0971-4693/94 Euro 10.00
Allelopathy Journal 39 (1): 43-54 (2016) *International Allelopathy Foundation* 2016
Table: 1, Figs : 7

Effects of wheat and soybean stubbles on soil sickness in continuous cropping of cucumber

T. FENG, Y.Y. WANG*, Y.H. ZHANG, X.H. SHI, C.H. QIN[2], S.A. ZHANG[1], S.C. JIN, H. ZHANG and J. ZHANG[1]
Institute of Economic Botany, Jilin Academy of Agricultural Sciences,
Gongzhuling- 136105, China.
E.mail: wyy6677@126.com

(Received in revised form : August 16, 2016)

ABSTRACT

In pot culture, we studied the effects of wheat and soybean stubbles in 3-ratios (0.5, 1, 2%) on the soil microbial community structure in cucumber (*Cucumis sativus* L.) and their effects on yield. The PCR-DGGE (Denatured gradient gel electrophoresis) was used to analyse the changes in the rhizosphere microflora. It was found that the bacterial diversity increased and the fungal diversity decreased, with the addition of wheat and soybean stubble. Besides, the addition of wheat and soybean crop stubbles also increased the yield of cucumber, the yield levels varied with the type and quantity of crop stubbles added

Key words: Bacteria, crop stubble, cucumber, fungi, *Glycine max*, PCR-DDGE, replanting soil, soil enzyme activity, soil microorganism, soybean, *Triticum aestivum*, wheat, yield.

INTRODUCTION

Cucumber is favourite Chinese vegetable. Hence, its cultivation has increased under protected cultivation in Green Houses and currently accounts for > 60% of the total area under vegetable cultivation (17,25). This crop when cultivated continuously in monoculture, is susceptible to soil sickness, which decreases its yield and quality (21,22,27). Studies have shown that continuous monocrop cultivation leads to an imbalance in soil nutrients, decline in physico-chemical soil properties, proliferation of soil pathogens and accumulation of autotoxins in soil (1,28). However, many researchers believe that the main problem in continuous cultivation is the accumulation of autotoxins in the soil (3,11,22). It is well known that the autotoxins, disturbs the balance in soil microbial flora and increases the soil sickness (8,9). When autotoxins accumulate in the soil, the normal microbial community structure and the micro-ecological environment in the rhizosphere is altered (11,18). Thus, measures to overcome the soil sickness in continuous cropping are required (23,24).

*Correspondence author, [1]Eco-Agriculture Research Center, Binzhou Vocational College, [2]Jilin Provincial Economic Management Cadre College, Changchun. China.

The addition of plant organic residues significantly increases the number and species of microorganisms in the rhizosphere soil and changes the community dominant species (6,7). In practice, organic materials have been applied since long, to alleviate the soil sickness in continuous monocropping. However no information is available, how the soil applied crop residues alleviates the soil sickness in cucumber replant soil. This study aimed to understand the effects of wheat and soybean stubbles on the micro-ecological environment of cucumber replant soil and to provide the scientific bases to eliminate the continuous cropping problem in cucumber cultivation.

METHODS AND MATERIALS

The study was conducted at Jilin Academy of Agricultural Sciences, Gongzhuling, China (Latitude: 43°48′, longitude: 125°19′, altitude: 224.14 m). Cucumber cv 'Jin Green No.3'. was used as the test crop. The wheat cv 'Kefeng No. 6' and soybean cv 'North 86-4', stubbles (Including roots) were collected after the harvest of these crops. The stubbles were cleaned and powdered with a grinder to 6 mm size particle. The nutrients composition of the stubbles is given in Table 1.

Table 1. The nutrients content in wheat and soybean stubbles.

Stubble	Total carbon ($mg \cdot g^{-1}$)	Total nitrogen ($mg \cdot g^{-1}$)	Total phosphorus ($mg \cdot g^{-1}$)	Total potassium ($mg.g^{-1}$)	C/N ratio
Wheat	328.57	7.38	1.71	9.52	83.63
Soybean	284.67	9.36	1.35	3.54	49.27

The experimental treatments consisted of wheat and soybean stubbles and their ratios: 0, 0.5, 1, and 2%. The treatments were replicated thrice in completely randomized design. The soil used was from a continuous cropping area in a greenhouse, in which cucumber was continuously grown for 6-years The soil without crop stubble was used as control (CK).

