	70.8	44.4	24.9	11.4	14.2	3.2	18.2	68.7	73.3	61.2	563.2

Table 2. Soil tillage methods in the major and secondary crop production.

Soil tillage methods	Soil tillage for winter wheat cultivation	Soi tillage for second crop soybean plant
Conventional tillage with residue (CTR)	<ul style="list-style-type: none"> • Chopping the residues • Plowing (30-33 cm) • Disk horrow (13-15 cm) (2 times) • Packing (2 times) • Wheat planting with a universal planter (4 cm) 	<ul style="list-style-type: none"> • Chopping the residues • Heavy disk horrow (18-20 cm) • Disk horrow (2 times) (13-15 cm) • Packing (2 times) • Soybean planting with Pneumatic-precision seeding machine (8 cm)
Conventional tillage with burnt residue (CTBR)	<ul style="list-style-type: none"> • Burning the residues • Plowing (30-33 cm) • Disk horrow (13-15 cm) (2 times) • Packing (2 times) • Wheat planting with a universal planter (4 cm) 	<ul style="list-style-type: none"> • Burning the residues • Chiseling (35-38 cm) • Disk horrow (13-15 cm) (2times) • Packing (2 times) • Soybean planting with Pneumatic-precision seeding machine (8 cm)
Reduced tillage with heavy disking (RTHD)	<ul style="list-style-type: none"> • Chopping the residues • Heavy disking (18-20 cm) (2 times) • Packing (2 times) • Wheat planting with a universal planter (4 cm) 	<ul style="list-style-type: none"> • Chopping the residues • Rotary tilling (13-15 cm) • Packing (2 times) • Pneumatic-precision seeding machine (8 cm)
Reduced tillage with rotary tiller (RTR)	<ul style="list-style-type: none"> • Chopping the residues • Rotary tilling (13-15 cm) • Packing (2 times) • Wheat planting with a universal planter (4 cm) 	<ul style="list-style-type: none"> • Chopping the residues • Rotary tilling (13-15 cm) • Packing (2 times) • Soybean planting with Pneumatic-precision seeding machine (8 cm)
No-Tillage with Heavy Disking (NTHD)	<ul style="list-style-type: none"> • Chopping the residues • Heavy disking (18-20 cm) • Doting (2 times) • Wheat planting with a universal planter (4 cm) 	<ul style="list-style-type: none"> • Chopping the residues • Herbicide application • Soybean planting with Pneumatic-precision seeding machine (8 cm)
No-Tillage with Direct Seeding (NTDS)	<ul style="list-style-type: none"> • Chopping the residues • Herbicide application • Wheat seeding with direct seeder (4 cm) 	<ul style="list-style-type: none"> • Chopping the residues • Herbicide application • Soybean planting with Pneumatic-precision seeding machine (8 cm)

Table 3. Basic soil properties of the study field (0-30 cm).

pH (1:2.5 H ₂ O)	Salt (mmhoscm ⁻¹)	CaCO ₃ (%)	Org. Mat. (%)	P ₂ O ₅ (kg.ha ⁻¹)	Nmin (kg.ha ⁻¹)	Nt (%)	Sand (%)	Silt (%)	Clay (%)	Texture
7.82	0.15	24.39	1.27	43.5	20.3	0.072	17.5	33.2	49.3	Clay

Table 4. Variance analysis results (P<0,05).

Variance analysis	Root weight (kg/ha)	Shoot weight (kg/ha)	Nodule number (no/plant)	Nodule weight (kg/ha)	Average nodule weight (mg/Nodule)	Root N %	Shoot N %
Mean square	49438.652	6432332.430	266.503	824.843	2.081	0.011	0.047
LSD values	211.8	2416	15.550	27.28	1.374	0.999	0.207

