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Studies of changes in chlorophyll content in *Artemisia annua L.* under the effects of biological and chemical fertilizers

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ABSTRACT

In order to consider impact of biological and chemical fertilizers (N, P) on chlorophyll contentnt which exist in Artemisia annua L. an experiment was carried out in factorial design in completely randomized design with 4 replications in Zabol University in 2011. Treatments included chemical fertilizers (N, P) in 4 levels (N0P0, N40P40, N80P40, N80P80) and biological fertilizers in 4 levels (control, Nitroxin [include bacteria which stimulus growth (Azotobacter and Azospirillum)], Bio-phosphorus [(include bacteria which stimulus growth (Bacillus and Pseudomonas)] and Vemricompost fertilizer. About chlorophyll content, applying biological fertilizers specially Vermicompost has better impact that chemical fertilizers and Vermicompost + N80P80 had better impact on chlorophyll content in comparison with other experimental treatments.

Key words: *Artemisia annua L.*, Nitroxin, Biophosphorus, Chlorophyll, Vermicompost.

INTRODUCTION

Artemisia annua (Asteraceae) is native to China, where it is known as qinghao (green herb) and has been used for over 2,000 years to treat symptoms associated with fever and malaria. It is known in the United States as sweet Annie, annual or sweet wormwood [1].

Malaria is a major health problem in many developing countries, mostly in Africa and Southeast Asia [2]. According to WHO report on malaria (2007), 40% world's population is living with risk of malaria, over 1.5 million death occur per year and the cost of malaria treatment is $1800 million US dollar. The first effective ant malarial drug was quinine, which was isolated from the bark of cinchona. Since then malaria has been treated with quinoline based drugs. However, Plasmodium falciparum developed resistant globally against two of the most common ant malarial drugs: chloroquine and the combination sulphadoxine/pyrimethamine [3].

One of the important elements for growth of plant is Nitrogen (N). This plant needs N in large amount which is a basic material for protein and nucleic acid. According to this fact that N participle in chlorophyll structure directly, it could be expected that there exist a positive and significant relation between leave's N and chlorophyll content [4].

Phosphorus (P) influence on cells structure and most of vital activities such as storage and transfer chemical energy as well. Need of P for favor growth is from 0.3 to 0.5% of dry weight is within growth and development stages [5]. Because N and P has been produced and used in chemical fertilizer form, its supply through using large content of chemical fertilizers in one of the water pollution in nature cycle and its production is expensive also, alternating this with organic fertilizers plays an important role [6]. So that, avoid of negative pressure to environment, it is needed to improve developmental programs which supply plant fertilizers requirements.

Improving soil quality could assesst according to quality and quantity index of biological society. As a result, using biological fertilizers is one of the effective managerial methods to keep soil quality in favorable level [7].

Using useful micro organism in agriculture had been begun since 60 years ago. Increasing this useful population can increase plant resistant against different environmental stresses such as lack of water, nutrition and heavy material toxicity [8].

Biological fertilizers are materials which include different micro creatures which have the ability to convert main nutrition elements from unavailable form to available form during biological processes [9] lead to develop better seeds' germination and root system [10].

In last decade biological fertilizers is applying as economically compatible compactly which lead reduction in using chemical fertilizers, improving soil fertility status to enhance plant production which is along with its biological activity in rhizosphere.

A group of bacteria which can be along with plant belong to Azospirillum, Azotobacter, Pseudomonas, Bacillus species [11]. Bacteria from Azotobacter and Azospirillum groups have the ability to make and leak some active and biological material such as vitamin B, Nicotinic acid, pentoterik acid, biotin, oxins, gebrelins etc in plant's root environment which have an effective and useful role in enhancement of root's absorbance [12]. Bacteria which work as solver of phosphate include a group of micro creatures most important species among this family is Pseudomonas and Bacillus [13]. Different species of Pseudomonas may cause to stimulate plant growth via different mechanisms such as antibiotics synthesis, plant hormone production, increasing P absorbance by plant, N stabling [14].