Pot experiments : Experiments were conducted in plastic pots (30 cm dia, 22 cm height). The soil was sieved through 6 mm sieve to remove the larger clods and plant materials etc. The powdered stubbles were added to the soil in ratio of 0.5%, 1% and 2% (W/W) (8,9) and evenly mixed. The mixture was then filled into the pots (8 kg/pot), adequately irrigated and then covered with plastic films. The plant residues were allowed to decompose for 21 days in the greenhouse. Three days before the cucumber seedlings were planted, 5 g diammonium phosphates was added per pot and mixed. The age of cucumber seedlings at transplanting was 55 days. One seedling was transplanted per pot on April 22, 2011.

Soil Sampling : After the cucumber seedlings were planted, two samplings were done at 20 days intervals. Three pots were randomly selected to collect the rhizosphere soil. First the top soil in the vicinity of cucumber plants was removed and the rhizosphere soil was collected from the depth of about 8-cms. The soil samples from 3- replicates were collected separately and immediately transferred to the laboratory in a foam ice box. These soil samples were mixed to obtain a composite sample, which was sieved through a 2 mm sieve. The soil samples were then kept in the freezer at -20^{o} C and later used to determine the changes in soil microbial flora (15,16).

Soil microbial analysis: The community structure of soil microorganisms was analyzed by using PCR-DGGE. Microbial genome DNA was extracted according to Zhang Ruifu (26). Crude extract of genomic DNA was purified by using Vitagen Gel Kit (Product code: 110610-05 Vitagene Biotechnology Co., Ltd.). This kit was jelled by using gel melting system, and selectively absorbed by using Silica membrane, DNA fragments of 100bp-30Kb were recovered from the gel (13,22,28). In amplification of bacterial 16SrDNA in gene V6-V8 area, F968 and R1401were selected as primers (Provided by Beijing Hua Mei Biotechnology Co., ltd.). Sequence: F968 : 5'-AACGCGGAAGAACCTTAC-3', R140: 5'-CGGTGTGTACAAG A CCC- 3'. Using purified genome DNA as a template, the primer with specificity for the majority of bacterial and archaebacteria 16SrDNA V6-V8 gene region of F968 and R1401 were amplified by using PCR (12,27).

Statistical analysis: The gray value of each bands was analyzed by using Quantity One software. The DGGE fingerprints was digitized, standardized by using BioEdit 7.0 software to get a digital matrix with the record of shift position and brightness of each one of bands in DGGE gel, and then we imported the digital matrix into MEGA3.1 software to make a clustering analysis, which was used to build the system tree. Each determination was repeated five times and the average and standard error were calculated. Excel (Microsoft, Redmond, WA, USA) and SAS 6.0 (SAS Institute, Cary, NC, USA) software were used to analyze the data. The values were considered to be significant at $P = 0.05$ level.

RESULTS AND DISCUSSION

DGGE fingerprint profile of bacterial community structure and its analysis

The results of DGGE profile of bacterial community structure showed that the number and grayscale of the bands of each treatment changed and the difference in number and grayscale, was very wide. This showed that adding crop stubbles greatly influenced the bacterial community composition and the number of bacteria in cucumber replanting soil (Fig. 1). Wheat stubble increased the number of bands and the grayscale of the bands was significantly darker than control. The band d1 was darker in 1% and 2% wheat stubble treatments, while lighter in 0.5% and 2% soybean stubble treatments and it did not appear in other treatments and control. Band d2 appeared only in 1% wheat stubble treatment, but

not in others. Band d3 appeared in all wheat stubble treatments, and it was darker in 0.5% wheat stubble treatment. Band d4 was darker in 0.5% soybean stubble treatment , while lighter in other treatments and it did not appear in the control. Band d5 was darker in in 2% soybean stubble treatment and control, while lighter in 0.5% soybean stubble treatment and it did not appear in other treatments. Band d6 was darker in 1% and 2% soybean stubble treatments and lighter in 0.5% wheat stubble treatment, while it did not appear in other treatments and control. Band d7 was darker in the control, while lighter in other treatments. Band d8 was darker in 2% and 1% wheat stubble treatments, while it did not appear in other treatments and control. The band d9 was darker in 1%, 0.5% soybean stubble treatments and the control and lighter in 1% wheat stubble treatment, while it did not appear in other treatments. Band d10 only appeared in 1% soybean stubble treatment, while it did not appear in other treatments and control.

