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ADVERSE EFFECTS OF DIFFERENT HUMIC + FULVIC ACID LEVELS ON BIOLOGICAL NITROGEN FIXATION ON GROUNDNUT

Gokcer Kirac, Ali Coskan
University of Suleyman Demirel, Isparta, Turkey

Abstract

Nitrogen is one of the key components of plant production and the nitrogen requirement of the plant supplied either by mineral nitrogen applications or by biological nitrogen fixation. Although the atmosphere contain huge amount of N_2, plants are not able to use that as a nitrogen source. Immediately usable forms of nitrogen are nitrate (NO_3^-) or ammonium (NH_4^+). The bonds between two nitrogen atoms are quite strong thus, reducing N_2 gas to mineral nitrogen forms needs considerable high amount of energy. However, in biological life, microorganisms may convert N_2 to mineral nitrogen sources in ambient temperature and pressure; therefore, biological nitrogen fixation is both environmental friendly and sustainable. Industrial nitrogen fixation and mineral fertilization leads both environmental pollution and economic impact. In this research, the effects of humic+fulvic acid (HFA) on nitrogen fixation were evaluated. For this purpose, a pot experiment in controlled environment was carried out. Peanut seeds were sawn in the hole prepared after dual application of HFA doses and rhizobium bacteria. Two times sampling was realized, one in the flowering and the other in harvest time. Results revealed that HFA application was effective on biologic nitrogen fixation; however, increasing HFA doses were adversely influenced determined parameters. Due to the soybean was cultivated as a forecrop at the field where the experimental soil is collected, nodulation was observed even at non-inoculated pots. Nevertheless, the higher values obtained from inoculated plants. Based on the results presented in the paper, HFA was positively effective on a number of parameters evaluated, yet the lower doses should be recommended.

Keywords: biological nitrogen fixation, peanut, humic+fulvic acid

INTRODUCTION

Soil productivity is mainly improved by mineral fertilizers which misuse of these salts leads to environmental problems as well as economic impacts. Among the nutrients provided by fertilizers, nitrogen has a special role which rather dynamically affects the soil, thus it's a critical limiting element for plant growth. Nitrogen is the main component of chlorophyll and amino acids, it's also found in important biomolecules such as ATP and nucleic acid (Wagner 2011). Actually, nitrogen is one of the most abundant elements in the nature; however, 95% of nitrogen utilized by the plants consisted of either nitrate or ammonium (Kacar and Katkat 2015, Coskan and Dogan 2011). Therefore nitrogen should be provided by either industrially fixed fertilizers or biologic nitrogen fixation. The biological nitrogen fixation was discovered by Martinus Beijerinck in 1901 (Wagner 2011), but even today, it is hard to say that farmers are paying attention to this environmental friendly techniques (Isler and Coskan 2009). The majority of the farmers are not aware of the potential risks of fertilizers on the environment; therefore, they are using excessive amounts of fertilizer without considering soil analyses (Coskan *et al.* 2012). Excessive or unbalanced fertilization contributes to deterioration of the biological value and quality of yields (Antonkiewicz and Labetowicz, 2016). With legume-bacteria symbiosis, the fixed nitrogen amount reported around 140 kg $N \cdot ha^{-1} \cdot year^{-1}$ (Burns and Hardy 1975), whereas in soybean – *Bradyrhizobium japonicum* relation, it may reach up to 300 kg $N \cdot ha^{-1} \cdot year^{-1}$ (Keyser and Li 1992). Discarding this system is causing more fertilizer usage which nitrite and some other compounds are carcinogenic to humans (Gok *et al.* 1991). Breimer (1981) reported nitrate accumulation as a result of excessive mineral nitrogen application on spinach and lettuce.

Humic substances (humic and fulvic acids-HFA) are natural organic substances (Andriesse 1988), they are complex and heterogeneous mixtures formed by biochemical and chemical reactions during the decomposition of plant and microbial debris (IHSS, 2016). Pena Mendez *et al.* (2005) reported that humic substances are still one of the strongest chelating agents among natural organic compounds; however, the structures of the humic substances are not clearly known. A number of researches reported beneficial effect of HFA as improving phosphorus availability (Delgado *et al.* 2002), increasing the number of flower stalks and branches as well as seed yield (Sugier *et al.* 2013) and enhancement shoot development (Fernandez-Escobar *et al.* 1996). Erdal *et al.* (2014) reported that the fulvic acid was more effective on plant dry weight, but no effect was observed at increasing doses. Moreover humic acide application and doses had no effect on the P, Mg, Mn and Zn contents of the plant (Leventoglu and Erdal 2014) reported that the effect of HFA is closely depends on different soil type. Antonkiewicz and Labetowicz (2016) addressed the quality of crop which is more important than the yield.

