EFFECT OF BIOFERTILIZERS ON THE MORPHOLOGICAL CHARACTERISTICS OF THE BURDOCK

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Abstract. Using eleven treatments, the present study aimed at investigating the influence that organic and inorganic fertilizers exert upon the germination percentage, the length and weight (i.e. dry and fresh) of the shoot and root, and number of leaves which are considered the morphological characteristics associated prominently with the burdock that is technically entitled *Arctium lappa*. Significant differences were observed between such treatments as urea, vermicompost, vermicompost combined with Nitroxin, 15% manure in comparison with the control treatment in terms of the germination percentage. Another salient feature of urea and vermicompost treatments was that they led to a decrease in the rate of germination. Vermicompost treatments were proven to be significantly different in terms of number of the plant leaves. Shoot and root length was shown to be significantly influenced by the vermicompost treatments utilized both individually and in combination with Nitroxin. Compared with the control treatment, the experimental treatments were more significant as far as the weight of the fresh root and shoot was concerned. Another source of significant difference in terms of dry root weight was identified to be correlated with the 15% and 30% vermicompost individually used as well as the 15% vermicompost and manure employed in combination with Nitroxin. As for the dry shoot weight, no significant difference was witnessed between Nitroxin and 30% manure treatments.
Keywords: *Arctium lappa, growth parameters, inorganic fertilizer, Nitroxin, vermicompost*

Introduction

Arctium lappa Linne, popularly known as burdock or bardana, is a member of the *Asteraceae* (*Compositae*) family and can be found worldwide (da Silva et al., 2013). This genus is native to Eurasia (Duistermaat, 1997). In Flora Iranica, three studied species are reported in Gilan, Golestan, Mazandaran, Tehran, Khorasan, Kerman, Lorestan and Azerbaijan provinces (Henry, 2002; Karimi et al., 2001). The morphological characteristics of this plant include the straight and branched stems covered with fluff, heart-shaped leaves, and same-genus heaps and the cluster inflorescences or pistil and almost spherical involucres, bracteoles in several bayonet and narrow rows, akene with cut and slightly compressed tip (Ghahreman, 1994). In addition, the root of burdock is long and fleshy, gray-brown outside and whitish inside, with a somewhat thick bark and soft wood tissue with a radiate structure. It has a sweet taste and crisp texture. Burdock (*Arctium lappa* L.) has long been cultivated and commonly consumed as a very popular vegetable in Asia (Imahori et al., 2010). The

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nutrients contained in *A. lappa* include inulin, polyphenols, chlorogenic acid, proteins, carbohydrates, vitamins, amino acids, minerals, and unsaturated fatty acids (Chang et al., 2009). Moreover, in folk medicine, *A. lappa* had been used to treat throat pain, arthritis, rashes, and various skin problems, and also as a diuretic, depurative, and digestive stimulant (Chan et al., 2011). The burdock beneficial effects observed are related to hypertension, gout, arteriosclerosis, hepatitis and other inflammatory disorders (Lieber, 1994; Tamayo, 2000). The several investigators have been demonstrated that *A. lappa* displayed hepatoprotective (Lin et al., 2002); antibacterial properties against gram-positive and gram-negative bacteria (Gentil et al., 2006; Pereira et al., 2005) and anti-inflammatory effects (Zhao et al., 2009), which might be due to its free radicalscavenging activity (Leonard et al., 2006). Many of the biological properties attributed to burdock, including antimutagenicity, anticarcinogenicity, and antiaging, may originate from the antioxidant ability of its component (Mclarty, 1997; Niki et al., 1997; Yang et al., 2001).

Roots from *A. lappa* are popular in the Asian cuisine being widely consumed, whereas the leaves are used as infusions or, externally, as a poultice (Jeelani and Khuroo, 2012). Although the leaves are rich in phenolic compounds (Lou et al., 2010), to which many health benefits are associated (Tamayo et al., 2000; Sun et al., 2011; da Silva et al., 2013), there is a prevalence of investigations on the roots and seeds. Extracts from roots containing several monocaffeoylquinic acids (MCQA) and dicaffeoylquinic acids (DCQA), including several isomers, were reported to have gastroprotective activity (da Silva et al., 2013; Santos et al., 2008). Other compounds were also found in *A. lappa*, including arctiin and arctigenin, caffeicacid, chlorogenicacid, cynarin, rutin, quercitrin, quercetin, luteolin, benzoic and p-coumaricacid (Lou et al., 2010; Liu et al., 2005). They are associated to the medicinal properties of *A. lappa*, such as the lignin arctigenin that exhibited antitumor and antidiabetic activities (Awale et al., 2006), these squilignans isolappaol C, lappaol (C,D,F) and diarctigenin, which have anti-inflammatory activity (Park et al., 2007). Therefore, improving the productivity and quality of *A. lappa* is an ultimate goal.

