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RESEARCH PAPER

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Effect of nettle manure on agronomic and biochemical parameters of green bean (*Phaseolus vulgaris* L.)

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Abstract

The main purpose of this study is to test the effect of biofertilizer of plant origin «Nettle manure» on agronomic and biochemical parameters of a green bean variety «*Phaseolus vulgaris* L. » grown under greenhouse. To achieve this, four foliar doses of biofertilizer (0% control, 5% 10% and 15%) were sprayed at different stages of crop development. Tested parameters were: height of plants, root length, number and weight of pods per plant, seeds weight per pod, as well as the amounts of chlorophyll (a), chlorophyll (b) and total chlorophyll (a+b) and total soluble sugars. The analysis of variance showed that there were significant differences between the four doses of biofertilizer for all the studied traits except the height of plants and the number of pods per plant. Whatever the studied traits, the concentration of 10% had given the most significant effect.

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Introduction

Organic farming has become a global priority (Mishra et al., 2013; Bhattacharje and Dey, 2014). The continued and abusive use of pesticides and chemical fertilizers for increased agricultural production has caused serious environmental and health problems (Vejan et al., 2016). Currently, the trend towards using natural products for crop fertilization is becoming more frequent. Plant and microorganismsbased fertilizers have become a promising substitute to chemical fertilizers (Popescu et al., 2014; Bhattacharjee and Dey, 2014; Win et al., 2018). They play a crucial role in improving food production to meet the needs of the growing world population without endangering natural resources in the context of sustainable development of agriculture (Pérez-Montaño et al., 2014; Win et al., 2018; Vishnu et al., 2022). Indeed, the use of organic fertilizers reduced amounts of energy consumption and contamination of soil and water, increased soil fertility, and promoted antagonism and biological control of phytopathogenic organisms (Carvajal-Muñoz et al., 2012). In recent years, plant-based biofertilizers have been widely used. They have a positive effect on plant growth and development and are effective for controlling crop pests and diseases (Rivera et al., 2010). Nettle (Urtica dioica L.) is rich in fatty acids, carotenoids, amino acids, vitamins, nitrogen compounds, mineral elements and phenolic substances (Joshi et al., 2014; Jan et al., 2017). Moreover, its extracts have shown fertilizer effect and plant growth stimulation (Goulfier, 2010). Green bean (Phaseolus vulgaris L.) is the most important legume for human consumption worldwide (Karavidas et al., 2022). It is an important source of vegetable proteins, minerals, antioxidants, bioactive compounds (Messina, 2014), vitamin C, fibre and carbohydrates (Chávez-Mendoza et al., 2017). In addition, it is rich in polyphenols and has a positive effect on human health by controlling obesity, diabetes and inflammatory processes in the body (Ganesan and Xu, 2017). As a legume, the common bean enriches the soil by fixing atmospheric nitrogen through the symbiosis with some soil bacteria (Mukherjee and Sen 2021; Karavidas et al., 2022), hence its interest in reducing the dependence of crop systems on nitrogen-containing chemical inputs. Moreover, it is an excellent rotational head or a good green fertilizer in some agricultural systems (Vertes *et al.*, 2010). Given the economic, agronomic and nutritional importance of green beans, it has been chosen in this study to test the effect of nettle manure on its growth and production.

Material and methods

Plant material

The experiment was conducted in a glass greenhouse at the experimental station of the University of Djilali Bouaama-Khemis Miliana. The plant material used is green beans (*Phaseolus vulgaris* L.), a dwarf variety, type « Mangetout », native to France with the trade name "Nelson". Every single plant was cultivated in pots with 5L capacity. A growing substrate containing 2/3 of soil whith1/3 of peat was used for each pot and for all treatments. Three foliar doses of stinging nettle manure were sprayed on the green beans plants and compared with the control (without biofertilizer).

Nettle manure (Urtica dioica L.)

