Journal of Applied Biological Sciences
Uygulamalı Biyoloji Bilimleri Dergisi
E-ISSN: 2146-0108, 11 (2): 35-38, 2017, www.nobel.gen.tr

Formation Of Bacteria (Rhizobium Spp.) In The Roots Of Bean Plants Grown In Aquaponics System

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Received Tarihi: May 10, 2017
Accepted Tarihi: September 18, 2017

Abstract
Current research was conducted to determine formation of bacteria (*Rhizobium spp*) in the roots of bean plants grown in aquaponics system. Aquaponics systems is a form of production made of both plant and fish farming by integrating the plants used in hydroponics to the closed circulation system which water is used over and over again and intensive fish farming is made. Microorganisms such as *Rhizobium spp*. increasing plant growth are effective in nitrogen fixation and disease control in a symbiotic environment with legume crops.
Studies were done under the aquaponics system. Randomized plot desing has been used. The research was arranged in two group and three replications. Formation of nodules was observed in the roots of bean plants in all replication in groups inoculated bacteria (*Rhizobium spp.)*. The highest nodule number was recorded in third group as 14.00±5.51 and the lowest one was recorded in second group as 12.33±3.53. The nodule number was determined as minimum 4 and maximum 23 in groups inoculated bacteria (*Rhizobium spp.)*.
Keywords: Aquaponics systems, bean plants, circulation system, *Rhizobium spp*., nodulation, nitrogen fixation.

INTRODUCTION

Aquaponics system is a form of production in which both plant and fish breeding are realized by integrating hydroponics to closed recirculation systems which water is used over and over again and intensive fish farming is made Rakocy et al (1993).

There were described three systems used in hydroponics system and adapted to aquaponics system: Nutrient film technique (NFT), Deep water or floating raft method and Media based systems (Resh 2013).

In media based system, organic (straw, bark, seaweed, sawdust and peat), mineral (sand, gravel, perlite, ceramic balls, red/black cinder and rock wool) and synthetic materials (expanded clay ball; polystyrene, polyurethane) are used for plant growbeds. Growbed materials in this system work as a bio-filter and was found to form more nitrate Lennard and Leonard (2004).

Aquaponics systems are based on the coexistence of fish, plant and nitrification bacteria in the same environment and on the production of animal and vegetable products that people can consume through mutual benefit of three living beings from each other. Due to the integration of secondary species benefitting from unused parts of nutrients or wastes of the main breeding species, it is possible to achieve more efficiency from unit area. In aquaponics system, there are many trials on vegetable production by conversion of the ammonia formed by the wastes of fish and nutrition to nitrite and then nitrate to be used by plants via nitrifying bacteria Diver(2000); Lennard (2004); Nelson (2008).

One of the most popular plant grown in aquaponics system is haricot bean. These are plants with a high protein content (% 17-35) Evans and Gridley (1979). Dried bean, one of the leguminous plants, has an important role in animal feeding beside human nutrition and also in crop rotation due to being source of nitrogen for soil Duc et al. (2010). Furthermore, growers are aware of bean's benefits to fertility and health of soils. Also depending on this situation, its cultivation is increasing Paull et al.(2011); Flores et al.(2013).

Leguminous plants are rich in minerals such as phosphorus, potassium and calcium as well as a good source of vitamin especially with regard to A and D vitamins (Allen, 1949). It is estimated to have approximately 27% of plant production worldwide Graham and Vance(2003).

Legume plants transform the free nitrogen of the air into a form that plants can use thanks to the rhizobium bacteria forming tubers in their roots. Rhizobium bacteria lives symbiotically with the plant. It takes the carbon compounds necessary for itself from the plant.

The tolerance of different rhizobium strains on soil to temperature and humidity fluctuations are different Boonkerd and Weaver (1982). In addition, the number, size and shape of the nodules vary depending on bacterial strains and plant varieties that they live together Stewart(1966).

By inoculating Rhizobium bacteria, it has been proven to increase yield and nitrogen content of the bean plantÖnder (1994); Küçük (2008).

The purpose of this research is to examine the formation of nodules in aquaponics system, after inoculation of rhizobium bacteria to the bean plants belonging to the leguminous plants grown extensively all over the World.

MATERIALS AND METHODS

In the study, tilapia (*O. aureus*) fry were used as fish material (Figure 1). The fry were provided with the fish stock formed in the study entitled "In Aquaponics System, TheEffects of The Different Stocking Rates of Tilapia Fish (*Oreochromis aureus*Steindachner, 1864) on Total Yield".

Fig. 1. Tilapia (*O. aureus*) fry used in the aquaponics system

In the experiment, wastewater of tilapia fish stocked at 50 kg/m^3 in the aquaponics system was used. The fish were fed with a trout diet of approximately 45% protein at 2% of their body weight for 112 days (TableI). There is no application on fish.