Biofertilizers are the formulation of living microorganisms which are able to make atmospheric nitrogen available for the plants (Subba Rao, 1993) and which contain microorganisms capable of transforming the nutritive elements from a non-usable form to a usable one through biological processes (Tien et al., 1979). Several bacteria that are associated with the roots of the crop plants can induce beneficial effects on their hosts and often are collectively referred to as PGPR standing for plant growth promoting rhizobacteria (Azarpour et al., 2011). Nitroxin is a biologic nitrogen fertilizer that contains *Azotobacter* and *Azospirillum* (Arun, 2007). *Azotobacter* and *Azospirillum* are the two most important non-symbiotic nitrogen-fixing bacteria in non-leguminous crops. Under appropriate conditions, *Azotobacter* and *Azospirillum* can enhance plant development and promote the yield of several agriculturally important crops in different soils (Okon and Labendera-Gonzalez, 1994). These beneficial effects of *Azotobacter* and *Azospirillum* on plants are attributed mainly to an improvement in root development, an increase in the rate of water and mineral uptake achieved by roots, a displacement of fungi and plant pathogenic bacteria and, to a lesser extent, biological nitrogen fixation (Okon and Itzigshohn, 1995). Furthermore, vermicompost is a product of biodegradation and stabilization of organic materials made by an interaction between earthworms and microorganisms. It contains microbial activities (Edwards, 1998). Vermicompost contains plant-growth regulating materials such as humic acids (Senesi

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et al., 1992; Masciandaro et al., 1997; Atiyeh et al., 2002) and plant growth regulators like auxins, gibberellins, and cytokinins (Krishnamoorthy and Vajrabhiah, 1986; Grappelli et al., 1987; Tomati et al., 1988), which are responsible for the increased plant growth and yield of many crops (Atiyeh et al., 2002).

Therefore, in reference to the mentioned issues, the growth characteristics including the percentage and rate of germination, the number of leaves, roots and shoot height, root and shoot fresh and dry weight of the burdock constituted the focal points of the current study. The effects of Nitroxin (mixed with *Azospirillium* and *Azotobacter*), vermicompost and cow manure bio-fertilizers; chemical fertilizers (i.e. urea) at two levels (15 and 30%) on the morphological characteristics were also examined.

Methods

Context and Materials of the Study

The design of the current study was completely randomized, and it was based on some experiments conducted throughout the study using 11 treatments and 4 replications in a greenhouse located in Isfahan (Khorasgan Branch), Islamic Azad University, Isfahan in Iran in 2013-2014. To achieve the main goal of the study, such fertilizers as vermicompost, Nitroxin (*Azotobacter* and *Azospirillium brasilense*), cow manure and urea as a chemical fertilizer were utilized along with some pots (16*19 cm) containing approximately 2 kg of soil and sand extracted from some farms near the university context mentioned above. The burdock is a biennial (lives 2 years), the most development of leaves and root occurs in second year. However, measurement of vegetative growth of seeds can be determining the deferent fertilizer treatments potential in first year in regard with the potted planting of burdock. In the bottom of pots the holes with a diameter of 1.5 cm is considered, it is to measure the roots that grow larger than the size of the pot. Moreover the pots embedded in an 80 cm height of ground surface (*Fig. 1*).

Figure 1. *Arrangement of pots in the greenhouses*

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Treatments

The pots 1-4, 5-8, 9-12, 13-16, 17-20, 21-24, 25-28, 29-32, 33-36, 37-40, and 41-44 were comprised of some soil and sand as the control sample of the study (i.e. 15% vermicompost and 85% soil, 15% vermicompost and Nitroxin and 85% soil, 30% vermicompost and 70% soil, 30% vermicompost and Nitroxin and 70% soil, Nitroxin and soil, 15% cow manure and 85% soil, 15% cow manure and Nitroxin and 75% soil, 30% cow manure and 70% soil, 30% cow manure and Nitroxin and 70% soil, urea and soil, respectively). After 25 burdock seeds were planted in each pot, and the seeds were covered with one centimeter soil, the pots were once irrigated every two days.