In this research, the possible stimulating effects of humic+fulvic acid (HFA) on nitrogen fixation were examined.

MATERIAL AND METHODS

A pot experiment was carried out at University of Suleyman Demirel according to randomized complete block design with 3 replicates. Two kg of dry soil equivalent fresh soil was sieved from 4 mm and placed to pots. Some soil properties are presented in Table 1.

Table 1. Some properties of experimental soil

Texture class	Organic matter (%)	pH (1:2,5 H_2O)	$CaCO_3$ (%)	Total N (%)	Mineral N (kg da^{-1})	P_2O_5 (kg da^{-1})	Fe (kg da^{-1})
SiC	1.28	8.11	26	0.029	3.8	6.2	2.88

To obtain humic + hulvic acid (HFA) 250 g of 40% HFA containing leonardite was placed to erlenmayer, final volume adjusted to 1 litre by heated 1N KOH at 50°C while slowly stirring via glass stick. Within the day constituent of erlanmayer was stirred frequently, after one night settling time were transferred to another container. The HFA concentration of the obtained solution was 10% (w/v) which determined via drying at 105°C and weighing sequence. COM peanut seeds were sown and 1, 2 or 4 ml of above mentioned solution, that equivalent to 0.1, 0.2 and 0.4 mg HFA pipetted to seed beds. Non-treated pots were also added to experiment as a control. The 0.1 ml of $2.15 \cdot 10^{10}$ *Bradyrhizobium japonicum* 380 containing solution was applied to each peanut seeds. All enumeration and cultivation practices were done using yeast mannitol broth (Jordan 1984).

Half of the pots were harvested at flowering stage, nodules, root and shoot were sampled whereas the root, shoot and seed was sampled from remaining pots which were harvested after seed maturation. All plant samples were dried at 65°C according to Kacar and Inal (2010). Chemical analyses were performed using the methods presented in the manuscript of Coskan *et al.* (2007). All nodules for each plants was weighed after oven dried as similar to plant materials, whereas one dry nodule weight value was obtained by dividing total nodule weight to nodule numbers.

Data obtained were statistically tested by MSTAT-C software (Crop and Soil Sciences Department, Michigan State University, Version 1.2) according to randomized complete block design.

RESULTS AND DISCUSSION

Nodulation

Roots were sampled at the flowering stage to determine nodule development; obtained results are presented in Figure 1. Nodule formation in newly introduced legumes is not expected if similar legume plants have not been cultivated earlier (Gok and Onac 1995). This phenomenon has been demonstrated at the Turkish Republic of Northern Cyprus by Biren (2002); however in this case, nodule formation was observed in both inoculated and non-inoculated variants. Soils used in this experiment were collected from the field where soybean was cultivated earlier; therefore bacteria were existed in the soil. This was the reason of nodule occurrence. Although the highest nodule number developed in bacteria inoculated HFA-0 dose, there were not statistical differences between inoculated and non-inoculated treatments ($p>0.05$) according to mean values. Mean of dose values clearly stated that the increasing HFA doses reduced nodule number. Total nodule weight values (Figure 1, upper right) are in correspondence with nodule number values, whereas the highest values are observed in inoculated HFA-0. Despite the lower values observed as a result of increasing HFA doses, differences between the treatments were not significant. Among the observed nodule-related parameters, one nodule number was the only one that positively influenced from increasing HFA doses, whereas the highest value was determined from inoculated and the highest HFA dose (HFA-4) applied variant. Similarly, inoculated and HFA-4 applied treatment resulted in the highest nitrogen contents (Figure 1, lower right). According to mean values, bacteria inoculation significantly improved nodule nitrogen contents. Since there was no conformity between the nodulation parameters, these results should be evaluated by plant biomass parameters to reach the reliable conclusions.

