

Urea Fertilization: Effects on Growth, Nutrient Uptake and Root Development of the Biodiesel Plant, Castor Bean (*Ricinus communis* L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

An indoor pot culture experiment was conducted in the growth chamber during the period of vegetative growth to evaluate the influence of inorganic nitrogen fertilizer in the form of urea on nutrient uptake, growth and root development of castor bean plant. The Nitrogen Fertilizer treatments imposed in the experiment were: Control (N0), no nitrogen and others at the rate and 60lb N/acre (N1), 90lb N/acre (N2) and 120lb N/acre (N3) respectively. Effect of higher nitrogen concentration indicated considerable increases in castor growth including vegetative growth and the plant components biomass. Elevated nitrogen fertilizer increased height and other morphological and physiological parameters (Leaf and petiole length, intermodal distance, root numbers) including the root, shoot dry wt, root/ shoot ratio, nitrogen and crude protein content in plants. Among the plant components, shoot, root dry weight and root shoot ratio had the greatest decrease under N deficiency, while root/shoot carbon ratio increased under N deficiency. No statistical difference was observed with treatments in shoot and root N% and shoot C% in plants although root carbon content was significantly higher with lowest nitrogen level compared to elevated levels. Significant

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increases of carbon content in plants at N0 showed some tendency of this crop to adjust with lower nitrogen levels. Also no statistical difference was observed in root and shoot N ratio, while the root and shoot carbon ratio was found significant at N0 compared to other treatments. However the concentration of carbon and nitrogen were found higher in shoot than root in all applied treatments. After harvesting the residual nitrogen effect in soil was also found significant with elevated nitrogen level compared with other treatments and control.

Keywords: *Castor bean; growth; nitrogen uptake; root/shoot ratio; urea.*

1. INTRODUCTION

Castor bean (*Ricinus communis* L.) is a non-food, drought resistant and an important oilseed bioenergy crop, especially for the production of biodiesel. This energy crop gaining attention for producing fuel in developed as well as in developing countries. Castor finds a place of prestige in the cropping systems of dry-land agriculture because of its deep root system, drought hardiness, and quick growth. It is widely cultivated throughout the world for fuel use as well as it has number of uses for domestic, and industrial purposes. It belongs to the family *Euphorbiaceae* and is also one of the medicinally important oil seed crops [1]. Castor oil, one of the oldest commercial products, was used in lamps by the Egyptians more than 4,000 years ago and seeds have been found in their ancient tombs [2,3]. Although castor commonly referred to as a "bean," it is not a legume and the plant has also been called the "castor oil plant." The plant may grow up to a height of 6 to 15 feet and can live for many years. The seeds contain 5.1-5.6% moisture, 12–16% protein, 46 to 60% oil and are a rich source of phosphorus. The oil consists of Oleic, ricinoleic, linoleic, stearic acids [4]. So as an oil bearing biomass feedstock, it can ensure an alternative source of energy and reduce our dependency on fossil fuel. Like other second generation biodiesel plant, *Jatropha*, this plant can grow anywhere including soil considered infertile for food production and can meet current and future energy demands with greater environmental benefits [5,6].

Castor plant is generally produced from seed and the time from planting to emergence was found to vary between 7 – 23 days, depending on soil temperature [7,3,8,9,10] and as well as regeneration by tissue culture, to receive disease free, large number of plants for commercial cultivation. Regeneration by tissue culture technique would also be a feasible alternative for improving the quality and to develop an efficient protocol for propagation of castor oil plant [11,12,13,14,15].

As a medicinally important plant, it is also purgative popular for the treatment or prevention of many ailments. The leaves have been used for treatment of rheumatic pains and as antibacterial and anti-inflammatory [1,16,17,18]. The oil also prescribed for infestation of intestinal worms. Infusion of the leaves was used as a remedy for rash, itch and eye inflammation. The decoction of leaves also used for skin diseases, diarrhea, kidney, urinary bladder infections [19] and the oil is used in mainstream medicine as a way to deliver chemotherapy drugs to cancerous tumors [20,21].

Production of castor plants has increased since the middle of the twentieth century [22]. Although, India, China and Brazil are the major world producers of castor oil, the plants are also grown commercially in many other countries, including the United States (New Mexico, Texas and the Midwestern United States), Philippines and Thailand. EU, China, Philippines, Paraguay, Brazil and United States sub-tropical regimes [23]. Production of castor is needed in the United States to supply castor oil for the hundreds of products using this versatile chemical raw material. Forty to forty five thousand tons of castor oil and derivatives are imported each year to supply the entire needs of domestic industries. The United States is the largest importer and consumer of castor oil in the world. The oil is classed as a strategic material critical to the United States national defense by the Agricultural Materials Act P.L. 98-284 passed by Congress in 1984 [24]. Castor bean yield in the US, however, dependent on the latitude and management practices [10,25]; maximum yield reported was 2690 kg ha⁻¹ in southern Nebraska under irrigated conditions [26]. In a recent planting date and location study in the US south-central region showed that castor bean mean yields were greatest at Memphis, TN, the northern most location (maximum, 1954 kg ha⁻¹) and lowest at Poplarville, MS, the southernmost location [27].