Measurement

The seedlings were counted once a week. On the fourth day, the germinated seeds were counted for 32 days. Finally, after 110 days, the shoot and root length was measured using a transparent ruler, and the normal seedlings were counted again. The leaves of each plant were counted as well. Afterwards, the roots and stems were washed, and the fresh weight was determined. To determine the root and shoot dry weight, the samples were dried in an oven for 48 hours at 72 °C. The dry weight was determined using a digital scale (accuracy level=one ten thousandth of a gram). The germination percentage was calculated using equation (1) below:

$$\mathrm{PG} = \frac{\mathrm{Ni}}{\mathrm{N}} \times 100 \qquad \text{(Eq.1)}$$

Where PG is the germination percentage, Ni indicates the number of the germinated seeds per N day, and N shows the number of seeds planted.

Statistical Analysis

All the experiments were repeated four times. The measured data (Tables of the Appendix 1) were analyzed using the analysis of variance (ANOVA), and the mean values of the treatments were compared using Duncan’s Multiple Range Test (DMRT) at $P \leq 0.05$. The 19th version of the SPSS was used to analyze the whole data.

Results and Discussion

The results gained from the ANOVA for the vegetative characteristics such as the number of leaves, root and shoot length, fresh and dry weight of root and stem and the germination percentage are shown in *Table 1* below.

Table 1. *Effect of the treatments on the vegetative characteristics of the medicinal burdock plant*

Treatment	% Germination	Number of leaves	Root length (cm)	Stem length (cm)	Root fresh weight (gr)	Shoot fresh weight (gr)	Root dry weight (gr)	Shoot dry weight (gr)
Control	38a	2.75c	12.7a	1.5ef	0.08f	0.09d	0.04f	0.03e
%15 Vermi	39bcd	4ab	28bc	2.87bcd	1.25bc	0.52bc	0.75bc	0.23bcd

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%15 Vermi. Nitroxin	35cd	3.25bc	17.75b	3.6ab	1.4b	0.82c	0.78ba	0.30b
%30 Vermi.	37bcd	4.5a	16.1b	4.25a	2.2a	1.3a	1.1bcd	0.56a
%30 Vermi Nitroxin	16e	3.5abc	15.75bc	3.37abc	0.89bcd	0.78b	0.5f	0.25bc
Nitroxin	24de	3bc	10.1c	0.75f	0.07f	0.07d	0.03def	0.02e
%15FYM	39bcd	2.5c	17.4b	2.1de	0.61def	0.29cd	0.26def	0.12cde
%15 FYM Nitroxin	53ab	3.5abc	17.25b	2.8bcd	0.8cde	0.47bcd	0.43 cde	0.2bcd
%30 FYM	48ab	2.5c	17.75b	2.25cde	0.20ef	0.32cd	0.10 ef	0.11de
%30 FYM Nitroxin	60a	2.75c	16.87b	2.87bcd	0.3def	0.26cd	0.12ef	0.13cde
Urea	0f	-	-	-	-	-	-	-

Means within the column with the same letter are not significantly different by Duncan multiple range test at ≤ 0.05.

Germination Percentage

According to the statistical analysis of the experimental data gained from the studied treatments shown in *Table 1* above, urea made the most significant difference among all the treatments tends by inhibiting the germination process. Urea has many advantages and is widely used as a nitrogen fertilizer throughout the world of agriculture; however, there are problems related to its use (Engelstad and Hauck, 1974). Some of these problems have adverse effects on germination, seed and early plant growth in soil (Gasser, 1964; Goyal and Huffaker, 1984). The adverse effect of urea on germination is due to the presence of impurities such as biuret (Wilkinson and Ohlrogge, 1960), due to cyanate formed by the isomerization of urea in aqueous solution, PH or high concentrations of ammonium ions due to hydrolysis of urea by soil urease (Widdowson et al., 1960; Court et al., 1964), Ammonia production from urea hydrolysis (Court et al., 1964), or nitrite production by nitrification and urea nitrogen by soil microorganisms (Court et al., 1964). Bremner and Krogmeier (1989) reported that the effect of urea fertilizer on seed germination of wheat, rye, barley and maize showed that the adverse effects of urea on seed germination in soil is due to ammonia formed by urease hydrolysis which is not related to urea. Impurities in urea as biuret or nitrite resulted from the nitrification of urea nitrogen. The studies compared the effects of germination on soils made by pure urea, urea and impure urea. Also, the compounds formed as a result of changes in soil microbial and enzymatic urea showed that ammonia volatilization from soils treated with urea completely inhibit the germination process.