Biomass development and nitrogen mass at flowering stage

Root and shoot dry weight values are presented in Figure 2. The highest values were determined at doses of inoculated HFA-1 and HFA-4 applications. In terms of general average values, inoculation increased root dry weight. Differences were not statistically significant between the HFA doses.

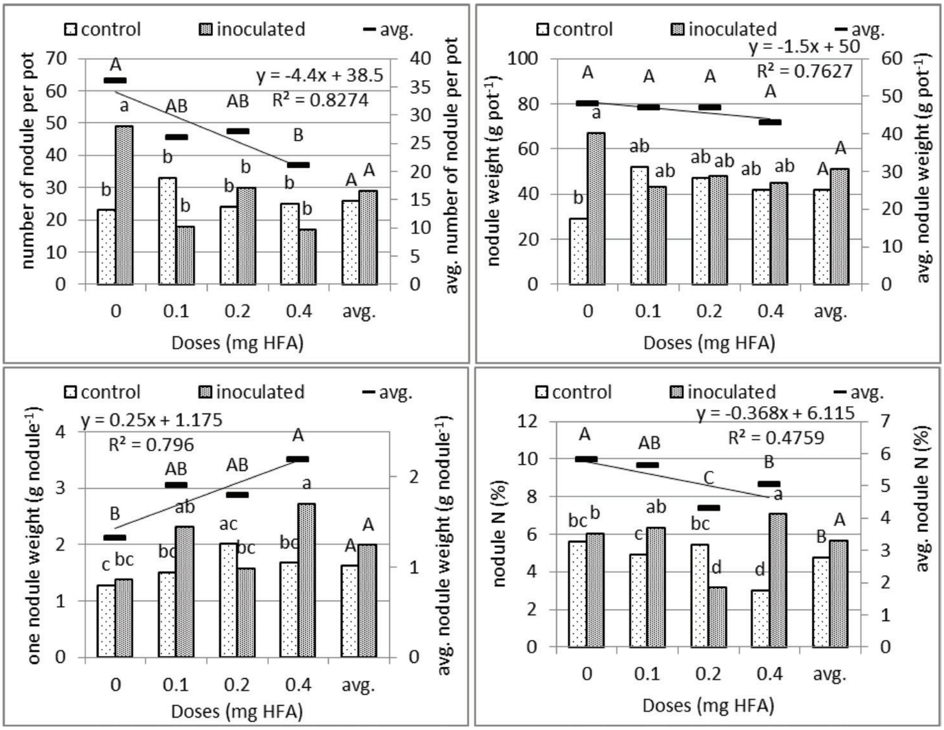

Figure 1. Number of nodule (upper left), total nodule weight as g (upper right), one nodule weight as g (lower left) and nodule nitrogen contents as % (lower right); note that the primary and secondary y axis ranges are different.

The dry weight values of shoot were partially in accordance with the root dry weight values, the highest value was determined in the HFA-1 dose of the inoculated treatment. The effects of HFA doses on shoot dry weights were not statistically significant while the weights of inoculated applications were higher in terms of mean values. HFA-1 dose along with bacteria inoculation is the best application among the treatments. Although HFA-1 dose increased biomass weight compared to HFA-0, the higher HFA doses reduced shoot biomass at flowering stage.

The total nitrogen mass which was calculated by considering biomass development and plant nitrogen concentration are presented in Figure 3. This value includes both the biological fixed nitrogen and the nitrogen uptake from the soil. Among the applications, the highest nitrogen mass value was obtained from the pots which inoculated and HFA-1 applied. There was no significant difference between the inoculated and non-inoculated variants in terms of mean values.

When the HFA dose averages were compared, it was determined that the first dose of HFA had a significant value increase compared to the 0 dose, while a decrease was observed at higher doses. This suggests that high doses of HFA have adverse effects on biological nitrogen fixation. Thus, in order to determine the optimal dose for biologic nitrogen fixation, it is necessary to carry out another experiment by setting new doses between HFA-0 and HFA-1 as well as HFA-1 and HFA-2. On the other hand based on existing results the highest recommended dose should not exceeded HFA-1 to be safe.