Vermicompost is an organic biological fertilizer and consists of biological mixture of very active bacteria, enzymes, plant rests, animal fertilizer and soil worm capsule which cause continuation of soil organic material analysis and development of microbial activity in plant cultivation bed [15]. We try to consider the effect of biological, chemical and their mixtures fertilizers on chlorophyll content in *Artemisia annua L.*

MATERIALS AND METHODS

In order to consider biological fertilizers (Nitroxin, Bio-phosphorus and Vermicompost) and chemical fertilizers (N, P) on chlorophyll content *Artemisia annua L.*, we had done an experiment in Zabol University green house in 2011. The plan of this experiment was factorial design in completely randomized design with 4 replications. Studding and considering chlorophyll content had been done in laboratory of biotechnology faculty of Jamia Hamdard University in India.

Experimented Factors:
A. Biological fertilizers in 4 levels: A_1: controls (without using fertilizer), A_2: Nitroxin (include *Azotobacter* and *Azospirillum*), A_3: Biophosphorus (include *Bacillus* and *Pseudomonas*) and A_4: Vermicompost (10 t/ha). There existed 10^8 live cell in each gr of Nitroxin liquid and 10^7 cells in each gr of Bio-phosphorus liquid.

To mix and Insemination the seeds, firstly we extend clean plastic under seeds and then sprayed the liquid fertilizers on them. Then we put Inoculated seeds in shadow for 1 hour, after drying they are ready for cultivation, 10 tons Vermicompost also had been used.
B. Chemical fertilizer of N and P in 4 levels: B_1: Control (without fertilizer), B_2: N40+P40, B_3: N80+P40 and B_4: N80+P80 (Kg/ha).

Before cultivation, all the P fertilizers and N fertilizers in 3 parts added to pots according to soil test. Harvesting, samples did in April 2011 when 90% of plant have flowers.

The content of chlorophyll present in the samples tested
Weight 100 mg of leaf tissue in fractions into vial containing 7ml Dimethyl sulphoxide (DMSO). Chlorophyll will extract into the fluid without grinding at 65 C by incubating for various times, depending on the degree of customization and thickness of the leaf. Transfer the extract liquid to a graduated tube and made up to a total volume of 10 ml with Dimethyl sulphoxide (DMSO), assay immediately or transferred to vials and stored between 0-4 ^{o}C until required for analysis. Take 3 ml of chlorophyll extract and transfer to curette.

Measure the optical density (OD) of the extract at the following wavelengths 645 and 663 nm using Dimethyl sulphoxide (DMSO) as a blank after 30 min, 1 hr, and incubation.

Calculate total chlorophyll as mg/g of tissue, using the following equations: [16]

$$\text{Chlorophyll A (mg/g)} = 12.7\,(OD663) - 2.69(OD645) \times (V/\,(1000 \times wt))$$

$$\text{Chlorophyll B (mg/g)} = 22.9\,(OD645) - 4.68(OD663) \times (V/\,(1000 \times wt))$$

$$\text{Total Chlorophyll (mg/g)} = 20.2\,(OD645) + 8.02(OD663) \times (V/\,(1000 \times wt))$$

Where;
OD: optical density at certain wave length (645 or 663 nm)
V: final volume (10 ml)
Wt: weight of sample (100 mg)

Statistical Analysis
Statistical plan considered as factorial in completely accidental plot with 4 repetitions. Data analysis did by MSTAT-C and SAS software and graphs drew by excel software. In addition means compared in Duncan test and 0.05% probable level.

RESULTS AND DISCUSSION

Results of experimental data's statistical analysis are in table 1 and results of comparing considered characteristics means are in table 2.

Table 1: result of variance analysis of chlorophyll a, b and total in *Artemisia annua L.*

(S.O.V.)	df	(Mean Square) Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
Bio-fertilizer (A)	3	0.478^{**}	0.388^{**}	1.664^{**}
Chemical fertilizer (B)	3	0.727^{**}	0.364^{**}	2.111^{**}
Bio-fertilizer × chemical fertilizer (A × B)	9	0.03^{*}	0.044^{*}	0.084^{**}
Error	48	0.005	0.006	0.012
C.V. %		3.52	8.29	3.65

Note: *and ** indicate significant difference at 5% and 1% probability level, respectively.