Both the vermicompost and the vermicompost combined with Nitroxin showed significant differences in comparison with the control treatment. Also, the two treatments 30% vermicompost combined with Nitroxin and Nitroxin indicated significant differences, but the germination was less than half in the combined treatment. Other treatments including the vermicompost treated both individually and in combination with Nitroxin were not observed to be significantly different from the control treatment. The 15% vermicompost treatment did not reduce the germination level. Once combined, the 15% vermicompost treatment turned out to exert a greater effect on the germination process. It is so likely that in the early stages the plants are susceptible to the negative effects of the vermicompost. The studies showed that the

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application of the vermicompost inhibits the germination and seedling growth so that when the concentration of the vermicompost increases, the growth decreases linearly (Ievinsh, 2011). Buckerfield et al. (1999) reported that radish seed germination decreases gradually once the concentration of the vermicompost increases, but a tenfold increase is observed in the 100% vermicompost as compared with its 10% counterpart.

In the early stages of growth, the plants were susceptible to the negative effects of vermicompost. Moreover, the stability and maturity are essential for successful application (Wang et al., 2004), Stability is related to microbial activity (Lasaridi and Stentiford, 1998). Puberty refers to the degree of decomposition of organic matter during composting and is produced in the absence of pathogens and weed seeds (Wu et al., 2000). Unstable compost leads to the creation of a competition for oxygen uptake between the biomass and the root or seed. The roots and seeds are deprived from oxygen; the H_2S and NO_2 reduce (Mathur et al., 1993). According to Nadi et al. (2011), the germination of pistachio in vermicompost cow manure treatments, compared with the control treatments, showed a significant increase owing to the growth of fungi and the disease in seeds in pots treated by the premature vermicompost. Bachman and Metzger (2008) stated that vermicompost were used to improve the growth and a lack of increase in the percentage or rate of germination caused by new seeds and the effect of vermicompost. Joshi and Vig (2010) reported that the highest germination percentage at 15% vermicompost treatments was observed to be up to 86%, and an increase in the concentrations of the 30 and 45% treatments that were gradually reduced to 60 and 55% owing to the presence of excessive nitrogen that inhibited the germination.

As Saleem et al. (2007) mentioned, Plant Growth Promoting Bacteria (PGPR) including the enzymes of amino cyclopropane and dideaminase carboxylic acid (ACC) facilitates the growth of the plant indirectly by reducing plant pathogens, but directly by facilitating the absorption of nutrients through production phytohormone such as auxins, cytokines and gibberellins and enzymes reducing the level of ethylene. They added that PGPR totally breaks the ACC ethylene precursor to utyrate and reduces ammonia and ethylene levels in growing plants. Throughout their study, Gallardo et al. (1994) demostrated that although a large amount of ethylene was not required for the germination of pea seeds, a certain amount was necessary. A comparison made between the cow manure and control treatments indicated that the 15% cow manure was significantly different from the control treatment, though no difference was found between other sorts of the cow manure treatment and the control treatment. However, the combination of the cow manure treatment and Nitroxin was found to be apt for germination in that the highest germination level was observed to be attributed to the %30 cow manure treatment combined with Nitroxin. Germination increased when the concentrations of the %15 to %30 cow manure treatments increased.

It should be mentioned here that the components of the manure treatment can enhance or hinder the germination process. Studies demonstrate that access to a higher level of nitrogen stimulates the germination of some species (Luna and Moreno, 2009). Using different doses of manure with the purpose of influencing the germination process, Carlos et al. (2013) argued for a threshold level above which cow manure could not be influential in the germination process.

Number of Leaves

In terms of the number of the leaves, no significant difference was found between 30% vermicompost treatment combined with Nitroxin, the 15% vermicompost and the

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15% manure treatments combined with Nitroxin. As indicated by *Table 1*, no significant difference was witnessed between the 15% and 30% cow manure treatments. Through the application of vermicompost for the growth of strawberries, Singh et al. (2008) showed that the vermicompost highly influenced such growth parameters as the leaf width and dry weight. This effect was due to the availability of plant growth regulation and humic acid, which is produced by increasing the activity of microbes in vermicompost (Arancon et al., 2004). Microbes such as fungi, bacteria, yeasts and actinomycetes that produce hormones like auxin and gibberellin make a significant amount of vermicompost (Brown, 1995; Arancon et al., 2004).

Stem Length

The results gained from a statistical analysis of the stem length presented in *Table 1* indicate a significant difference made by the vermicompost and the composition of vermicompost and Nitroxin. The 30% vermicompost treatment which turned out to exert the most significant influence on the stem length exhibited no significant difference from the vermicompost composition, though a significant difference was observed as far as the 15% vermicompost treatment combined with other treatments was concerned. The greatest impact on the stem elongation was found to be related to the 15% and 30% vermicomposts combined with Nitroxin and the 30% vermicompost treatment. The 15% vermicompost was more effective once combined with Nitroxin. Joshi and Vig (2010) demonstrated that the application of 15%, 30% and 45% vermicompost treatments increased the height of tomato more significantly than their control treatments. Gutiérrez-Miceli et al. (2007) contended that a significant increase in the average stem diameter and plant height was caused by the use of different doses of sheep manure vermicompost. High microbial activities in the vermicompost are due to fungi, bacteria and autonomists (Tomati et al., 1988). Microorganisms such as bacteria, fungi, yeasts, algae and actionists cause the production of plant growth regulators such as auxin, gibberellins, cytokinin and ethylene (Frankenberger and Arshad, 1995) and vermicompost showed a positive effect on the growth of *Begonias* and *Coleus* (Tomati et al., 1988).