Nettle manure used as biofertilizer in this experiment is an extract of a mixture of leaves and stem fragments from the prickly nettle (*Urtica dioica* L.), freshly harvested after flowering. This extract is obtained after maceration at a temperature of 20°C for fifteen days in source water until fermentation. After the bubbles have disappeared, the resulting product (nettle manure) is filtered and diluted at different concentrations.

Experimental design

The experiment was performed using a total randomized design. The dose of the biofertilizer (the nettle manure) was the only studied factor with four replications. Also, four treatments (solutions) were performed: 0 %(control) 5 %, 10% and 15% of biofertilizer.

Applied treatments

Four foliar doses of nettle manure were sprayed as a liquid biofertilizer and applied at different

concentrations (0%, 5%, 10%, and 15%) at different plant stages development of growing green beans.

Dilutions of the stock solution (nettle manure extract)

(To): Control (0%) of bio fertilizer (water).

(T1): Dose of 5 % equivalent to a dilution of 50 ml of nettle manure $\frac{1}{1L}$ water.

(T2): Dose of 10% equivalent to a dilution of 100 ml of nettle manure extract/ 1L water.

(T3): Dose of 15% equivalent to the dilution of 150 ml nettle manure extract /1water.

Stages of application of nettle manure on beans plant

Beans seedlings were transplanted into the pots after twenty-four (24) days of the nursery. The application of biofertilizer treatments has been carried out at each stage of the development of green beans.

Vegetative stage: corresponding to 3 and 4 leaves (07 days after transplantation);

At the beginning of flowering: corresponding to 10 % of flowering (34 days after transplantation);

Full blooming or flowering: corresponding to 75% of flowering (37days after transplantation);

Fructification: (49 days after transplantation);

Swelling of fruits: 58 days after the transplantation.

Analysed parameters

Two kinds of traits were observed: Agronomic and

biochemical traits. The Agronomic traits were: height of plants, root length, root weight, number of pods per plant, weight of pods per plant and weight of the seeds per pod.

The biochemical traits were: chlorophyll a, chlorophyll b and chlorophyll (a+b), which are determined using the Francis (1970) method and soluble sugars using the Dubois *et al.* (1956) method.

Data analysis

For statistical analysis, means for different analysed traits of *Phaseolus vulgaris* under different concentrations of biofertilizer were calculated and subjected to one-way analysis of variance (ANOVA) using the R software (R Core Team, 2020). Fisher's least significant difference (LSD) method calculated using the R software was used to detect significant differences among means. Correlation (Pearson's method) was performed using the 'facto extra' package in the R software. All tests were considered significant at p = 0.05.

Result and discussion

To evaluate the effects of different concentrations of biofertilizer (Nettle manure) on the development of a green bean variety, *«Phaseolus vulgaris* L. », different agronomic and biochemical parameters were tested. The results of all the analysed parameters are shown in Tables 1, 2 and 3.

Table 1. Results of analysis of variance, showing effects of fertiliser doses for agronomic and biochemical traits.

Dependent traits		df	Mean square
Height of plants (cm)	(HP)	3	9.89ns
Root lenght (cm)	(RL)	3	14.5^{*}
Root weight (g)	(RW)	3	3.00**
Number of pods per plant	(NPP)	3	22.56ns
Weight of pods perplant (g)	(WPP)	3	625.8***
Seeds weight per pod (g)	(SWP)	3	0.04**
Chlorophyll a (µg/gMF)	(Chl a)	3	0.01*
Chlorophyll b (µg/gMF)	(Chl b)	3	0.05***
Chlorophyll (a+b) (µg/gMF)	(Chl a+b)	3	0.09***
Soluble sugars (mg/gMF)	(SS)	3	0.38***

df: degree of freedom; *, **, ** = Significant at P< 0.05, P< 0.01 and P<0.001; ns= not significant.