Table 2. Comparison of experimental treatments' simple effects and interaction means on measured characteristics

Treatment	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
Bio-fertilizer (A)			
Control (A_1)	1.89d	0.749d	2.639d
Nitroxin (A_2)	2.21b	0.921b	3.13b
Bio-phosphorus (A_3)	2.04c	0.826c	2.866c
Vermicompost (A_4)	2.273a	1.11a	3.384a
Chemical fertilizer (B)			
Control (N0P0) (B_1)	1.87d	0.718d	2.585d
N40P40 (B_2)	1.993c	0.851c	2.844c
N80P40 (B_3)	2.203b	0.973b	3.177b
N80P80 (B_4)	2.347a	1.064a	3.411a
Bio-fertilizer × Chemical fertilizer (A ×B)			
Control (A_1B_1)	1.749m	0.663m	2.407l
N40P40 (A_1B_2)	1.848k	0.713l	2.561j
N80P40 (A_1B_3)	1.946h	0.787jk	2.733h
N80P80 (A_1B_4)	2.02g	0.835hi	2.855g
Nitroxin × N0+P0 (A_2B_1)	1.90i	0.729l	2.636i
Nitroxin × N40+P40 (A_2B_2)	2.154e	0.812ij	2.966f
Nitroxin × N80+P40 (A_2B_3)	2.316d	0.937e	2.253d
Nitroxin × N80P80 (A_2B_4)	2.458b	1.21c	3.663c
Bio-phosphorus × N0+P0 (A_3B_1)	1.783l	0.701l	2.484k
Bio-phosphorus × N40+P40 (A_3B_2)	1.876j	0.85gh	2.726h
Bio-phosphorus × N80+P40 (A_3B_3)	2.093f	0.873fg	2.966f
Bio-phosphorus × N80+P80 (A_3B_4)	2.404c	0.882f	3.285d
Vermicompost × N0+P0 (A_4B_1)	2.04g	0.779k	2.819g
Vermicompost × N40+P40 (A_4B_2)	2.092f	1.025d	3.118e
Vermicompost × N80+P40 (A_4B_3)	2.456b	1.299b	3.755b
Vermicompost × N80+P80 (A_4B_4)	2.505a	1.338a	3.843a

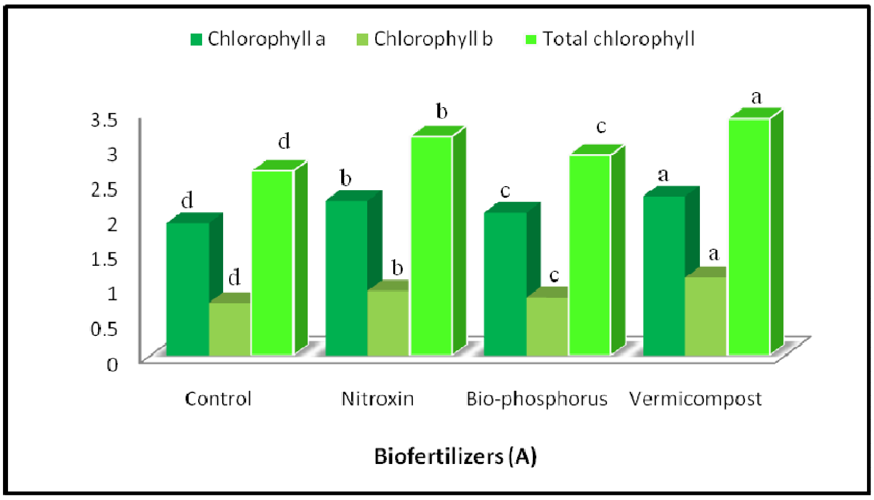
Note: Similar letters in each column hadn't any significant statistical difference.

The effect of chemical, biological fertilizers and interactions on the Chlorophyll content (a, b and total) content

Results of variance analysis table indicate that using biological fertilizers has significant impact on chlorophyll in 1% statistic level (table 1). Results of means comparison in Duncan way showed that (table 2) among different level of biological fertilizers, the most chlorophyll content (a, b and total) relates to Vermicompost (A_4) that are (2.273, 1.11 and 3.384 mg/g) and the less chlorophyll contents relates to control (A_1) that are (1.89, 0.749 and 2.639 mg/g) (Figure 1).