Nitroxin treatment, as indicated by *Table 1* above, was not significantly different from the control treatments, but it significantly reduced the stem length. The average nitrogen rate of soil or nitrogen by nitrogen fixation is not a barrier to nitrogen fixing but its high levels reduce nitrogen fixing (Marschner, 1995).

The cow manure and its combination with Nitroxin were not observed to be significantly different. This composition had a greater impact on the stem length than the individual application of the cow manure treatment. The differences between the 15 % and 30% cow manure and control treatments were not significant.

Shoot Fresh Weight

As for the fresh weight of the shoots, the vermicompost treatments (i.e. the vermicompost itself and its combined form) were shown to be significantly influential. Specifically, the 30% vermicompost was found to play the most critical role in the weight than other treatments of the study. According to other studies (Peyvast et al., 2008), the greatest impact on the plant growth and yield was demonstrated to be made by low ratios of vermicompost (20 to 40 %). A reduction in shoot fresh weight was observed in the Nitroxin treatment, but there was no significant difference between this

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treatment and the control one. The cow manure treatments were found not to be significantly different in this respect. They had not been different from the control treatment neither. However; the stem was much more significantly affected by this treatment than the control treatment of the study.

Shoot Dry Weight

As for the shoot dry weight, a significant difference between the vermicompost itself and its combined form was found. In addition, the 30% vermicompost was more significantly influential than other treatments. The Nitroxin treatment decreased the shoot dry weight in comparison with the control treatment, yet the difference was not significant. This treatment was significantly different from other treatments, but the difference existing between the 30% cow manure treatments applied individually and the one utilized in combination with Nitroxin was not significant. The greatest impact on stem dry weight was found to be correlated with the combination of the 15% cow manure and Nitroxin.

Root Length

According to *Table 1*, significant differences between the experimental and control treatments were observed. The root length increased due to the 15 and 30% vermicompost treatments. The plants respond differently to different doses of vermicompost due to the production of growth enhancing materials in lower doses than higher doses (Arancon et al., 2004). No significant difference was found between the 15% vermicompost and 15% vermicompost combined with Nitroxin, as well as between the 30% vermicompost treated solely and the one combined with Nitroxin. A root length reduction was observed in 15% and 30% vermicompost treatments combined with Nitroxin. Nitroxin treatments were demonstrated not to be significantly different from the 15% and 30% vermicompost treatments combined with Nitroxin, but the difference was significant between the Nitroxin and other treatments. Also, a significant reduction in the root length occurred due to the experimental treatments in comparison with the control one. Vessey (2003) opined that an increase in gibberellins can increase cell elongation influenced by biological fertilizers. He asserts that rhizosphere bacteria are likely to cause the synthesis and release of auxin as a matter of secondary metabolites in roots.

Cow manure treatments applied individually or in combination with Nitroxin didn’t exert a significant influence on the root length in comparison with the control treatments. Verlinden et al. (2010) conducted a study on the effect of biofertilizers on increasing pasture vegetative organs. The availability of food increased the population and colonization of bacteria in the root of the organic fertilizers combined with *Azotobacter* and *Azospirillium* (Siddiqui, 2004).

Root Fresh Weight

As presented by *Table 1*, the vermicompost treatment applied both individually and in combination with Nitroxin made a significant influence upon the fresh weight of the roots in comparison with the control treatment. The 30% vermicompost was the most significantly influential in the root fresh weight among all the treatments of the current study. This significance can lie in the increasing amount of available nutrients, the number of beneficial microorganisms, enzyme activities and plant growth promoters

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such as gibberellin, cytokinin and auxin. Kadam and Pathade (2014) showed that using the proper proportions of vermicompost, root fresh weight increased more significantly in the experimental treatment than its control counterpart. In their study, the Nitroxin treatment showed no significant differences, but it led to a higher reduction in the root fresh weight in comparison with the control treatment. The cow manure treatments were greatly but insignificantly effective in the fresh weight as compared with the control treatments.