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Agronomic parameters

There were significant differences between applied doses of bio-fertiliser except for plant height (HP) and number of pods per plant (NPP) which indicated that there is a non-significant difference between different treatments (Table1). Plant heights vary between 19.5 \pm 2.88cm at To and 23.25 \pm 2.87cm at T3, while those of the number of pods per plant are between 15.50 \pm 4.35 pods obtained at T3 concentration and 19.50 \pm 0.57 at T2 (Table 2). The results obtained for the root length parameter (RL) showed significant differences between the different treatments applied (Table 1). The Fisher test ranked the averages and showed the existence of two homogeneous groups for this

character. The shortest value of root length was observed at To treatment whith 13.50 ± 0.57 cm while the highest value was observed at T2 treatment (17.50 ± 2.08 cm) which corresponds to the 10% foliar dose, equivalent to the concentration of (100 ml/L) (Table 2). In terms of root weight (RW), analysis of variance revealed a very highly significant effect between treatments. The Fisher test at the 5% threshold showed two homogeneous groups. The best mean root weight (4.19 ± 0.98 g) was obtained with T2 treatment (10% dose), while the lowest root weight was observed at T3 treatment with 2.18 ± 0.82 g (Table 2). Obtained results showed that the used biofertilizer had promoted root growth and development.

Table 2. Effect of biofertilizer concentrations on height of plants (HP), root length (RL), root weight (RW), number of pods per plant (NPP), weight of pods per plant (WPP) and seeds weight per pod (SWP).

	Agronomic traits					
Treatments	HP(cm)	RL(cm)	RW(g)	NPP	WPP(g)	SWP(g)
To (0%)	22.00 ± 1.15	13.50±0.57b	2.58±0.45b	15.50 ± 4.35	19.42±6.23b	0.25±0.03b
T1 (5%)	22.00±1.63	17.37±3.40a	2.97±0.06b	18.00±4.89	15.49±1.68b	0.28±0.03b
T2 (10%)	19.50±2.88	17.50±2.08a	4.19±0.98a	19.50±0.57	42.17±4.18a	0.45±0.11a
T3 (15%)	23.25 ± 2.70	17.00±1.41a	2.18±0.82b	14.25±3.30	17.17±3.89b	0.20±0.08b

Mean \pm SD in a column superscripted with different lowercase letters indicate significant (p < 0.05) differences among means within bio fertilizer effect.

These results converge with those of Draghi (2005), who has asserted that nettle manure stimulates plant growth. Indeed, Nettle-based extracts (Urtica dioica L.) are known for their many virtues due to their richness in organic components (Billotte et al., 2014). Also, according to Rivera et al. (2012), aqueous nettle extract is rich in nitrogen, phosphorus, calcium, magnesium and iron and promotes plant growth. Fresh nettle leaves being rich in protein, fat acids, carbohydrates, vitamins, minerals and trace elements (Sekeroglu et al., 2006; Rutto et al., 2013; Pradhan et al., 2015) make this plant extract an elicitor and a Phyto-stimulant (Goulfier, 2010; Moro, 2011). Similarly, Safiddine et al. (2019) reported that plant bio-fertilizers significantly affected root growth. The results for weight of pods per plant (WPP) showed that there was a very highly significant difference between the different applied concentrations of biofertilizer (Table1). Fisher's test confirmed two homogeneous groups and revealed weights ranging from 15.49±1.68g to 42.17±4.18g (Table 2). Except for T1 (5% dose), weight of pods per plant was improved compared to the control (Table 2). It is noted that the T2 corresponding to the concentration of 10% nettle manure has particularly achieved the best yield (more than double compared to the control). This could be explained by the fact that the green bean plants would have benefited from better mineral nutrition, via foliar spraying of liquid bio-fertiliser. In fact, the direct assimilation of nutrients through the stomata of the leaves would be rapid and efficient, hence the availability of solutes during the filling of the pods. These results are in accordance with previous work by Marschner (2012) and Mohanty et al. (2013), who reported that plant responses to the nutrients provided by foliar sprays are faster than when applied as a soil amendment. In addition, Shaaban (2001) has interpreted the good results of foliar fertilization by improving nutrient uptake and regulating the physiological mechanisms of plants. On the other hand, the leaves of the stinging nettle would have contributed to the weight increase of the pods per plant because they are rich in mineral elements and trace elements (Kavalali, 2003, Pradhan *and al.*, 2015). Minerals can account for up to 20% of the dry mass (Chrubasik *et al.*, 2007; Pradhan *et al.*, 2015), as for proteins, 30% of the dry mass (Pradhan *et al.*, 2015). Furthermore, several authors have confirmed the presence of many bioactive compounds, especially in nettle leaves (DiVirgilio *and al.*, 2015). These compounds, also present in other biofertilizers such as seaweed extracts, can positively affect cell metabolism and condition plants advantageously (khan *et al.*,2009; Li *et al.*,2011). In the same direction, studies on field crops of wheat and corn carried out in the United States (Wisconsin) also showed the role of fertilizing nettle manure. In fact, yields were higher than a control crop following a better development of the root system of plants (Billotte *et al.*, 2014).