Castor bean is successfully cultivated in tropical and sub-tropical regimes to reduce the dependency of fossil fuel. The plant grows as an annual in cold and arid areas. But in the tropics and sub tropics, the plant is a perennial [28]. The oil contains a very high percentage of hydroxy fatty acid [29] known as ricinoleic acid. Ricinoleic acid can be treated by dehydration. This dehydrated castor oil is in demand for paints and varnishes because of its non-yellowing quality. The oil has a great promise in the field of biodiesel production or in addition the oil can be used in biodiesel studies [30,31]. Besides being used as a source of biodiesel the oil is also used in polishes, ointments, waxes, printing inks, cosmetic, hair dressings and disinfectants. The oil is also used for the synthesis of soaps, linoleum, printer's ink, nylon, enamels and electrical insulations, dyeing and finishing of fabrics and leather, preservation of leather and production of Rilsan-polyamide nylon-type fiber [32,33,34,35,30,36]. The oil has also been used as a lubricant in all parts of machinery and for internal combustion engines in airplanes and in making of explosives [37,38,39]. It is also used as an illuminant with a steady flame and burning much longer than any other vegetable oil.

Castor with a global average yield with about 40–60% oil is a more suitable and viable biodiesel crop than the traditional oil seed crops such as soybean and sunflower with 25.5% of oil and with 18% oil, respectively [40,41]. In recent years, the oil is largely used in the specialty chemical industry worldwide, and the growth of its consumption is limited by insufficient and unreliable feedstock supply rather than by the industry demand [42]. Therefore, castor bean crop can become a cash crop in modern agriculture, in particular, for non-food uses including bioenergy purposes [43,44]. Although increased castor production in the world can be obtained with the use of varieties and hybrids with higher genetic potential and an improved crop management. The transition from a low-input/low-yield to a high yielding crop will require a deeper understanding of castor plant growth and development. This knowledge is the key for the breeding of high yielding varieties adapted to each growing environment and for the optimization of crop management in order to express the maximum yielding potential of the crop [45]. In addition, compared to soybean and sunflower, little work has been done so far in castor bean, and with additional agronomic production and plant breeding studies [27,46],

the crop may have more potential than presently realized.

For agronomic production, Nitrogen is most important macronutrient that all plants need because this fertilizer is an integral component of amino nucleic acids, proteins, nucleotides, chlorophyll, chromosomes, genes, ribosome and is also a constituent of all enzymes. This wide range of different nitrogen containing plant compounds explains the important role of nitrogen for plant growth [47]. In plants, nitrogen present in the chloroplasts, which are the molecules within plants, perform photosynthesis for making food. If plants do not have enough nitrogen, they turn yellow, in part because the chloroplasts are not functioning properly [48]. Also, if the soil is deficient in nitrogen, *Alternaria* leaf spot (disease) is more severe in nitrogen-starved plants [49]. The most important factor in fertility level is the supply of nitrogen in the soil [50].

Different abiotic stresses cause extensive losses to agricultural production worldwide [51,52]. Among those stresses, the availability of nitrogen (N) is the major limiting factors in crop growth, development, and finally economic yield [53,54]. Farm managers generally apply N on majority of the crops except N-fixing legumes and the amount and application time dependent on crop growth stage, weather, tillage practice, and on soil N status. The major requirements are for the production of seed [55] and forage [56]. Nitrogen is the most critical element of plant development as the plant growth is affected differently by various N sources [57]. The different sources of N using for plant indicated that the growth (fresh or dry weight) is always more in nitrate than various reduced N sources [58].

So, responses of plants to N fertilization are of considerable importance in agriculture. Crop yields have been steadily increased over the last 60 years by a combination of improvements in genetics and management practices including N application [40,59]. Although adequate supply of N to crops is fundamental to optimize crop yields, mismanagement of N, such as excessive N application, can result in contamination of groundwater [60]. Applying different nitrogen levels (0, 40, 80 and 120 kg ha⁻¹) indicated highest seed yield, and biological yield under application of 80 kg ha⁻¹, whereas highest seed weight (thousand) was achieved under 120 kg ha⁻¹. The oil percent decreased under increased nitrogen application [61]. Functional relationships

between plant nitrogen (N) and crop growth processes are not available in many crops including castor bean plant [62].

The application and effects of different doses of N fertilization on castor bean depend on soil and climatic condition of different countries. 30 kg ha⁻¹ nitrogen are considered as best dose for *R. communis* in Egyptian condition whereas, 50 kg ha⁻¹ and 100 kg ha⁻¹ considered under Belgium and Ankara conditions respectively [63,64,65]. However N frequently limits growth and development of several crop species under field conditions, the precise mechanisms by which the limitation occurs are complex and variable [66], depending on species, developmental stage and environment. Limited N supply also decreases rates of cell division and cell expansion [67], photosynthesis, leaf production, tillering [68,69,70,71,72,66,73,74, 75,76], and yield [77]. N deficiency affects more strongly the leaf area development than photosynthesis [78,79,71]. The effects of low N nutrition on plants as causing lower photosynthetic rates and slower leaf area expansion resulting from lower hydraulic conductivity as altered responses to water stress [80,73,81]. So plant species often exhibit substantial variation in rates of nutrient gain and resource use. Developing alternate crops and the utilization of bioenergy crops produced on American farms as a source of renewable fuels are concepts with great relevance to current ecological and economic issues at both national and global scales. Castor bean (*Ricinus communis* L.), as a fast growing C3 plant is now being investigated as a new source for biodiesel and as well as an industrial crop [27] because of its high oil yield and various potential uses. N requirements, in castor is relatively high compared to other oil seed crops such as soybean, an N-fixing leguminous plant and variable depending on the soil organic matter [26].

Critical leaf N levels, functional relationships between leaf N and growth and developmental processes and developing rapid and less expensive diagnostic tools for spatial and temporal estimation of plant N status for castor bean crop are needed for producers to manage this crop for optimum yields under varied growing conditions. Castor bean plant N uptake and nutrient flow [26,82,83] and N effects on leaf ontogeny and growth have received some attention now a days [67].