Root Dry Weight

Regarding the root dry weight, the vermicompost treatments, especially the 30% form, had a more significant influence than the control treatment. Bachman and Metzger (2008) examined the effect of 10% and 20% vermicompost treatments on marigold, revealing that the latter brought about a greater increase in shoot and root dry weight than the former. In their study, the Nitroxin treatment showed no significant differences as compared to the control treatment. The cow manure treatments, especially the 15% form, and their combination with Nitroxin were more significant than other treatments in this respect.

Conclusions

The results gained from the current study showed different effects of application of vermicompost, biological, cow manure and urea fertilizers on the germination process. The germination percentage of the vermicompost and Nitroxin decreased in comparison with the control treatment. The cow manure treatment and its combination with Nitroxin increased the germination percentage. Furthermore, a higher dose of the vermicompost treatment was found to be more influential in the number of the leaves. Both levels of the cow manure treatment turned out to be negatively influencial in the number of the leaves. A negative effect was found to be made by the Nitroxin treatment on the shoot fresh weight. Regarding the shoot dry weight, all the study treatments had a great effect except for Nitroxin. In terms of the root length, all treatments were significantly different in comparison with the control treatment. The 15% vermicompost applied individually, the 15% vermicompost used in combination with Nitroxin, and the 30% cow manure were witnessed to be the most significantly influential, but the Nitroxin was found to have a negative influence. Of all the treatments included in the present research study, the 30% vermicompost treatment had the highest effect on the root fresh weight which was differently influenced by the experimental treatments (i.e. vermicomposts) and the control treatment. The root dry weight was shown to be positively significantly influenced by the 15% and 30% vermicomposts, and the 15% cow manure treatment combined with Nitroxin. More importantly, the vermicompost treatments applied individually and in combination with Nitroxin had the greatest effect among all the treatments. The 30% vermicompost was observed to be the highest in this respect. The organic manures and their combination with biofertilizers played a crucial role in the growth of burdock. As for the number of the leaves, stem length, fresh and dry weights of the roots and shoots, the higher doses of the vermicompost 30% were more influential than the 15% vermicompost treatment. Regarding the characteristics such as the germination percentage, the number of the leaves, and the length of the roots, the 15% vermicompost assumed the most significant role, whereas the combined version of the vermicompost treatment was most influential in other parameters.

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The individual and non-individual application of the 15% vermicompost treatment had a negative effect, so this level was proved to be far from optimal as far as the morphological characteristics were concerned. The negative impact of Nitroxin treatment (containing *Azotobacter* and *Azospirillium*) was probably due to the negative interplay between two bacteria. The 15% cow manure applied both individually and non-individually was significantly influential in all parameters except for the root length, but the 30% counterpart had the highest impact on all the features of the plant. Consequently, it is suggested that a lower dose of the animal manure be applied in order to enhance the growth of burdock. However, as for the germination percentage, and the shoot and root dry weight, the combined form is highly recommended. A lower dose of other treatments combined with Nitroxin is suggested once other parameters are intended to be taken into consideration.

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APPENDIX

Appendix 1. *Tables of burdock biometric variation in different treatment*

Pot	Root height (cm)	Stem height (cm)	Root fresh weight (gr)	Stem fresh weight (gr)	Dry root weight (gr)	Dry stem weight (gr)	Number of leave	Germination%
1	8	1.5	0.07	0.06	0.03	0.04	4	44
2	17	1.5	0.11	0.12	0.05	0.05	3	40
3	15.5	2	0.11	0.11	0.08	0.05	2	40
4	10.5	1	0.06	0.07	0.01	0.01	2	28
5	34	3.5	1.2	0.53	0.71	0.2	4	32
6	22	3.5	1.12	0.65	0.73	0.24	4	36
7	29	2.5	1.6	0.45	0.84	0.23	4	40
8	27	2	1.11	0.48	0.73	0.27	4	48
9	17	3	1.12	0.56	0.6	0.22	3	32
10	18.5	3.5	1.45	0.76	0.74	0.25	3	36
11	17.5	4	1.85	0.82	1.1	0.31	3	32
12	18	4	1.23	1.15	0.69	0.44	4	40
13	18.5	4.5	2.12	0.95	1.15	0.42	5	52
14	15	4	2.85	1.12	1.54	0.54	5	36
15	16.5	4.5	1.46	1.4	0.71	0.49	4	32
16	14.5	4	2.46	1.9	1.39	0.79	4	28
17	15.5	3.5	2.21	1.64	1.25	0.46	3	20
18	17.5	3.5	0.31	78.2	0.17	0.22	3	16
19	18	5	0.83	0.51	0.41	0.25	5	16
20	12	1.5	0.21	0.22	0.17	0.09	3	12
21	9.5	0.5	0.09	0.11	0.05	0.02	3	40
22	7.5	0.5	0.02	0.03	0.01	0.01	2	4
23	9.5	1	0.08	0.09	0.03	0.05	4	28
24	14	1	0.09	0.06	0.04	0.03	3	24
25	13	1.5	0.25	0.16	0.15	0.05	3	60
26	24	2.5	0.68	0.31	0.38	0.11	2	52
27	18.5	2	0.78	0.26	0.21	0.14	2	20
28	14	2.5	0.74	0.46	0.32	0.18	3	24
29	13.5	2.5	0.74	0.35	0.43	0.16	4	40
30	13.5	3	0.72	0.36	0.39	0.15	3	60
31	17	3	0.81	0.62	0.41	0.25	3	60
32	25	3	0.95	0.57	0.49	0.26	4	52
33	22	3	0.36	0.54	0.21	0.19	3	48
34	20.5	2.5	0.15	0.39	0.09	0.11	3	56
35	17.5	3	0.31	0.35	0.11	0.14	3	48
36	11	0.5	0.01	0.02	0.001	0.001	1	40
37	15.5	2	0.47	0.34	0.25	0.23	2	64
38	20	2.5	0.39	0.27	0.16	0.11	3	60
39	17	3	0.31	0.21	0.06	0.05	3	56
40	15	4	0.03	0.23	0.01	0.12	3	60