Table 3. Effect of biofertilizer concentrations on Chlorophyll a (Chl a), Chlorophyll b (Chl b), Chlorophyll a+b(Chl a+b) and soluble sugars (SS).

	Biochemic Traits					
Treatment	Chl (a)(µg/gM)	Chl(b) (µg/gM)	Chl(a+b) (µg/gM)	SS (mg/gMF)		
To (0%)	0.46±0.10b	0.07±0.01c	0.53±0.12b	0.32±0.12c		
T1 (5%)	0.51±0.01ab	0.35±0.08a	0.87±0.09a	0.53±0.10b		
T2 (10%)	0.58±0.00a	0.24±0.01b	0.83±0.02a	1.01±0.20a		
T3 (15%)	0.55±0.01a	0.28±0.03ab	0.83±0.02a	0.42±0.08bc		

Mean \pm SD in a column superscripted with different lowercase letters indicate significant (p < 0.05) differences among means within biofertilizer effect.

Concerning the seeds weight per pod (SWP) traits, the results of the analysis of variance showed highly significant differences between concentrations of liquid biofertilizer applied to green bean plants (Table 1). The Fisher's test has indicated the presence of two homogeneous groups. The recorded weights of seeds per pod are between $0.20g\pm0.08g$ and $0.45g\pm0.11g$. The biofertilizer has allowed an increase in the weight of the seeds per pod except for the treatment T3 (dose 15% equivalent to 150mL/L), which remains the smallest weight (Table 2). This implies that high doses of nettle manure have had an inverse effect on this parameter. These results are in agreement with those of Draghi (2005), who has confirmed that higher concentrations of nettle manure produced the opposite effect and inhibited plant growth. Thus, according to the same author, the optimal dilution in watering seems to be 10% for plants in vegetation. The same result was obtained in this study, where the best weight was achieved with T2 treatment (10% dose). This dose of biofertilizer is economically more profitable than high doses.

Biochemical parameters

The results relating to chlorophyll showed that nettle manure had a positive impact on the amount of chlorophyll a, b and total (a+b) in green bean leaves. The analysis of variance concerning the amount of chlorophyll (a) in bean's leaves revealed a highly significant difference between the applied treatments and also, there were very highly significant differences between biofertilizer's treatments for the amounts of chlorophyll b and total chlorophyll (a+b) (Table 1).

The Fisher's test has shown the presence of three homogeneous groups for chl (a), four groups for chl (b) and two homogeneous groups for chl (a+b) (Table 3). The best amount of chl (a) was obtained with T2 treatment (dose 10%) followed by T3 treatment (15%) corresponding to ($0.58\pm0.00\mu g/gMF$) and ($0.55\pm0.01\mu g/gMF$) respectively. However, the best value of chl (b) ($0.35\pm0.08\mu g/gMF$) was recorded in the T1 treatment (5%). Similar results were found for the total amount of chl (a+b), with a value of (0.87 $0.09\mu g/g MF$). It should be noted that the lowest amounts of chlorophyll (a), (b) and (a+b) were recorded with the control.