Nitrogen has been considered as the most critical and is a very essential component for plants growth as plants use nitrogen in the creation of specially proteins and nucleic acids, which are then used for the construction of leaves, other important structures and finally fruits to seeds. The nitrogen can then help the plant produce larger crops because of the increase in the nitrogen available for creating these structures. Urea fertilizer containing 46% of Nitrogen promotes healthy growth by providing essential nitrogen to plants and also allowed an increased N utilization as compared to other fertilizer sources as well as better than other reduced N sources and appeared to be the best source which gave the highest dry matter yield compare to other nitrogen sources are used [9,84].

The objectives of this present study were to investigate the effects of urea on a biodiesel plant growth, development and physiology, and to derive functional relationships between root, shoot and responses of N in controlled environment including characterize urea uptake and assimilation by castor seedlings by following N metabolites of seedlings with exposure to different levels of N (urea) fertilization regimes and to examine possible interactions between N and carbon transport to shoot and root at various growth stages.

2. MATERIALS AND METHODS

A pot experiment was conducted under controlled condition at the Growth Chamber, Department of Crop and Soil sciences, Washington State University during summer season in 2013 to evaluate the effect of different doses of inorganic nitrogen fertilizer in the form of urea on growth, nutrient uptake and root development of castor bean, Zanzabaries variety during the period of vegetative growth and the different parameters were measured weekly till the final harvest.

The experiment was arranged in a Randomized Complete Block Design (RCBD) with 4 treatments and 4 replications. The treatments imposed on the experiment were: Control (N₀), no nitrogen and others at the rate of 120 lb/acre (N₃), 90lb/acre (N₂) and 60lb/acre (N₁) by applying N fertilizer with 391 mg/pot (N₃), 293 mg/pot (N₂) and 195 mg/pot (N₁) respectively. The experiment was carried out by applying pure nitrogen from source of urea (46% N) as chemical fertilizer were maintained for 4 weeks

(28 days) in this condition to grow the plants. The soil used in the experiment was the following physical and chemical properties: Sand 46.08%, silt 46.09%, clay 7.83%, Bulk density 1.8, EC 0.39 mmos/cm, pH 8.0, organic matter 0.75%, Moisture 3.2%, CEC 10.3 meq/100 g soil and Total N 0.02%.

Seeds of castor Zanzibaries, were obtained from local seed mass of Oregon, USA. Homogenous seeds were soaked in wet paper towel for 24 hours before sowing and were sown in plastic pot (7inches x 6 inches) containing 6.6 lbs (3 kgs) of Silt loam soil at the growth chamber. The soil was collected from Prosser, Washington State and the collected soil was air dried and sieved to pass a 2 mm mesh screen.

One seed of castor was sown in each plastic pot and were germinated after 12 days of sowing in the growth room (approximately 14h light/10 h dark) providing photosynthetically active radiation of 80 $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ with day/night temperature of 75°F and 57% relative humidity. The plants were grown under this controlled growth chamber conditions during the vegetative growth for a period of approximately one month (28 days) or four weeks. The plant samples were collected every week to measure the morphological and physiological parameters during the growing period. Finally at the end of the experiment after treatment for 28 days (4 weeks) the castor bean plants were finally uprooted carefully, made free from soil particles and sampled for growth (plant height, leaf and petiole length, intermodal distance, root number), nutrient concentrations in root and shoot and plant biomass were measured. After harvesting the plants were first washed with tap water to remove the soil particles from the root and then with distilled water. Plant parts (roots and shoots) were separated and the fresh weight was determined for each plant. The plants were then dried for 48 hours at 47 degree Celsius to receive the dry wt of samples. The samples were than grind for chemical analysis and used for determination of nitrogen, carbon and crude protein content in plants.

Plant heights were measured as the distance between soil surface and the main-stem apex. Leaf and petiole length were estimated non-destructively by measuring the distance from the point of petiole attachment to the leaf tip of the center lobe on all main-stem leaves and intermodal space by measuring distance between internodes. For determination of root

number on pot grown roots, soil with intact plants and roots were taken up carefully from the soil depth with a knife. The soil cores were immersed in water overnight in darkness at 5°C. The roots were then removed carefully using a kitchen sieve.

Fresh and dry wt of plants were measured in every week and at the end of the experiment were made after harvesting the plants at 28 DAE. Plant height (cm/plant), shoot dry weight (g/plant), root dry weight (g/plant), and root no (for 3 weeks) were recorded. Total biomass (g/plant) and root: shoot ratio were derived from basic data. After harvesting the data were statistically analyzed using Duncan's multiple range technique (DMRT), to determine the significant differences of the results.

So, the aim of the present study was to investigate the effects of fertilizer on this oilseed bioenergy crop, castor bean seedlings at the 6 leaf stage, subjected for 28 days under controlled growth chamber condition, taking in consideration the morphological and physiological quality of the underground and aboveground parts of the plant under different doses of urea.

3. RESULTS AND DISCUSSION

The results were presented in response of different growth, biomass and nutrient concentration parameters to different levels of Nitrogen at vegetative growth for a period of 4 weeks, and their responses to N increasing from 195 to 391 mgs/pot and control. The improvement of each parameter due to increased and reduced N at different growth stages were measured over control level values, and the maximum responsive growth stage to different levels of N was identified.

During the vegetative period, the growth, and other parameters were measured weekly as well as plant components biomass from 1st to 4th week (final harvest at 28 DAE). Maximum growth and developmental rates were achieved in 4th week at N3 compare to other treatments and all growth rates declined and were more sensitive at controlled followed by increasing rate of fertilizer application (Figs. 1-2) like N1, N2 and N3 respectively. Although Plant dry weight and root/shoot ratio had the greatest increased under higher nitrogen and lowest under deficiency. Among the plant components plant height,

intermodal space addition, leaf and petiole length were higher at N3.