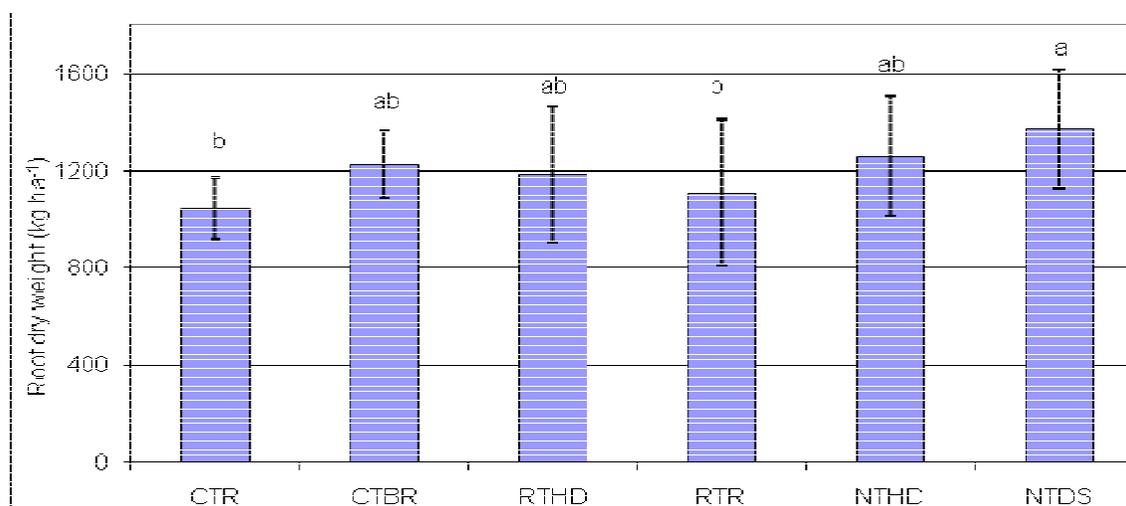


Figure 1. The effects of different soil tillage methods on secondary crop soybean in terms of dry root weight.

Soil analyzes before the experiments

Soil samples, taken from research area, have been applied to texture analyze with hydrometer method according to Bouyoucos (1951), lime has been obtained with Scheibler calcimeter (Caglar, 1949), 1:2.5 mixture of soil- water of total salt, has been determined with electrical conductivity method, and soil reaction has been determined in the same solution with Beckman pH meter (U.S. Salinity Laboratory Staff, 1954). Basic soil properties of the research field have been given in Table 3.

Plant analysis

The dry weights of biomass (kg/ha), number of nodules (number/plant), nodule weight (kg/ha), average nodule weight (mg/nodule), and nitrogen concentrations (%) of root and shoot were determined from the samples taken at the blooming stage of soybean.

Statistical analysis

Variance analysis has been carried out on the data by using

MSTAT-C package programme (Crop and Soil Sciences Department, Michigan State University, Version 1.2). The data was classified by using Duncan Test (Bek, 1983). A Complete Randomized Design (CRD) was implemented within the field.

RESULTS

This study was conducted to investigate the effects of different soil tillage methods on nitrogen fixation parameters to the secondary crop soybean. The variance analysis results are given in Table 4.

Biomass weights of root and shoot

According to the Figure 1, in which the effects of different soil tillage methods to the dry root weight are shown, the highest value (kg/ha) was detected in NTDS as 1372, whereas the lowest was 1044, obtained with stubble CTR. These statistical differences have been found

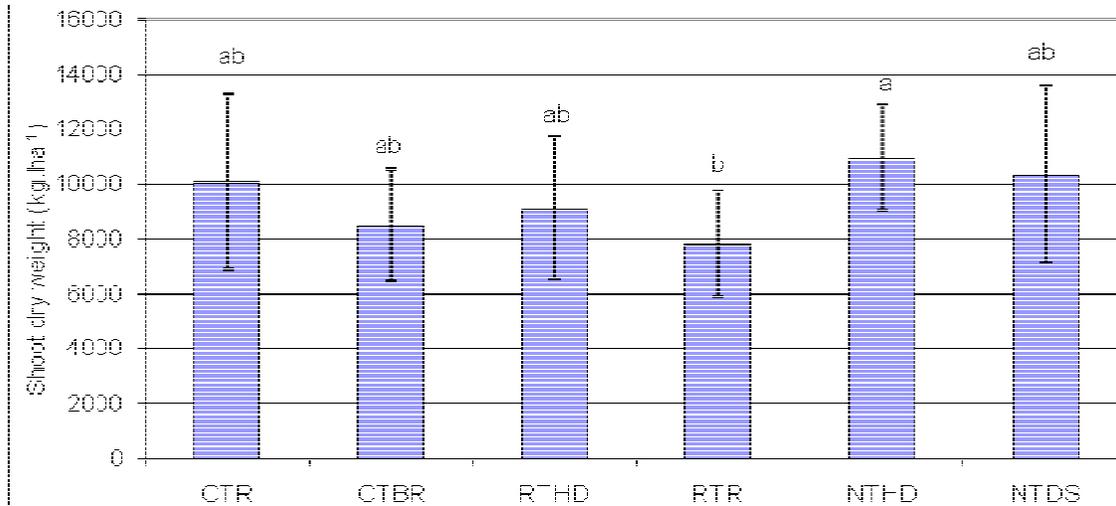


Figure 2. The effects of the different soil tillage methods on the secondary crop soybean plants shoot weight.