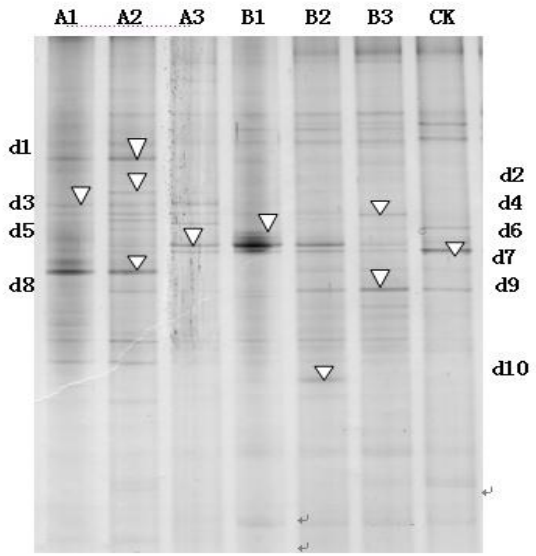

Figure 1. DGGE fingerprint profile of the bacteria from different treatments.
A1: 2% wheat, A2: 1% wheat, A3: 0.5% wheat, B1: 2% soybean, B2: 1% soybean, B3: 0.5% soybean, CK: Control

Using the DGGE fingerprint profile of bacterial population composition in cucumber rhizosphere soil, the cluster analysis was done using the MEGA3.1 software, to analyse the genetic distance and similarity of the bands (Fig 2). The genetic distance in 2% wheat stubble treatment was closer to that of 1% wheat stubble treatment and the two had a high degree of similarity. The genetic distance of 1% soybean stubble treatment was closer to control, while, 0.5% wheat stubble treatment was distantly related to 1% soybean stubble treatment. The DGGE fingerprint profile of bacteria in cucumber rhizosphere soil was converted to the concise bands by using the Bio Rad Quantity One software, easily compare and analyze them and calculate their richness indices (Fig 3). In each treatment, the richness index varied greatly. The higher richness indices were observed in 0.5% and

1% wheat stubble treatments, which were significantly higher than other treatments and control, followed by 2%, wheat stubble treatments and 0.5% soybean stubble treatment. However the richness indices had no significant difference among them. By contrast, 2% soybean stubble treatment had the lowest richness index.

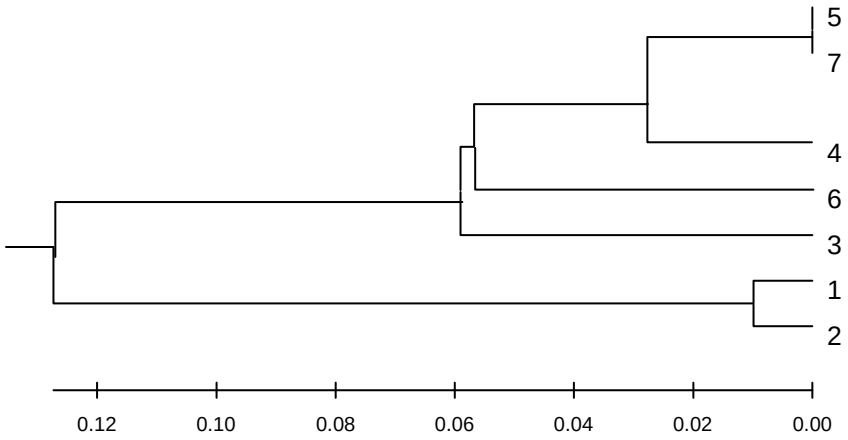

Figure 2. Cluster analysis of 16SrDNA band fingerprint profile of bacteria communities from different treatments.
1: 2% wheat, 2: 1% wheat, 3: 0.5% wheat, 4: 2% soybean, 5: 1% soybean, 6: 0.5% soybean, 7: Control.