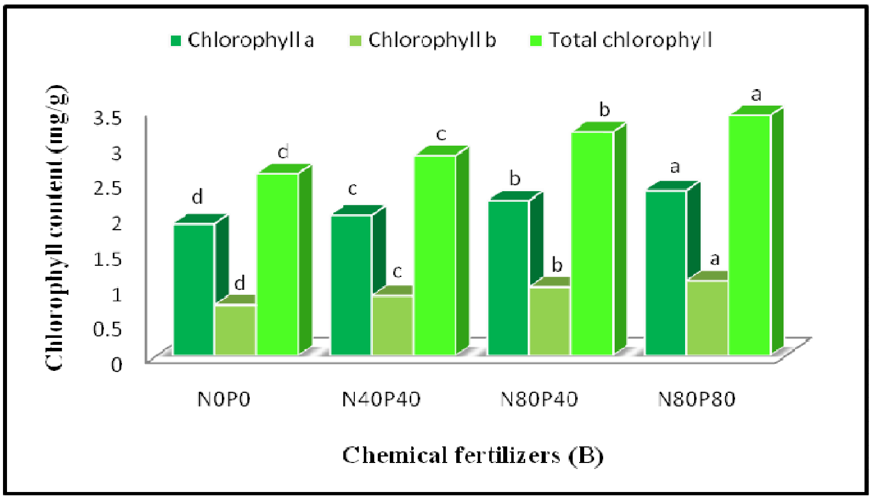
Results show that the ability of biological fertilizers and using bacteria which stimulus growth are cause of enhancement chlorophyll in this treatment by fertility and by using bio-fertilizers we can increase chlorophyll content (a, b and total) and decrease chemical fertilizers usage significantly as well.

Figure 1: Effect of different content of Bio-fertilizer on chlorophyll content



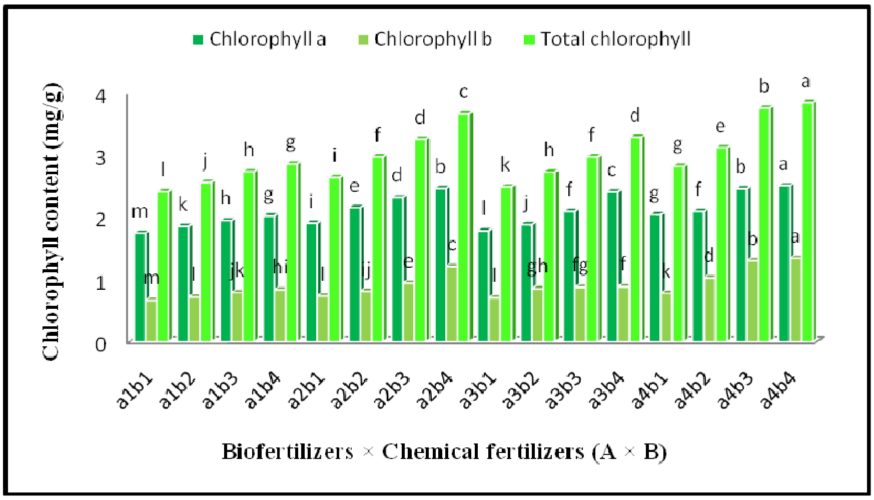
As you see in table 1, effect of chemical fertilizers on chlorophyll content was significant in 1% statistical level. Among different level of chemical fertilizer (table 2) the most contents of chlorophyll a, b and total were 2.347, 1.064 and 3.411 mg/g orderly. (Figure 2).

Figure 2: Effect of different contents of chemical fertilizers (N, P) on chlorophyll content



In addition, interactions of biological and chemical fertilizers on chlorophyll content were significant in 5% statistical level (table 1). By comparison means comparison table (table 2) we observe that among treatments, the most chlorophyll content (a, b and total) relate to apply Vermicompost + N80+P80 (A_4B_4) which were (2.505, 1.338 and 3.843 mg/g). After that Vermicompost + N80+P40 (A_4B_3) with the chlorophyll content of (2.456, 1.299 and 3.755 mg/g) and Nitroxin + N80+P80 (A_2B_4) with (2.458, 1.21 and 3.663 mg/g) leaded to the most chlorophyll content (Figure 3).