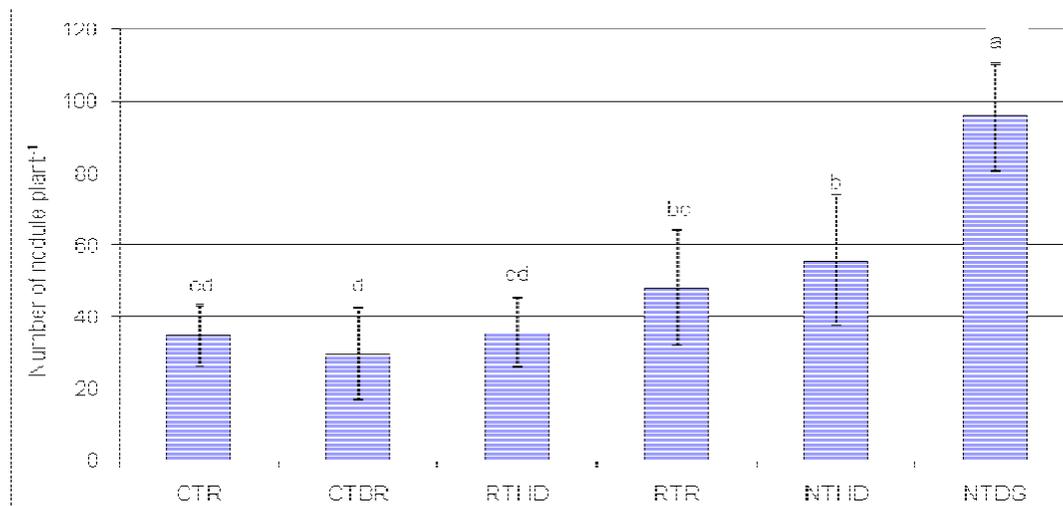


Figure 3. The effects of different soil tillage methods on secondary crop soybean plant.

significant. The similar results were obtained with the other tillage methods.

RTHD gave statistically higher dry root weight (10945 kg/ha) when compared with the other applications (Figure 2). The lowest dry root weight value (7796 kg/ha) was obtained in plots of RTR. Results of plant biomass weight have been affected positively and gave higher values in NTHD and NTDS tillage methods. Similar studies showed that the soil tillage methods not only affect the soil biological properties positively but also affects the plant growth positively (Sidiras et al., 1982; Ferreira et al., 2000).

Nodulation

The effects of the tillage methods on the number of

nodules can be seen in the Figure 3. The number of nodules which is the most important parameter of nitrogen fixation is significantly affected by the soil tillage methods. According to the mean values, the highest nodule number was 96 per plant in NTDS plots. The nodule numbers with the other applications were changed between 30 number/plant (CTBR) and 56 number/plant (NTHD). The low rates of nodules on the plots with burnt residue (CTBR) may have been explained by the destruction of Rhizobium bacteria in dormant form. As it is known, when the residue is burnt the higher temperatures in soil surfaces adversely affect the soil organisms, the amount of organic matter, the availability of soil nutrients, and consequently the soil fertility.

According to the values shown in Figure 3, in the reduced tillage systems (RTHD and RTR) the nodule