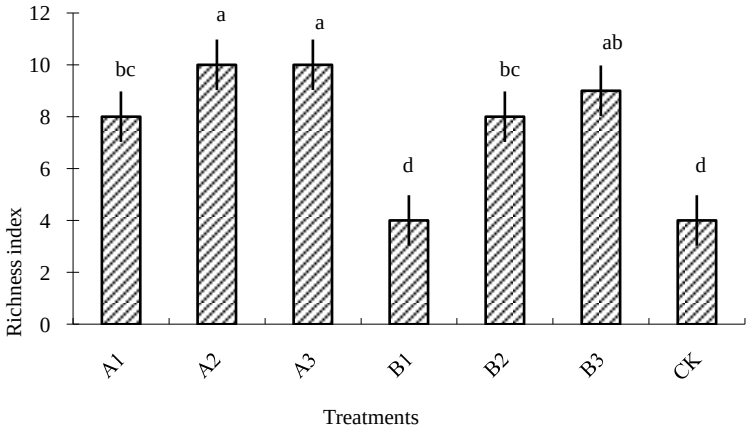

Figure 3. Effects of different treatments on the richness index of bacterial communities.
A1: 2% wheat, A2: 1% wheat, A3: 0.5% wheat, B1: 2% soybean, B2: 1% soybean, B3: 0.5% soybean, CK: Control

DGGE fingerprint profile of fungal community structure and its analysis

Adding crop stubbles decreased the number and the grayscale bands compared with control and different crop stubbles had variable effects. This suggests that adding crop stubbles changes the community composition and the number of fungi in the cucumber replant soil (Figure 4). The number and grayscale of bands in each treatments varied. The band e1 appeared in all soybean stubble treatments, but not in control and was lighter in grayscale. The band e2 appeared only in the control and did not appear in other treatments. The band e3 was darker in grayscale in 2% soybean stubble treatment and 0.5% wheat stubble treatment and the control, while lighter in other treatments. The band e4 and e5 showed similar regularity. The band e6 appeared in 2% wheat stubble treatment, 1% and 0.5% soybean stubble treatment and the control and not appear in other treatments, It was very lighter in grayscale in the control. The band e7 only appeared in control and was very lighter in grayscale, while did not appear in other treatments. band e8 appeared in 1% and 0.5% soybean stubble treatment, it did not appear in the other treatments and control. Band e9 appeared in all treatments, but not in control and it was lighter in grayscale**.** The number and grayscale of bands varied with the types and the amount of stubbles added.

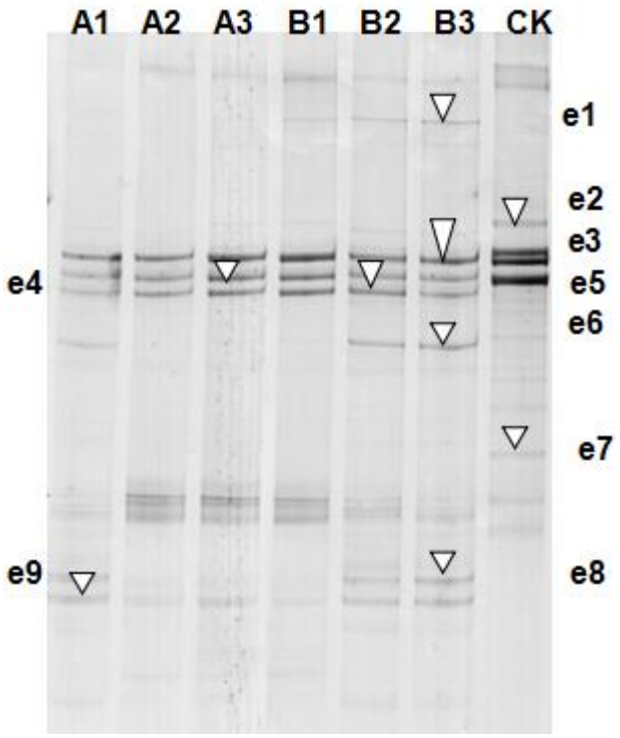

Figure 4. DGGE fingerprint profile of fungi from different treatments.
A1: 2% wheat, A2: 1% wheat, A3: 0.5% wheat, B1: 2% soybean, B2: 1% soybean, B3: 0.5% soybean, CK: Control