Table I. Amount of nutrients used in the experiment, %

Nutrient quantities (%)	
Protein %	45.0
Fat %	20.0
Moisture %	8.5
Ash %	11.0
Cellulose %	3.0
NFE %	12.5
Phosphorus %	1.5
GE (Gross energy) kcal/kg	5124
DE (Digestible energy) kcal/kg	4125
ME (Metabolizable energy) kcal/kg	3742
DP/DE g/MJ	22.15
Vitamin A IU/kg	5.000
Vitamin D IU/kg	1.500
Vitamin E IU/kg	100
Vitamin K IU/kg	20

The experiment was conducted in three replications random blocks (Tablo II).

Each repetition in experimental groups were organized as separate closed recirculation system. During the feeding period, the temperature is set equally via the thermostat heaters.Ventilation is provided by a blower.Temperature and oxygen values were checked daily to avoid differences in temperature and oxygen values.In the experiment, 6 aquariums of about 95x30x40 cm were used and media based system was designed.In the system, approximately 60x40x30 cm plastic tanks were used for the beds where plants were placed.Hydroton (baked clay) as a plant bed and the Bio-Digest (French brand) as nitrification bacterial culture was used.Aquarium type water pumps capable of providing 400 litres of current per hour for water circulation were utilized. A tap was placed at the outlet of each pump for equal flow of water.The amount of water in the aquarium was adjusted as to be 50 litres in order to comply with the fish stocking density. Attention was paid for amount of water not to change. Tap water rested for at least 48 hours was used.Marble in the size of pebbles were used to regulate the pH. The average water quality parameters in the aquariums during the trial are given in Table III. Daily water change was approximately 2%. It was utilized natural light for lighting.

Domestic climbing variety bean seeds were used in the experiment (Figure 2). Bacterial strains belonging to the bean plants were obtained from Ankara Soil and Fertilizer Research Institute.The seeds, before planting are kept in pure alcohol for 10 seconds and washed with sterile distilled water. After being kept in 10% commercial sodium hypochlorite for 30 minutes, they were washed 3 times with

Table II. Research trial plan

Group	Replications	Stock quantities kg/m^3	Number of fish (piece)	trial period (day)
I	1.1	50	7	112
	1.2	50	7	112
	1.3	50	7	112
II	2.1	50	7	112
	2.2	50	7	112
	2.3	50	7	112

Table III. Average water quality parameters in aquariums during the trial

Temperature (°C)	pH	Oxygen (mg/l)	NH_4(mg/l)
25.33 ± 0.284	6.42 ± 0.254	6.48 ± 0.224	1.99 ± 0.722

sterile distilled water.Before planting, half of the moist seeds were mixed with 1% bacterial inoculants. Seedling beans were placed in the aquaponics system (Figure 3).

Fig. 2. Domestic climbing varieties bean seeds used in the experiment

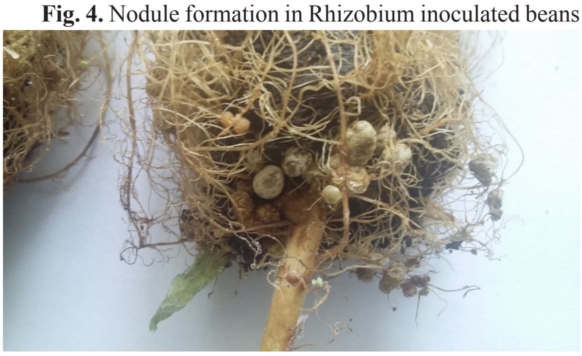
Fig. 3. Climbing varieties bean in an aquaponics system

RESULTS

At the end of the ripening period of the beans plant, the number of nodules on each plant was measured. In addition, growing roots and leaves was observed. Data on nodule numbers is given in Table IV.

At the end of 112 days, among repetitions the minimum number of nodules was 4.00 and the highest number of nodules was 23.00.

Fig. 4. Nodule formation in Rhizobium inoculated beans



It was observed that the roots of the beans in the nodule-forming group were well-developed, while the roots of the beans in the non-nodule group were weaker (Figure 5).

Fig.5. Root development in nodule-forming and non-nodular beans

In the experiment, some leaves of bean plants were observed yellowing. This situation is thought to be caused by lack of iron.

CONCLUSION

In the study, nodule formation in aquaponics systems was examined by inoculating rhizobium bacteria in bean plant.It is expected that the results of this research will make significant contributions to the researches to be done later. It is needed to be answered many questions such as

- The effects of rhizobium bacteria on height, body structure, number of leaves, seed yield of plants in aquaponics system,
- The determination of rhizobium strains suitable for the aquaponics system,
- Influence of rhizobium on nitrogen compounds in system water,
- The effect of the conditions in the aquaponics system on rhizobium

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