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Appendix 2. *The effect of treatment on burdock growth parameters*

Pot	Root height (cm)	Stem height (cm)	Root fresh weight (gr)	Stem fresh weight (gr)	Dry root weight (gr)	Dry stem weight (gr)	Number of leaves	Germination%
1	34	3.5	1.2	0.53	0.71	0.2	4	32
2	22	3.5	1.12	0.65	0.73	0.24	4	36
3	29	2.5	1.6	0.45	0.84	0.23	4	40
4	27	2	1.11	0.48	0.73	0.27	4	48
5	28	2.875	1.2575	0.5275	0.7525	0.235	4	39
6	9.5	0.5	0.09	0.11	0.05	0.02	3	40
7	7.5	0.5	0.02	0.03	0.01	0.01	2	4
8	9.5	1	0.08	0.09	0.03	0.05	4	28
9	14	1	0.09	0.06	0.04	0.03	3	24
10	10.125	0.75	0.07	0.0725	0.0325	0.0275	3	24
-	-	-	-	-	-	-	-	-
11	13	1.5	0.25	0.16	0.15	0.05	3	60
12	24	2.5	0.68	0.31	0.38	0.11	2	52
13	18.5	2	0.78	0.26	0.21	0.14	2	20
14	14	2.5	0.74	0.46	0.32	0.18	3	24
15	17.375	2.125	0.6125	0.2975	0.265	0.12	2.5	39
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
16	13.5	2.5	0.74	0.35	0.43	0.16	4	40
17	13.5	3	0.72	0.36	0.39	0.15	3	60
18	17	3	0.81	0.62	0.41	0.25	3	60
19	25	3	0.95	0.57	0.49	0.26	4	52
20	17.25	2.875	0.805	0.475	0.43	0.205	3.5	53
-								
21	22	3	0.36	0.54	0.21	0.19	3	48
22	20.5	2.5	0.15	0.39	0.09	0.11	3	56
23	17.5	3	0.31	0.35	0.11	0.14	3	48
24	11	0.5	0.01	0.02	0.001	0.001	1	40
25	17.75	2.25	0.2075	0.325	0.10275	0.11025	2.5	48
26	15.5	2	0.47	0.34	0.25	0.23	2	64
27	20	2.5	0.39	0.27	0.16	0.11	3	60
28	17	3	0.31	0.21	0.06	0.05	3	56
29	15	4	0.03	0.23	0.01	0.12	3	60
30	16.875	2.875	0.3	0.2625	0.12	0.1275	2.75	60

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Appendix 3. *Cumulative data of pot replication in different treatment*