For total soluble sugars (SS), there was a very highly significant difference between the biofertilizer's doses for this biochemical parameter. The Fisher's test revealed four homogeneous groups. Soluble sugar values range from $(0.32\pm0.12mg/gMF)$ to $(1.01\pm0.20mg/gMF)$. The best soluble sugar value was obtained with the T2 treatment (10%). However,

the lowest value was recorded with the To control $(0.32\pm0.12 \text{ mg/gMF})$. The results relating to biochemical parameters, namely, the amount of chlorophyll (a), (b) and total (a+b) and total soluble sugars, have shown that nettle manure has stimulated photosynthetic activity and, therefore, the synthesis of total sugars. According to the literature, nettle leaves contain a significant amount of chlorophyll (a) and (b) (Draghi 2005; Tissier 2011); they also contain many bioactive compounds, including nine carotenoids (Di Virgilio *et al.*, 2015).

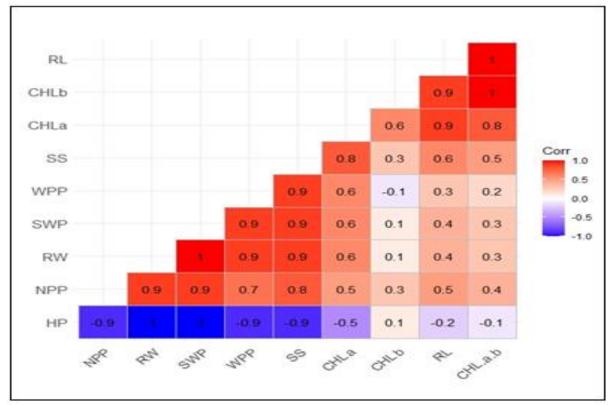


Fig. 1. Pearson's correlation matrix for agronomic and biochemical traits.

These organic pigments, present in chloroplasts and chromoplasts (Wijesinghe *et al.*, 2012), are very powerful antioxidants (Li and Kim, 2011) that protect plants from the degradation of chlorophyll (Whapham *et al.*, 1993; Tarakhovskaya, 2007). In addition, the richness of nettle manure in mineral elements (Billotte *et al.*, 2010) magnesium, particularly in nettle leaves (Pradhan *et al.*, 2015), would also contribute to the stimulation of photosynthesis. Indeed, magnesium plays a pivotal role in the plant's metabolism by being involved in the synthesis of chlorophyll (Veeranan *et al.*, 2018)

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and by participating in the distribution and use of photoassimilates (Cakmak et Yazici, 2010). Our results disagree with those of Garmendia *et al.* (2018), who found no significant effect of nettle manure on the chlorophyll content of potato leaves.

However, they coincide with the work of Uthirapandi *et al.* (2018), who demonstrated that the application of seaweed extracts improved growth and biochemical parameters of basil (*Ocimum sanctum*), including chlorophyll (a), (b), and total content, as well as sugars. In the same context, John Peter Paul *et al.*

(2017) has found that the application of 10% of a biofertilizer based on a brown macroalga (*Sargassum linearifolium*) on corn (*Zea mays*) gave good results on carbohydrates and total chlorophyll.

Correlation

There were negative significant correlations between height of plants (HP) and both root weight (RW) and seeds weight per pod (SWP). In contrast, there were positive significant correlations between chlorophyll a+b (Chl a+b) and both chlorophyll b (Chl b) and root length (RL). Furthermore, root weight (RW) was positively correlated with seeds weight per pod (SWP) (Fig. 1).

Conclusion

This present study has shown that foliar spraying with nettle manure applied at various stages of development of the green bean variety "Nelson", has had a beneficial effect on most agronomic and biochemical studied parameters. The obtained results showed that the treatment which gave the best results on the growth and development of green beans (Phaseolus vulgaris L.) was the one with a concentration of 10% (100mL/L) of biofertilizer. Indeed, biofertilizers prepared from nettle manure represent an appreciable source of mineral elements and bioactive substances, allowing the improvement of the growth and production of crops. Therefore, the use of nettle manure of (Urtica dioica L.) as a green fertilizer would be an interesting solution to counteract the harmful and costly effects of chemical inputs. Therefore, it contributes to sustainable and environmentally friendly agriculture since it is biodegradable and avoids pollution.

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