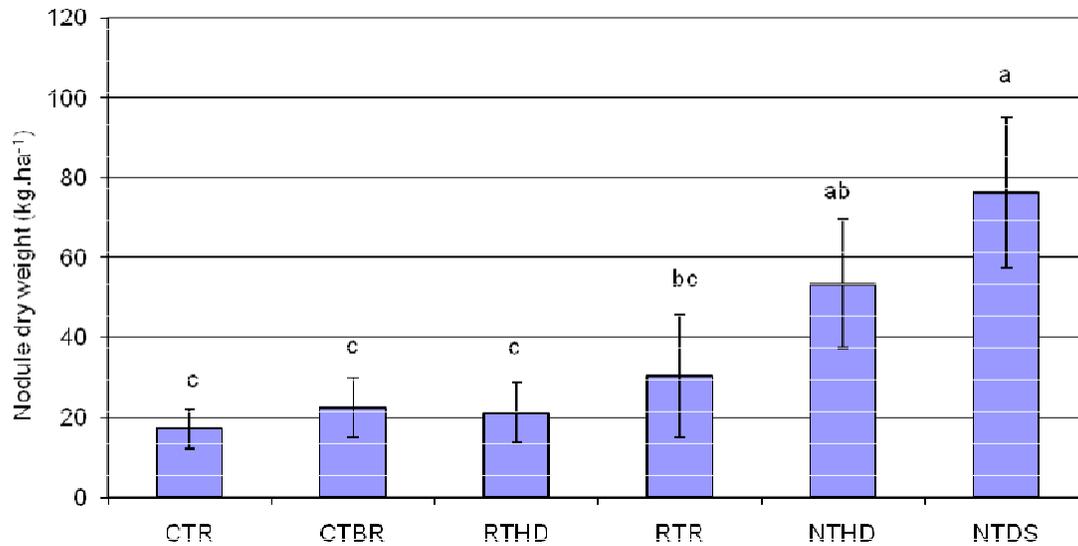


Figure 4. The effects of different soil tillage methods on mean nodule weight.

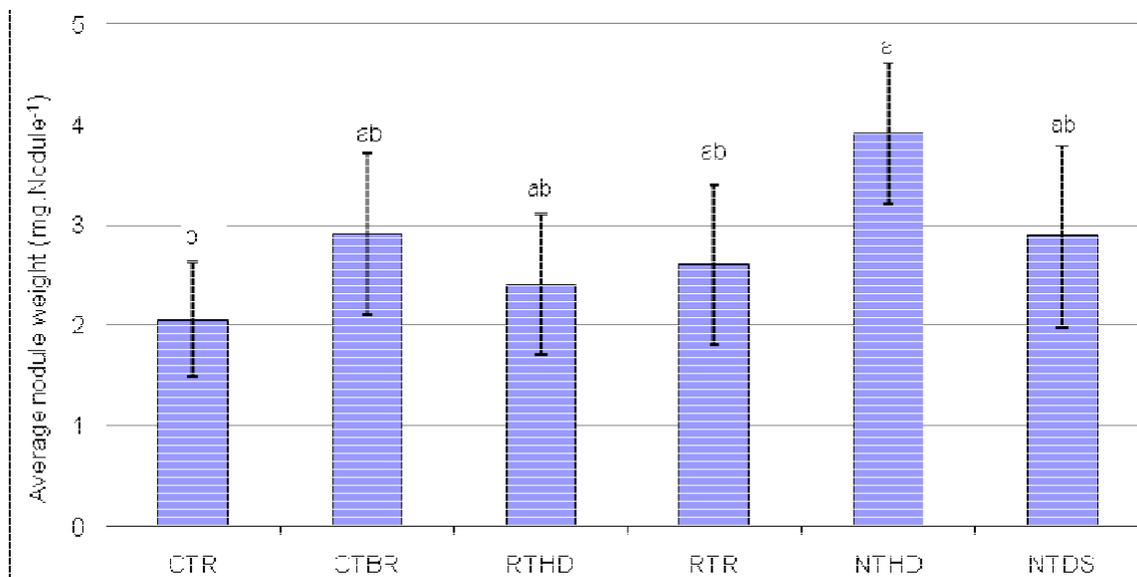


Figure 5. The effects of different soil tillage methods on soybean plant by means of mean nodule weight.

numbers were increased compared to the conventional tillage systems. As the number of tillage operation and the tillage depth are increased, the nodule numbers are decreased.

Many similar studies conducted in different conditions and locations were resulted that no-tillage systems had positive effects on soil microbial activities (Olivares, 2004; Urzua, 2005; Hassen et al., 2007).

Figure 4 shows the dry weights of nodules for different tillage systems. It can be seen that tillage systems have significant effects on the nodule weights. The highest dry nodule values have been observed in the NTDS plots

with 0.763 kg/ha whereas, the lowest values have been observed in CTBR plots and NTHD plots with 17.06-22.40-21.07 kg/ha, respectively.