Pot number	8	12	16	20	24	28	32	36	40	sum	gmt
1	0	2	3	2	1	1	1	0	0	10	
1	0	24	48	40	24	28	32	0	0	196	19.6
2	0	3	2	2	1	1	1	1	0	11	
2	0	36	32	40	24	28	32	36	0	228	20.72727
3	1	1	0	1	2	2	2	1	0	10	
3	8	12	0	20	48	56	64	36	0	244	24.4
4	0	2	2	0	1	1	1	0	0	7	
4	0	24	32	0	24	28	32	0	0	140	20
5	1	1	1	2	1	2	1	0	0	9	
5	8	12	16	40	24	56	32	0	0	188	20.88889
6	0	1	1	1	0	1	1	2	2	9	
6	0	12	16	20	0	28	32	72	80	260	28.88889
7	0	1	3	1	2	1	2	1	0	11	
7	0	12	48	20	48	28	64	36	0	256	23.27273
8	0	1	1	1	2	3	1	1	1	11	
8	0	12	16	20	48	84	32	36	40	288	26.18182
9	0	1	1	2	0	1	1	1	1	8	
9	0	12	16	40	0	28	32	36	40	204	25.5
10	0	1	1	0	1	1	2	3	0	9	
10	0	12	16	0	24	28	64	108	0	252	28
11	0	1	1	2	1	1	2	0	0	8	
11	0	12	16	40	24	28	64	0	0	184	23
12	0	1	1	0	2	2	3	1	1	11	
12	0	12	16	0	48	56	96	36	40	304	27.63636
13	0	1	1	1	1	2	3	4	0	13	
13	0	12	16	20	24	56	96	144	0	368	28.30769
14	0	1	1	1	1	2	3	1	0	10	
14	0	12	16	20	24	56	96	36	0	260	26
15	0	0	1	1	1	2	2	1	0	8	
15	0	0	16	20	24	56	64	36	0	216	27
16	0	1	1	2	2	1	1	0	0	8	
16	0	12	16	40	48	28	32	0	0	176	22
17	0	0	1	1	1	1	2	0	0	6	
17	0	0	16	20	24	28	64	0	0	152	25.33333
18	0	2	1	0	0	1	1	0	0	5	
18	0	24	16	0	0	28	32	0	0	100	20
19	0	1	1	0	0	1	2	0	0	5	
19	0	12	16	0	0	28	64	0	0	120	24
20	0	1	0	0	0	0	1	1	0	3	
20	0	12	0	0	0	0	32	36	0	80	26.66667
21	0	1	2	1	1	3	2	1	1	12	
21	0	12	32	20	24	84	64	36	40	312	26
22	0	1	1	0	0	0	0	0	0	2	
22	0	12	16	0	0	0	0	0	0	28	14
23	0	2	1	1	0	2	1	1	0	8	
23	0	24	16	20	0	56	32	36	0	184	23
24	0	1	1	1	1	2	1	0	0	7	
24	0	12	16	20	24	56	32	0	0	160	22.85714
25	0	2	4	3	0	3	2	2	1	17	
25	0	24	64	60	0	84	64	72	40	408	24
26	1	1	1	3	3	4	1	0	0	14	
26	8	12	16	60	72	112	32	0	0	312	22.28571

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27	0	1	1	1	0	2	0	0	0	5	
27	0	12	16	20	0	56	0	0	0	104	20.8
28	0	1	1	1	2	1	1	0	0	7	
28	0	12	16	20	48	28	32	0	0	156	22.28571
29	1	1	1	2	3	1	1	1	0	11	
29	8	12	16	40	72	28	32	36	0	244	22.18182
30	1	2	1	2	1	3	2	1	2	15	
30	8	24	16	40	24	84	64	36	80	376	25.06667
31	1	1	4	3	2	1	2	1	1	16	
31	8	12	64	60	48	28	64	36	40	360	22.5
32	3	3	2	2	1	0	1	1	0	13	
32	24	36	32	40	24	0	32	36	0	224	17.23077
33	3	2	1	1	1	0	0	0	0	8	
33	24	24	16	20	24	0	0	0	0	108	13.5
34	3	2	2	1	2	2	2	1	0	15	
34	24	24	32	20	48	56	64	36	0	304	20.26667
35	1	1	1	2	2	2	3	1	0	13	
35	8	12	16	40	48	56	96	36	0	312	24
36	1	2	1	1	2	1	2	1	0	11	
36	8	24	16	20	48	28	64	36	0	244	22.18182
37	2	3	2	2	2	1	2	2	1	17	
37	16	36	32	40	48	28	64	72	40	376	22.11765
38	0	2	3	2	1	3	2	1	1	15	
38	0	24	48	40	24	84	64	36	40	360	24
39	2	2	2	2	1	1	2	1	0	13	
39	16	24	32	40	24	28	64	36	0	264	20.30769
40	2	3	2	3	2	2	1	1	0	16	
40	16	36	32	60	48	56	32	36	0	316	19.75
41	0	0	0	0	0	0	0	0	0		
42	0	0	0	0	0	0	0	0	0		
43	0	0	0	0	0	0	0	0	0		
44	0	0	0	0	0	0	0	0	0		

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