Nitrogen in the form of urea influenced the vegetative growth of the plants. The greatest plant height (17.1 cm), dry weight (1.0 g) of shoot, leaf length (6.2 cm), petiole length (12.1 cm), intermodal distance (2.5 cm), number of roots (22), and root dry wt (0.55gm) were obtained highest with the application of 391gm N/plant treatment. Nitrogen affected the growth, nutrient uptake and root development significantly influenced the vegetative growth of the plant showed that plant height significantly varied with treatments (Figs. 3-6). Maximum plant height was recorded at N3 followed by 16.3 cm at N2 and 16.0 cm at N1, whereas the lowest value 14.5 cm was recorded at control.

The results indicated that application of urea in a concentration of 120lbs/acre or 391mgs/pot showed the best effect on root development and other parameters. Functional relationships between leaf Maximum growth and developmental rates were also achieved at this treatment. Among the plant components, plant dry weight and root/shoot ratio had the greatest decrease under N deficiency. Significant difference was not observed among the treatments in any of the parameters like shoot nitrogen and carbon uptake and root Nitrogen uptake, except the root carbon percentage which was highest at N0 and lowest at N3 were measured. However, no significant difference was observed in root/shoot nitrogen ratio under the nitrogen fertilizer treatments and at control condition (Tables 1-2).



Fig. 1. Effect of different N doses on plant growth and development: L (N₀-N₃), R (N₃-N₀)

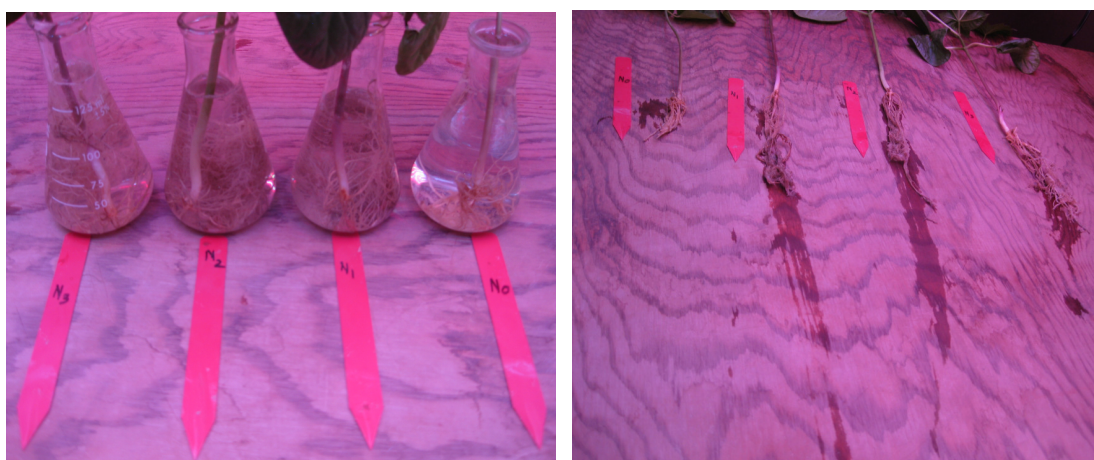


Fig. 2. Root development under different rates of nitrogen: L (N₃-N₀) and R (N₀-N₃)

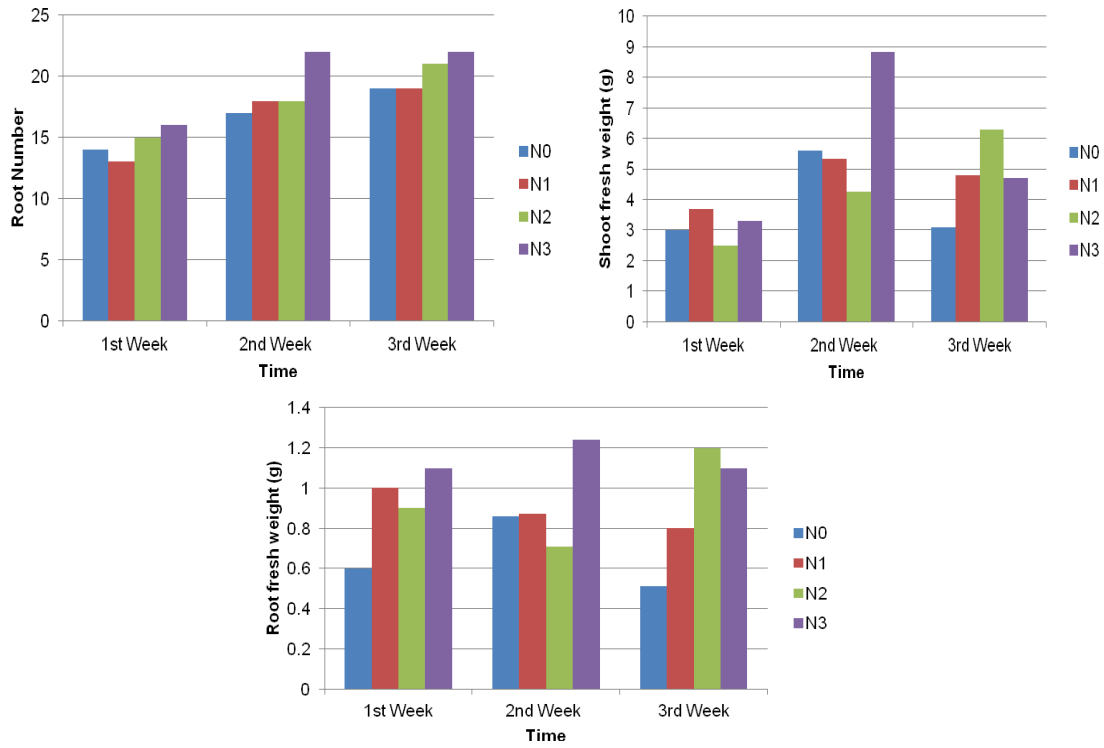


Fig. 3. Effect of urea on weekly development of root and shoot fresh wt and root numbers