Figure 3: Effect of biological and chemical fertilizers on chlorophyll content



Nitrogen is closely related to the synthesis of chlorophyll [17]. Rubisco enzyme acts as a catalyst in CO3 fixation that plants need for photosynthesis [17, 18]. Therefore, total nitrogen content in plants can influence the outcome of photosynthesis via the photosynthetic enzymes and chlorophyll formation. In plants, nitrogen initially is in the form of ammonia and subsequent ammonia has been changed into glutamic acid, catalyzed by the enzyme glutamine synthetase [19]. Glutamic acid serves as the base material in the biosynthesis of amino acids and nucleic acids [20]. Robinson [21] called glutamic acid as a precursor for the porphyrin ring in formation of chlorophyll.

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REFERENCES

[1] JFS. Ferreira, J. Janick. *International Journal of Plant Science*., **1995**, 156, 807-815.
[2] RW. Snow, CA. Guerra, AM. Noor, HY. Myint, SI. Hag. *Nature*., **2005**, 434, 214-217.
[3] RG. Ridley. *Nature*., **2002**, 415, 686-693.
[4] KG. Cassman, MJ. Kropff, ZD. Yan. *IRRI. Los Banos, Philippines*., **1996**, Pp: 81-96.
[5] H. Ebrahim zadeh. Plant physiology (topic of nutrition and absorption)., Tehran University Press, Iran, **1994**; 689 pages.
[6] BR. Chandrasekar, D. Ambrose, N. Jayabalan. *Journal of Agricultural Technology*., **2005**, 2, 223-234.
[7] N. Kokalis–Burelle, JW. Kloepper, MS. Reddy. *Applied Soil Ecology*., **2006**, 31, 91-100.
[8] SC. Wu, ZH. Cao, ZG. Li, KC. Cheung. *Geoderma*., **2005**, 125, 155-166.

[9] K. Rajendran, P. Devaraj. *Biomass and bioenergy*., **2004**, 26, 235-249.
[10] YI. Bi, XL. YI, P. Christie. *Chemosphere*. **2003**, 50, 831-837.
[11] MA. Selosse, E. Baudoin, P. Vandenkoornhyse. *Competes Rends Biologist*., **2004**, 327, 639-648.
[12] MA. Kader. *Journal of Biological Sciences*., **2002**, 2, 259-261.
[13] KVBR. Tilak, N. Ranganayaki, KK. Pal, R. De, AK. Saxena, C. Shekhar Nautiyal, Shilipi Mittal, AK. Tripathi, BN. Johri. *Current Science*., **2005**, 89, 136-150.
[14] C. Abdul-Jaleel, P. Manivannan, B. Sankar, A. Kishorekumar, R. Gopi, R. Panneerselvam. B: Biointerfaces., **2007**, 60, 7-11.
[15] Y. Bashan, G. Holguin. Can. J. Microbial., **1997**, 43, 103-121.
[16] HK. Litehtenhaler, AR. Wellburn. Biochem. Soc. Trans. 603rd Meeting Liverpool., **1983**, 591-592.
[17] FB. Salisbury, CW. Ross. Plant physiology, 4th ed., Wadsworth Publ. Co, Diah R. Lukman dan Sumaryono (translate), ITB Press, Bandung, Indonesia, **1995**; pp. 241.
[18] E. Zamski, AA. Schaffer. Photoassimilate Distribution in Plants and Crops: Source-Sink Relationships., Marcel Dekker Inc. **1996**; pp. 928.
[19] JB. Harborne. Phytochemical methods, guiding the modern way to analyze plant., Penerbit ITB, Bandung, **1987**.
[20] MY. Nyakpa, AM. Lubis, MA. Pulung, AG. Amrah, A. Munawar, BH. Go. Kesuburan Tanah, Universitas Lampung, **1988**; 258 hal.
[21] T. Robinson. The organic constituents of higher plants, 4th ed., Cordus Press. North Amherst, **1980**