The dry weights of nodules have been investigated and it was determined that there are some differences among the soil tillage methods (Figure 5). The highest mean nodule weight has been measured on NTHD plots with the rate of 3.91 mg/nodule, whereas the lowest value has been measured on CTR plots with the rate of 2.06 mg/nodule. Other applications (CTBR, RTHD, RTR and NTDS) gave similar results and the mean nodule weights for these applications are 2.91, 2.41, 2.61 and 2.89

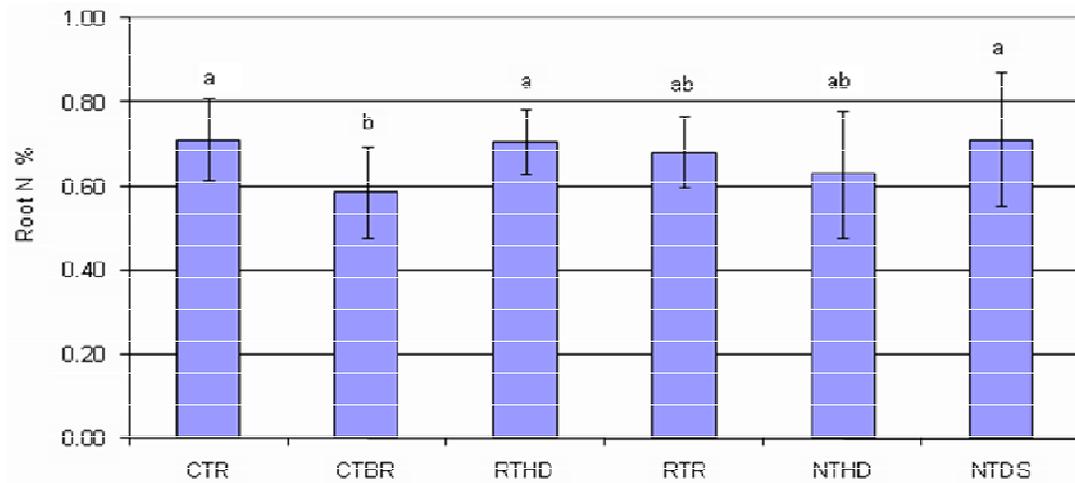


Figure 6. The effects of different soil tillage methods on the concentrations of root N.

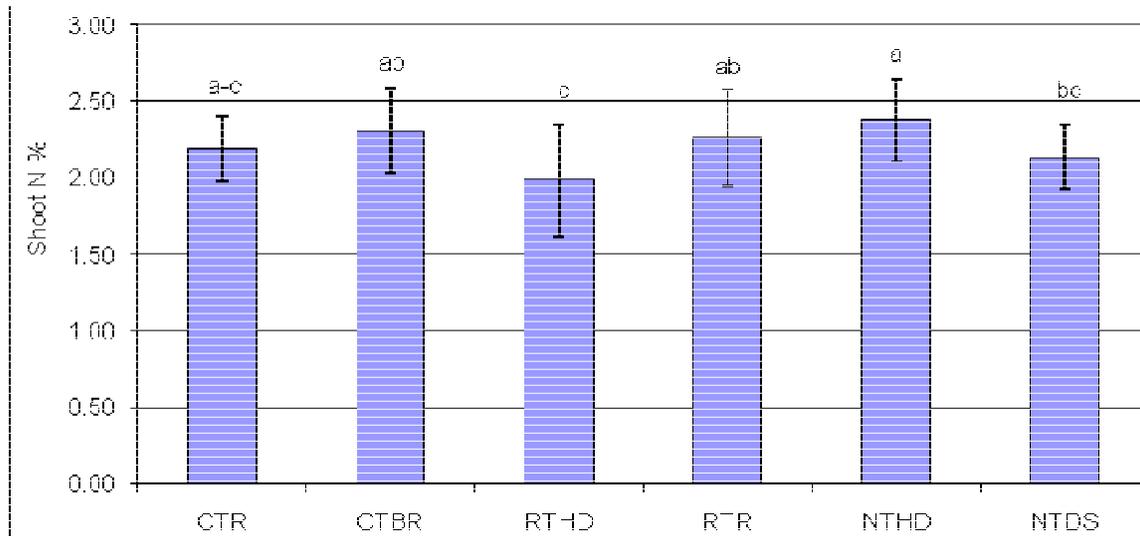


Figure 7. The effects of different soil tillage methods on the concentrations of up-root N.

mg/nodule, respectively.