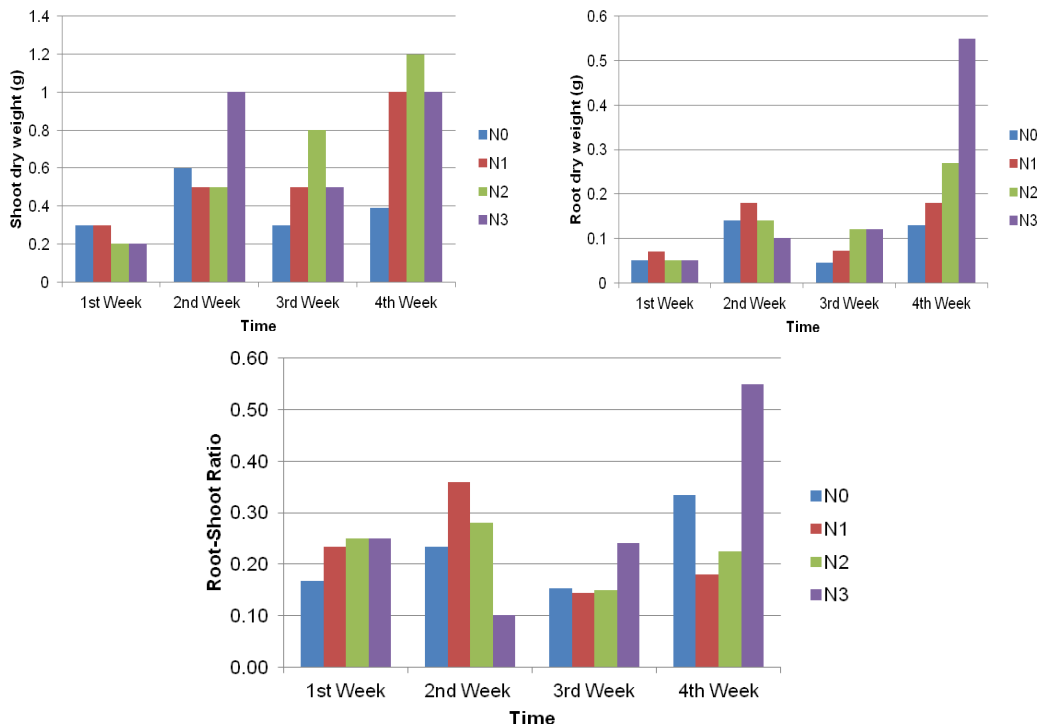


Fig. 4. Effects of different doses of nitrogen on dry wt and root shoot ratio

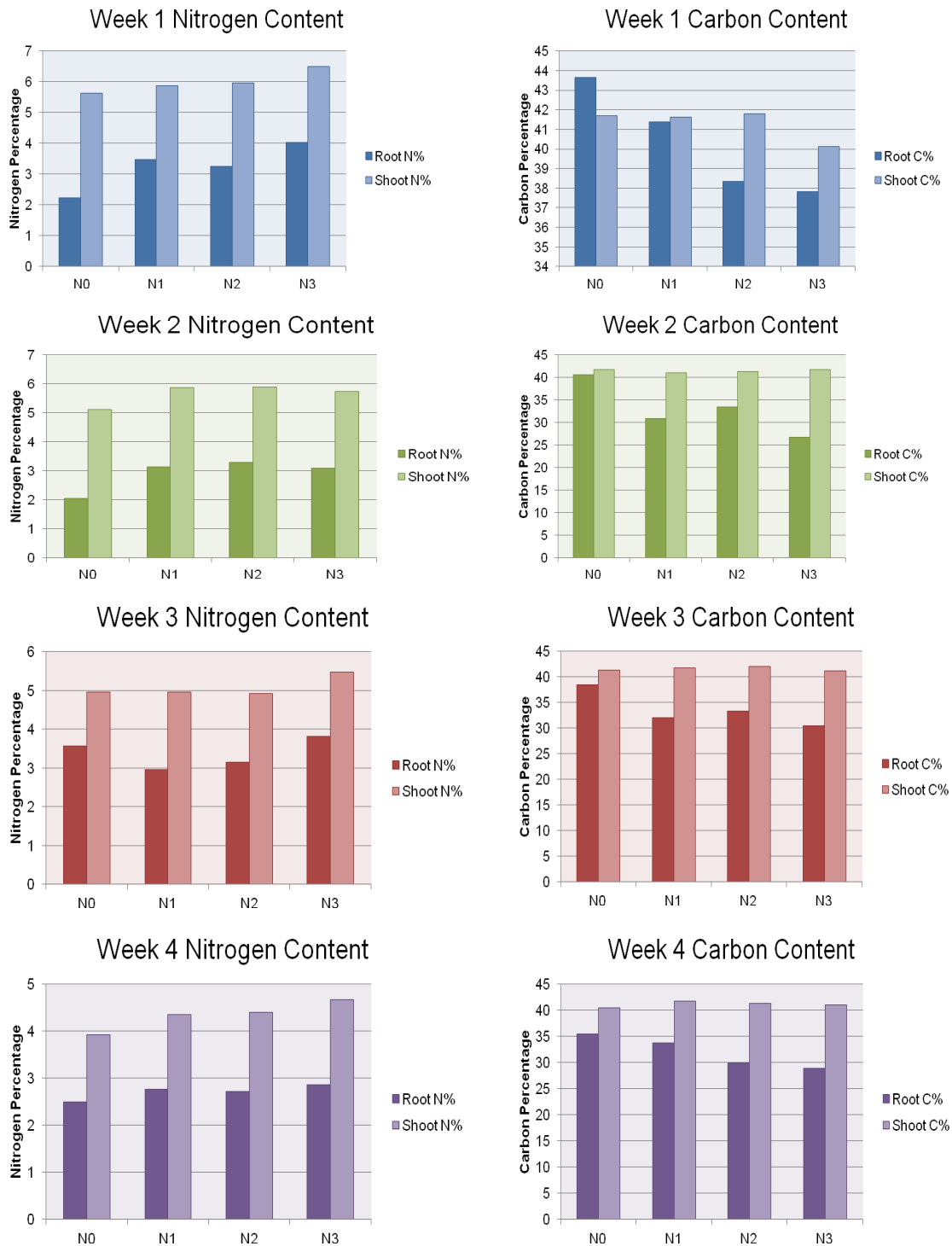


Fig. 5. Effect of different doses of urea on nitrogen and carbon uptake by plant at different time intervals

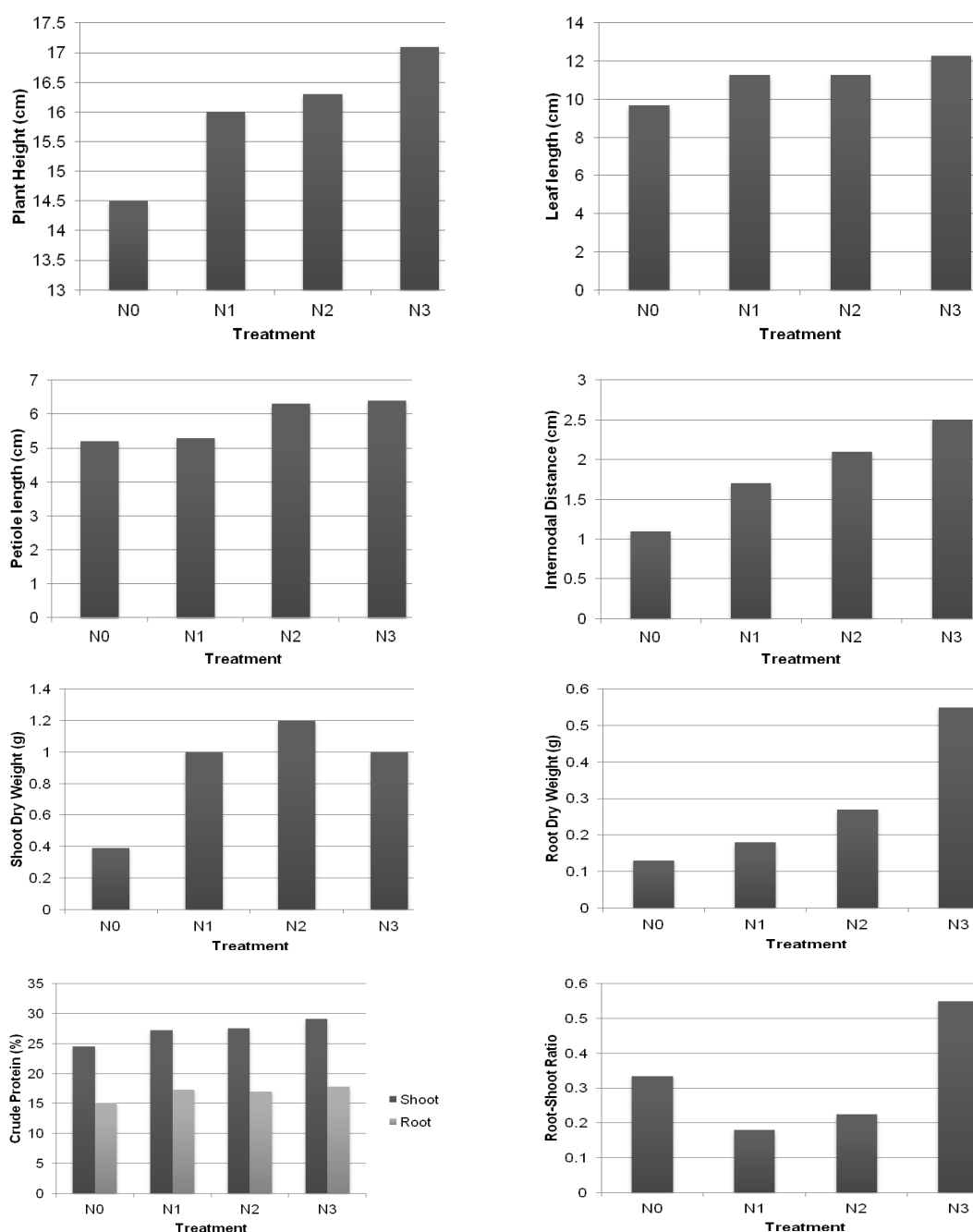


Fig. 6. Effect of different doses of urea on growth and other characteristics in plant (4th week)

The results indicated that the plant growth and other morphological parameters were improved by applying higher dose of N (Fig. 1). Also the root/shoot ratio, nutrient uptake, Crude protein concentration and residual N% in soil increased. The lowest root carbon uptake was observed in plants treated by higher urea. Application of higher dose of urea substantially improved the

plant height, leaf and petiole length, intermodal space addition, shoot and root dry weights, and number of roots. While the lowest root no was observed in plants treated by control (Fig. 3). This improved growth with higher nitrogen fertilizer in plant is mainly due to nutrient availability in the fertilizer at N3 and uptake by plants. Adding nitrogen to the soil provides a

surplus of food at the plant's disposal that allows it to support more biological processes at an accelerated rate. As a result, the nitrogen allows the plant to grow much faster than it might otherwise without the nitrogen introduced.