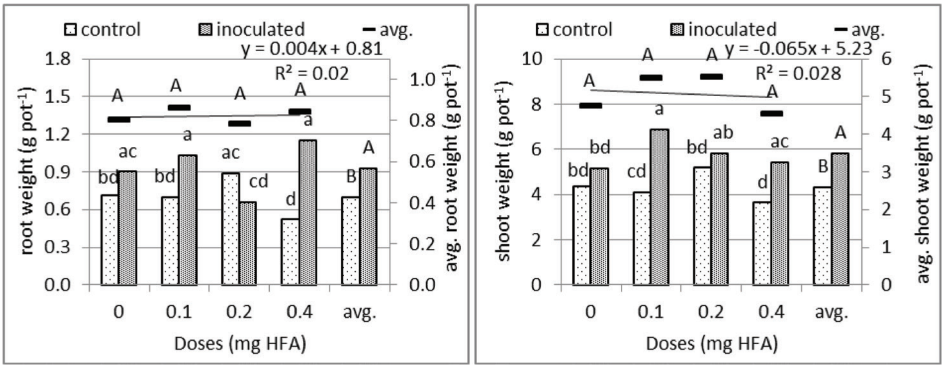

Figure 2. Root (left) and shoot (right) dry weight values obtained at flowering stage; note that the y axis ranges are not equal

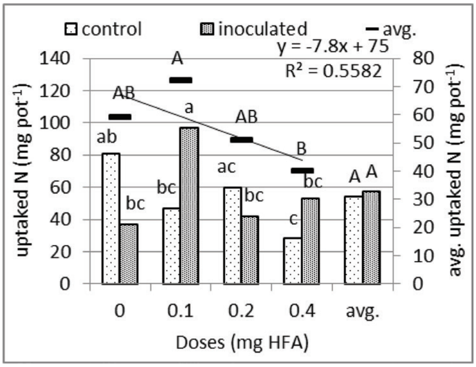

Figure 3. Total nitrogen mass in nodule + root + shoot

Seed yield and seed nitrogen content

The values of seed yield and seed nitrogen content are presented in Figure 4. Contrary to expectations, the highest seed yield was obtained from the untreated control pots. Both HFA applications and bacterial inoculation had a negative effect on the yield.

As clearly stated in the literature, bacterial inoculation is an application that has considerably positive effects on yield and nitrogen contents. However, it is also known that the stress factors may adversely influence this symbiotic relationship (Coskan *et al.* 2003). Although the obvious stress conditions were not observed, the decrease in the seed weight would be the indication of any stress factors. This idea was supported by mean values of inoculation practices that higher value obtained from non-inoculated pot. HFA applications also have adversely effected the yield which decreased by increasing HFA doses.

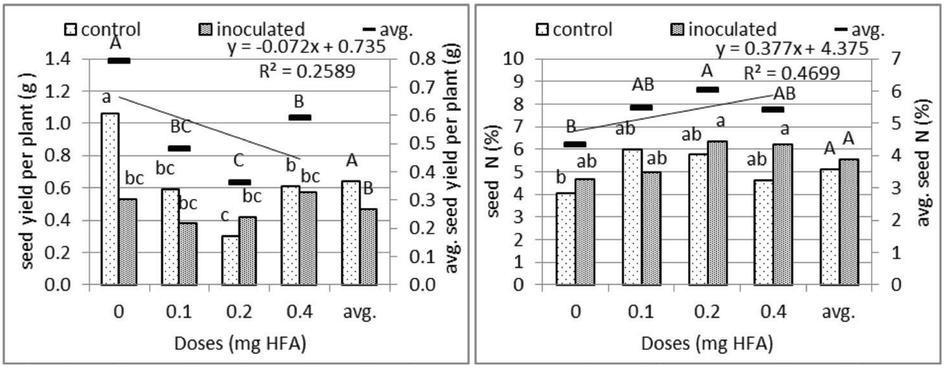

Figure 4. Seed yield (left) and seed nitrogen content (right)

Nitrogen contents of the seeds were influenced from both inoculation treatment and HFA applications. The highest nitrogen contents were observed in inoculated HFA-2 and HFA-4. However, there was no statistical difference between inoculated and non-inoculated applications in terms of mean values. When HFA doses are compared, it is seen that increasing doses increased the nitrogen content, but the highest dose cause partial reduction. On the other hand, the lowest nitrogen content was observed in the pot in which the highest yield was achieved. Recent studies revealed that the yield by itself is not the adequate parameter to predict the most convenient practices, considering recent concerns such as “food safety” and “secret hunger”.