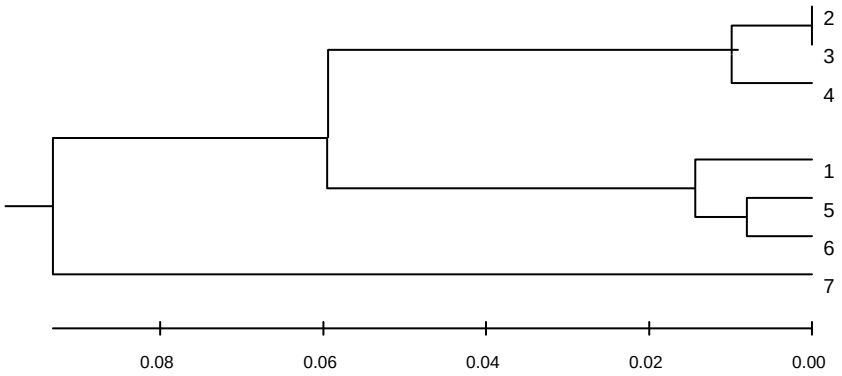

Figure 5. Cluster analysis of 16SrDNA band fingerprints of fungi communities from different treatments.
1: 2% wheat, 2: 1% wheat, 3: 0.5% wheat, 4: 2% soybean, 5: 1% soybean, 6: 0.5% soybean, 7: Control.

Using DGGE fingerprint profile of fungal population composition in cucumber rhizosphere soil, the cluster analysis was done using the MEGA3.1 software and the genetic distance and similarity of the bands was analysed (Fig 5). The genetic distance of 1% wheat stubble treatment was closer to 0.5% treatment and the two had high degree of similarity. Similarly, there was a closer gene distance among 2% wheat, 0.5% and 1% soybean stubble treatments and the three were similar, while the control was distantly related to the other treatments.

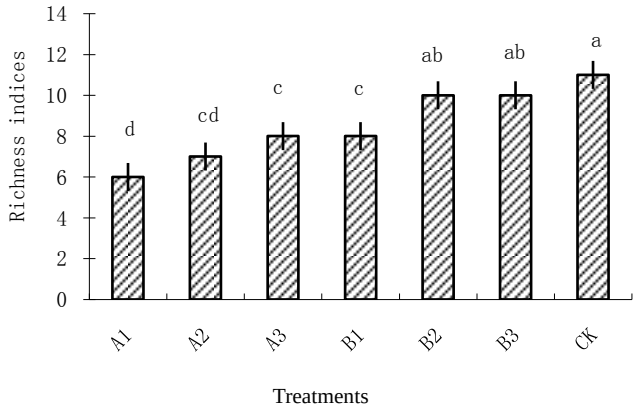

Figure 6. Effects of different treatments on the richness index of fungal communities.
A1: 2% wheat, A2: 1% wheat, A3: 0.5% wheat, B1: 2% soybean, B2: 1% soybean, B3: 0.5% soybean, CK: Control

The DGGE fingerprint profile of fungi in cucumber rhizosphere soil was converted to the concise bands by using the Bio Rad Quantity One software, to easily compare and analyze them and calculate their richness indices (Fig 6). In each treatment, the richness index changed greatly. The greatest richness index was observed in control, which was significantly higher than all treatments, followed by 0.5% and 1% soybean stubble treatments, but there was no significant difference between the two. The lowest richness index was observed in 2% wheat stubble treatment, which was significantly lower than other treatments and control. In wheat stubble treatments, the richness index decreased with the increase in the amount of stubble added, while in soybean stubble treatments, the richness index had no obvious regularity.