So, effect of higher concentration of urea indicated considerable increases in castor growth including vegetative growth and the whole plant fresh and dry weight (Figs. 3-6). At 28 DAE at harvesting, dry weights of plant components were measured and found shoot and root dry weight had the greatest increase under elevated nitrogen supply and had decrease under N-deficiency. The highest values of nutrient especially nitrogen and crude protein contents in plants were also obtained at N3. However, lower nitrogen applications had no significant effect on most of the investigated plant parameters, except for root/shoot C ratio and uptake of carbon by roots. Effects of different nitrogen doses on some plant physiological characteristics after harvesting are given in Table 2.

Root number increased throughout the vegetative growth period, i.e. from 7 to 21 days after emergence and the highest root no was recorded at 21 days and the number under elevated N was higher at all stages when compared with control (Fig. 3). Among the elevated N levels, at N3 showed a better response than N2. At different growth stages the increment in root number varied from 16 to 22 with N3 from 1st to 3rd week when compared with control and the maximum response was recorded at 3rd week for treatment N3. So,

during the vegetative growth the root no was highest at 21 days after emergence and at elevated nitrogen N3 treatment followed by N0 and N1, however the root and shoot fresh wt was found highest in 2nd week at the same treatment (Fig. 3).

In the last week (4th week) of vegetative growth, finally the plant height was measured and the highest height was observed at N3 followed by N2, N1 and lowest at N0. Leaf length was also found highest at N3, same at N2 and N1 and lowest at N0 (control). In case of petiole length, same trend was found, which was highest at N3 and N2, followed by same at N1 and control. Root and shoot fresh wt were higher at N3 and highest value were obtained in 2nd week compared to 1st and 3rd week. Intermodal distance was also found highest at N3 followed by N2, N1 and N0, which was lowest compare to other treatments. Shoot dry wt was found higher at N2 and followed by N3, N1 and lowest at N0, whereas Root dry weight was highest at N3 and followed by N2, N1 and N0. Crude protein concentration and root shoot ratio were also highest at N3 treatment compare to others and the Root shoot ratio was also found highest at N3 treatment followed by N0, N2 and N1 (Fig. 6).

It is also evident from the Fig. 1, that Root number increased with elevated nitrogen application and with time (weekly) and the highest root no was found in 3rd week followed by 2nd and lowest in 1st week. Although the no of root was found highest in 3rd week with elevated nitrogen level, however the number

Table 1. Nutrient uptake and their significant response to N levels (N1, N2, N3) over control treatment (N0)

Treatment	Shoot N%	Root N%	Shoot C%	Root C%
N0	4.411a	2.657a	40.70a	37.27a
N1	4.356a	2.775a	41.81a	33.75b
N2	4.406a	2.858a	41.37a	32.05b
N3	4.879a	2.978a	40.45a	28.32c

* Means within column followed by the same letter are not significantly different at 5% level by DMRT

Table 2. Effect of different doses of N on shoot & root N and C ratio and residual N% in soil

Treatment	Root and shoot ratio (N)	Root and shoot ratio (C)	Soil residual N%
N0	0.5727a	0.9157a	0.02310d
N1	0.6375a	0.8076b	0.02657c
N2	0.6717a	0.7750b	0.03465b
N3	0.5871a	0.7024b	0.06122a

* Means within column followed by the same letter are not significantly different at 5% level by DMRT

could not be determined in 4th week, due to the limited space for root development in the pot. Again shoot and root fresh wt were highest at N3 in second week, compared to 1st and 3rd week. However the shoot N% in first week was higher at N3 treatment and lowest at N0, and followed by the sequence (N3>N2>N1>N0). At N3 treatment in 4th week, the concentration of nitrogen in root and shoot were increased compared to N0 treatment but no statistical difference were observed among the treatments. No statistical differences in Carbon value was also observed in shoot among the treatments, so percentage of carbon is statistically same in all treatments, and no differences are observed from N0 to N3 treatments whereas in case of root carbon value, opposite picture was found and the highest value was obtained at N0 and lowest at N3, and followed a reverse sequence as N0>N1>N2>N3 (Table 1). The results in Table 1 also indicated that the uptake of nitrogen and carbon were higher at shoot than that of root in all the treatments imposed in the experiment.

Also in 3rd week, the shoot N% was highest at N3 and followed by same at other treatments N2, N1 and N0. Although the root N% was also highest in 3rd week at same treatment, it followed the sequence N0, N2 and N1. Also in final or 4th week shoot N content followed the same sequence as the 3rd week and was found higher at N3 followed by N2 and N1 as same and lowest at N0. The same trend was found at final stage in root nitrogen conc. as highest value was obtained at N3, followed the same as shoot Nitrogen uptake. Finally it is observed that at different growth stages of castor in growth chamber from 1st to 4th week (final week) in all treatments showed lower carbon and nitrogen concentration in root compared to shoot N and C concentration. Compare to N% and C%, shoot containing more Nitrogen and Carbon than root in all treatments and every week till the final harvest.