Biomass development and nitrogen mass at harvest

Root and shoot dry weight values are presented in Figure 5. Statistical differences were determined between the applications in terms of root dry weights at the harvesting stage. The highest values were determined in inoculated

HFA-1 and non-inoculated HFA-2 doses whereas the lowest value was found in the non-inoculated HFA-0 dose.

There was no difference between the inoculated and non-inoculated applications according to mean values. The highest value among the HFA doses was obtained from the second dose of HFA. The highest shoot weight value was in inoculated HFA-1 application which nearly doubled compared to non-treated control pots, whereas the lowest were in non-inoculated HFA-0 and inoculated HFA-4 doses. HFA-1 dose gave the highest value according to mean values of HFA; however, further increasing doses caused decrease in shoot weight, while there was no difference between the mean values of inoculation applications.

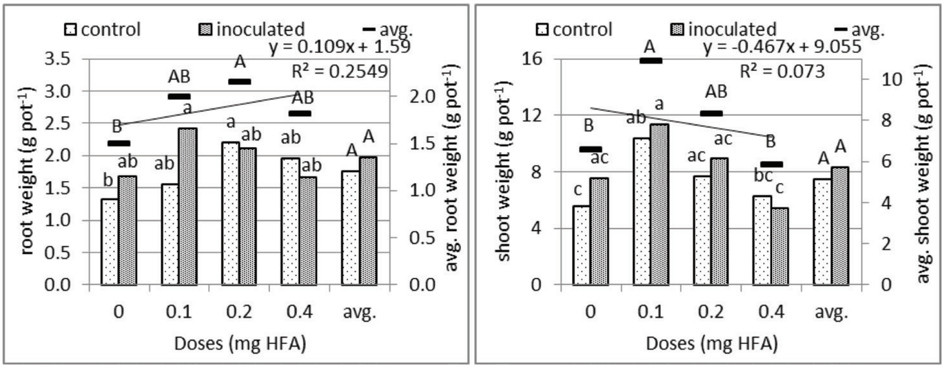

Figure 5. Root (left) and shoot (right) dry weight values obtained at harvest stage; note that the y axis ranges are not equal

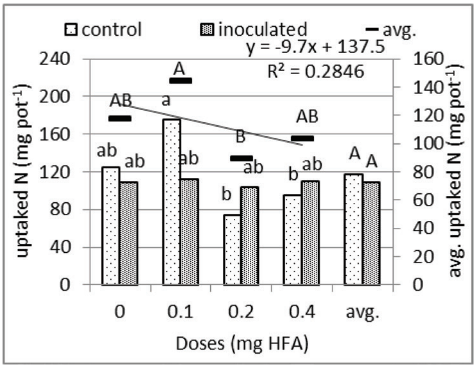

Figure 6. Total nitrogen mass in nodule + root + shoot

The total nitrogen mass at harvest stage is presented in Figure 6. As mentioned earlier, this value includes both the biological fixed nitrogen and the nitrogen uptake from the soil. The highest value, among all, was determined in the HFA-1 dose of the non-inoculated application. There was no difference between inoculated and non-inoculated variants based on the mean values. Among the HFA doses, the highest value was obtained from HFA-1 dose.

CONCLUSSION

Based on the overall results, it can be said that increasing HFA doses have a negative impact on nodule number and nodule weight whereas have a positive impact on average one nodule weight and nodule nitrogen contents. When all values are examined together, it has been assessed that the inoculated HFA-1 dose is the best application among the treatments. These results should be further examined by field experiments; however, utmost HFA dose should not exceed HFA-1 on transferring these results to the field experiments. Moreover, it concluded that the surface area of the pot is critical for entrance to the soil in peanut cultivation in the pot.

This study also showed that the presence of nodules in field does not guarantee the effective nitrogen fixation where nodulations were observed in all treatments. The existing rhizobium bacteria in the soil are infected to groundnut in this experiment; however, these bacteria have not been as effective as the newly introduced one. Yet bacterial inoculation is the promising practice on legume cultivated areas.

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Corresponding author: Prof. Dr Ali Coskan
Gokcer Kirac
University of Suleyman Demirel
Faculty of Agriculture, Department of Soil Science and Plant Nutrition
Isparta, Turkey
e-mail: alicoskan@sdu.edu.tr

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