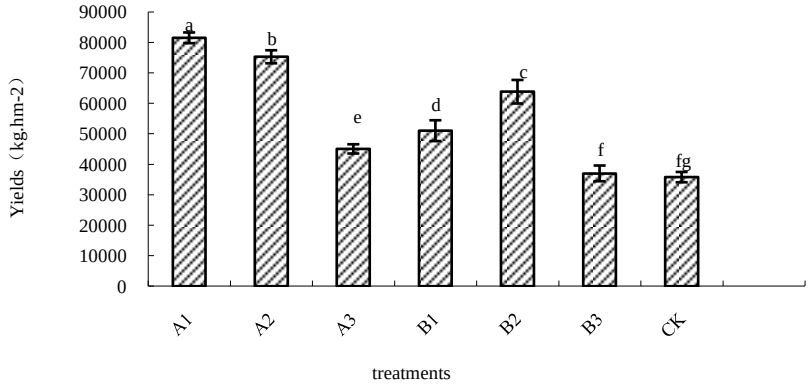

Figure 7. Effects of different treatments on cucumber yield.

Cucumber yield: Wheat and soybean stubbles promoted the growth of cucumber and also increased the yield of cucumber (Fig.7). With wheat stubble treatments, the yields increased with the increase in amount of wheat stubble. The 1% and 2% doses markedly increased the yield of cucumber, the highest yield was with 2% wheat stubble. Soybean stubbles also increased the cucumber yield but the increase was smaller than wheat stubbles, the highest yield was observed with 1% soybean stubble treatment. However, there was no increase in the yields with soybean stubble dose.

DISCUSSION

Many studies have indicated that the decomposing crop residues affect the number and population structure of rhizosphere microorganisms (4,8). In the present studies, the effects of different crop stubble on diversity of soil microbial community were studied by using denatured gradient gel electrophoresis (DGGE). The results of DGGE profile

showed that the diversity of bacteria increased with the addition of wheat and soybean stubble, while the diversity of fungi decreased. This showed that wheat and soybean stubble could promote the bacterial growth and increase the microbial diversity. Irrespective of the stage of cucumber growth, 1% and 2% wheat stubble treatments increased number of bands of bacteria, and the grayscale of the bands was significantly darker than the control. This suggests that adding higher amounts of wheat stubble was more conducive to increase the community composition and the number of bacteria in cucumber replanting soil. However, compared with the wheat stubble, soybean stubble had a smaller promotion effect on the diversity of soil microbial community. This may be related to the degree of soybean stubble decomposition in the soil. Hence the treatments with soybean stubble showed no promoting effects on microbial growth.

Using higher amounts of crop stubble stimulated the soil microbial community. This may be because the higher amounts increased the nutrients in the soil, which improved the environment for microbial growth. Similar results were reported by Wu *et al.* (20), who reported that adding garlic stubble increased the diversity of rhizosphere soil microorganisms.

PCR-DGGE is a new method used to analyze the soil microorganisms, however, there are few studies (4,16,19). In present studies, PCR-DGGE technique was used to study the community structure of soil microorganisms, The results showed that the decomposed wheat or soybean stubble significantly influenced the microbial community, which promoted the growth of cucumber.

According to many investigations, straw added to the soil promotes the growth of crops and increases with crop yields by 5-10% (17,21). In our experiments, wheat and soybean stubbles promoted the growth and yield of cucumber in continuously cropped soil. The highest increase in yield was with 2% wheat stubble treatment. The response to wheat stubbles was better than to soybean stubbles. Addition of wheat and soybean stubble increases the soil microbial activity, which might have reduced the soil toxicity in continuous cropping of cucumber.

CONCLUSIONS

The addition of wheat or soybean stubble increased the soil enzyme activity, the numbers and activity of soil microorganisms, leading to improved micro ecological environment in replanting soil. Soil enzyme activities varied with the types of crop stubble and its added quantity. Wheat stubble at 2% greatly improved the soil enzyme activities, which improved the micro-ecological environments in replant soil. The DGGE analysis showed that the addition of wheat and soybean stubbles increase the number of bacteria and the biggest increase was with 2% wheat stubble. Wheat and soybean stubbles promoted the growth and yield of cucumber. The 2% wheat stubble treatment had the biggest increase in yield. Thus, to reduce the continuous cropping problem in cucumber, one can improve the biological characteristics of continuous cropped soil by adding wheat or soybean stubbles in the replant soil.

ACKNOWLEDGEMENTS

This project was financially supported by the Innovation Fund of Agricultural Science and Technology of Jilin Province and by Shandong Provincial Natural Science Foundation.

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