Root and shoot N content (%)

According to the results shown in Figure 6, which represent only root (without nodule) nitrogen concentration, significant difference have been come out among the applications and while CTR, RTHD and NTDS applications have the highest values, the lowest values have been observed in CTBR plots as 0.59%. RTR and NTHD plots have nitrogen concentration values of 0.68 and 0.63%, respectively. The most important finding is, if the wheat stubble is burned before soybean, the root nitrogen concentration is decreased. This can be

explained by the destruction of rhizobial activities with the burning process. Moreover, destruction of organic substances in the soil, and benefits of food elements may be another factor. Different soil tillage applications have significant effects on up- root nitrogen concentrations (%) and the highest value of 2.38%, have been observed on NTHD plots. The lowest value of 1.98% has been measured on RTHD plots (Figure 7).

DISCUSSION

In this study, the effects of six different soil tillage methods on some parameters related with nitrogen

fixation have been investigated. Every effect that soil is subjected has positive or negative reactions. Soil is alive and dynamic system whose physical, chemical, and biological properties are constantly in interaction with each other and changes in any properties affects other properties. By soil tillage, the chemical and biological characteristics of soil are also affected due to physical manipulation that the soil subjected. Such organisms like rhizobium and mycorrhiza providing many significant benefits together with the plants and their activities are considerably affected by the changes in surrounding area (Urzua, 2005; Hassen et al., 2007; Dogan et al., 2007).

According to the findings of the research in the NTDS plots, root weights (6.9 g/plant), number of nodules (96 number/plant), weight of nodules (0.318 g/plant) and root nitrogen content (0.7%) are found to be statistically higher than with the other tillage applications. In the Reduced tillage with rotary tiller (RTR) plots, the values of up-root dry weight (51.3 g/plant), mean nodule weight (3.91 mg/nodule), root N content (2.38%), are found higher on the lands than in NTDS plots.

Among the applications, in the plots of Reduced Tillage (RTHD and RTR) rhizobial nitrogen fixation parameters have been found considerably higher compared with the other applications. However, some soil tillage methods used in this study negatively affected some soil parameters. For the RTR plots the dry root weight (4.8 g/plant), up-root weight (35.7 g/plant) and root N content (0.68%) values and for the Conventional Tillage with Burnt Residue (CTBR) plots, number of nodules and weight of nodule values were found to be lower than in the other tillage applications. The values of dry nodule weights, like in CTBR were low in the plots of CTR and RTHD with the values 0.071 and 0.088 g/plant, respectively. Besides, the lowest mean nodule weights (2.06 mg/nodule) have been observed in CTR plots and the lowest up root N content (1.98%) have been observed in RTHD plots.

The results of the study have been showed that, parameters of nitrogen rhizobial fixation has been affected negatively by the conventional tillage methods in which 3-5 tillage operations are applied and soil is disturbed. There were differences among the tillage methods and these differences were found to be statistically significant. In general, the best results related with rhizobial activity have been obtained with NTDS and RTHD. However, other soil tillage methods decreased the nitrogen fixation. Similar studies have also showed that zero and reduced soil tillage methods have increased the soil microbial activity and population (Alvarez et al., 1995; Gassen and Gassen 1996; Ferreria, 2000).

Generally, soil microbial activity is affected negatively by soil tillage. (Hussain et al., 1999; Kladienko, 2001; Sagar et al., 2001; Jinbo et al., 2007; Hernandez and Hernandez, 2002). Rhizobial activity can also be affected negatively by soil tillage (Ferreira et al., 2000; Urzua, 2005; Hassen et al., 2007). Soil organic matter decreased by soil tillage operations is also important for the vital

activities of soil microorganisms. The decrease of organic matter in the soil can also cause decreases in soil microbial activity (Elliot et al., 1984; Hussain et al., 1999; Sagar et al., 2001). As it can be seen in the similar studies, the effects of soil tillage methods may differ depending on climate, regional, and environmental factors. These factors must be taken into consideration before applying tillage methods. Otherwise, biological activity, fertility and sustainability of soil will be destroyed.

This study showed that for this region and for secondary crop soybean, nodulation is affected positively with RTHD and NTDS methods. Thus, by considering the nodulation is an important parameter of Rhizobial nitrogen fixation, similar studies must be conducted in different soil types. It is recommended that RTHD and NTDS methods can be used for secondary product soybean plant instead of CTR and CTBR.

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