So in the comparison of different N doses, tests showed that higher level of N application (391mg urea/pot) to castor bean plants had more of an effect on the dry weight and other morphological and physiological parameters of the plant as well as soil residual N effect. Although high level of N application do not show any positive effect on root carbon uptake by plants. The nitrogen contents in roots of plant grown in soil containing N3 was generally lower than the nitrogen contents of stem. However, nitrogen applications had no significant affect on most of the

investigated physiological plant parameters, like, root and shoot nitrogen and shoot carbon uptake and root/shoot nitrogen ratio. Effects of different nitrogen doses on some physiological characteristics of castor bean are shown in Tables 1 and 2.

In this experiment nutrient uptake and growth of aboveground and belowground parts of castor were ascertained in seedlings at various vegetative growth stages until the end of the experiment. Our results indicated that application of urea at the rate of 120lb/acre or 391 mg/pot showed the best effect on castor plant development. Increased shoot and root growth was only at the highest N level applied. No statistical difference was observed between shoot and root N, root carbon uptake and root/shoot N ratio whereas there were statistical differences in root carbon and root/shoot carbon ratio which was highest at control, although the residual N concentration in soil was highest at application of elevated nitrogen levels compared with other treatments and control (N0).

It is therefore evident from the results, that the Castor bean plant showed significant response under elevated Nitrogen levels in terms of growth, biomass and uptake of nutrients when compared with control. Castor responds very well to N nutrition due to its indeterminate growth habit and reduced N resulted in reduced growth and nutrient uptake and root development. In addition, N deficiency decreased plants growth leading to lower biomass accumulation in plants. Partitioning of biomass to roots decreased more than the other plant components especially shoots under N deficiency.

Root dry weight followed the same trend as root number and it increased from 7 to 28 DAE. Initially, root dry weight increased slowly in the 1st week but from 21 to 28 DAE it showed the maximum increase (Fig. 4). The highest root dry weight was recorded at 28 DAE in N3 compare to all the treatments.

Effect of nitrogen fertilizer on root and shoot, nitrogen and carbon uptake and root/shoot nitrogen carbon ratio per plant in 5 % probability level was significant. On the other hand, effect of nitrogen fertilizer treatments on root and shoot nitrogen, root carbon uptake and root /shoot nitrogen ratio was non-significant. Comparison between nitrogen levels show that the highest plant height, root no, leaf length, root dry weight and root shoot ratio were obtained by application

of higher dose of pure nitrogen in the form of Urea (Figs. 3 and 6). Similar results were also obtained by [84] who also reported increased growth and development of plants by application of urea.

The interaction effect of nitrogen treatments was highly significant in root carbon absorption. Lower Nitrogen application provides higher carbon uptake in roots. This result also confirms the findings of [85] who postulated the carbon content in roots increases upon nitrogen deprivation means higher carbon uptake by reduced nitrogen application. The nutrient uptake parameters are presented in Table 1 and the data show that no significant difference was observed in nitrogen uptake in shoot, root and also carbon uptake in shoot among the treatments, except the root carbon absorption difference among the treatments which was highest in control. Also N0 contributed better root shoot carbon ratio compared to other 3 treatments (Table 2). These three treatments (N1-N3) did not show significant differences among themselves. The results in Table 2 also clearly demonstrate that there was no significant difference in root shoot nitrogen ratio in all the treatments (N0-N3). However the results after harvesting the plants revealed the lowest residual Nitrogen in soil in the control treatment compared to highest nitrogen dose at N3 treatment in pots.

The different physiological and morphological parameters were affected by N0 (control) compared to N3, probably restricts metabolism activities affecting castor plant Growth. In contrast, at N3 crude protein concentration was also higher in the plant due to elevated nitrogen levels (Fig. 6) and provides the optimum growth of the plant. The result of the current experiment also revealed that the concentration of crude protein in castor plant increased with increasing doses of urea. This result also agrees with the findings of [86,65], who reported that higher nitrogen application increased the plant crude protein content in plants.

The growth of aboveground and belowground parts of castor seedlings at different growth stages until the end of the experiment indicated that application of urea at a higher dose showed the best effect on plant development. This result also confirming the findings of [87], who reported increase in castor plant yield due to elevated level of nitrogen. The residual N effect in soil was also highest with elevated nitrogen level (N3)

followed by medium, lowest (N2, N1) and control (Table 2).

Maximum growth and developmental rates were achieved at N3. Even though all growth and developmental rates declined with lower N level, leaf length expansion rate was more sensitive to plant N followed by rates of petiole elongation and intermodal space addition. Among the plant components, shoot and root dry weight had the greatest decrease under N deficiency while root/shoot carbon ratio increased under N deficiency These results also confirm the findings of [9,87,84] who reported increase in growth, development, yield and nutrient uptake in castor and other bean with increasing age and application of elevated level of nitrogen.

4. CONCLUSION

On the average, evaluation of castor bean show that most of the investigated plant parameters were significantly affected by different N application and were more sensitive to N Deprivation. The plant was more suitable for cultivation with higher followed by moderate nitrogen application. Moreover, 120 lb/acre application was found the most suitable N application that can be suggested for castor bean in Washington State.

From the preceding discussion, it is concluded that the application of higher nitrogen per acre per year in the form of urea should be applied to castor plant for optimal growth, plant biomass and nutrient uptake in plants. Nitrogen requirement identified in this study could be used to estimate castor growth and development thus might be useful in managing crop N during the growing season. Nitrogen affects morphological and physiological processes in plants and accurate detection of plant N requirement can help farm managers to make appropriate N management decisions. In conclusion, the potential use of castor bean seeds for biodiesel production might be preceded by a much detailed work to be conducted until the stage of seed production.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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