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The effect of biological and chemical fertilizers on protein content in *Artemisia annua L.* leaves

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ABSTRACT

Artemisia annua L is an aromatic annual herb endemic to the northern parts of Chahar and Suiyuan provinces in China. In order to consider impact of biological and chemical fertilizers (N, P) on protein content that 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 protein content, the result showed that applying biological fertilizers specially Vermicompost has better effect than chemical fertilizers, also Vermicompost + N80P80 had better impact on protein content in comparison with other treatments.

Key words: *Artemisia annua L.*, Biophosphorus, Nitroxin, Protein, 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 nutrition for growth of this plant is nitrogen (N). This plant needs N in large content that is a basic material for protein and nucleic acid.

Phosphorus (P) interferes with cells structure and most of vital activities such as storage and transfer chemical energy as well. Need for P in favor growth from 0.3 to 0.5% of dry weight is within growth and development stages [4]. 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 [5]. So that, avoid of negative pressure to environment, it is needed to improve developmental programs which supply plant fertilizers requirements'.

Improving soil quality could assess 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 [6].

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 [7].

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 [8] lead to develop better seeds' germination and root system [9].

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 [10]. 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 [11]. Bacteria which work as solver of phosphate include a group of micro creatures most important species among this family is Pseudomonas and Bacillus [12]. 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 [13].

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 [14]. We try to consider the effect of biological, chemical and their mixtures fertilizers on protein 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 protein 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 protein 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 2 parts added to pots according to soil test.

The content of protein present in the samples tested

Concentration of protein was determined by using bovine serum albumin as standard [15]. Fresh leaves (0.5 g) were ground with 1 cm^3 phosphate buffer (0.1 M, pH 7.0) in mortar and pestle and kept in ice. The material was centrifuged at 5 000 *g* for 10 min. Supernatant (0.5 cm^3) was mixed with 0.5 cm^3 TCA and again centrifuged at 3 300 *g* for 30 min. The supernatant was discarded and the pellet left was washed twice by double distilled water (DDW) and dissolved in 0.1 M NaOH and mixed with 0.5 cm^3 Bradford reagent and kept for 1/2 h. Absorbance was measured at 595 nm on spectrophotometer.

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 and 3.

Table 1: result of variance analysis of protein content in *Artemisia annua L. (mg/g)*

(S.O.V.)	df	Anova SS	Mean Square	F Value
Bio-fertilizer (A)	3	66.92	22.308**	55.2
Chemical fertilizer (B)	3	57.88	19.29**	47.74
Bio-fertilizer × chemical fertilizer (A × B)	9	9.19	1.022*	2.53
Error	48	25.23	0.52	
C.V. %			10.53	
*Note: *and ** indicate significant difference at 5% and 1% probability level, respectively ns is not significant*				

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

Treatment	Mean	Std-Dev
Bio-fertilizer (A)		
Control (A_1)	4.718	D
Nitroxin (A_2)	6.498	B
Bio-phosphorus (A_3)	5.501	C
Vermicompost (A_4)	7.431	A
Chemical fertilizer (B)		
Control (N0P0) (B_1)	4.809	D
N40P40 (B_2)	5.522	C
N80P40 (B_3)	6.504	B
N80P80 (B_4)	7.312	A
***Note**: Similar letters in each column hadn't any significant statistical difference.*		

Table 3. Comparison of experimental treatments' interaction effects means on measured characteristics

Treatment	Mean	Std-Dev
Bio-fertilizer × Chemical fertilizer (A ×B)		
Control (A_1B_1)	4.086i	1.01
Without bio-fertilizers × N40P40 (A_1B_2)	4.423h	1.08
Without bio-fertilizers × N80P40 (A_1B_3)	4.932g	0.98
Without bio-fertilizers × N80P80 (A_1B_4)	5.43f	1.22
Nitroxin × N0+P0 (A_2B_1)	5.027g	0.72
Nitroxin × N40+P40 (A_2B_2)	5.99le	0.56
Nitroxin × N80+P40 (A_2B_3)	7.049c	0.39
Nitroxin × N80P80 (A_2B_4)	7.925b	0.36
Bio-phosphorus × N0+P0 (A_3B_1)	4.561ih	0.48
Bio-phosphorus × N40+P40 (A_3B_2)	5.14g	0.23
Bio-phosphorus × N80+P40 (A_3B_3)	5.869e	0.81
Bio-phosphorus × N80+P80 (A_3B_4)	6.437d	0.36
Vermicompost × N0+P0 (A_4B_1)	5.565f	0.48
Vermicompost × N40+P40 (A_4B_2)	6.533d	0.61
Vermicompost × N80+P40 (A_4B_3)	8.167b	0.78
Vermicompost × N80+P80 (A_4B_4)	9.46a	0.54
Note: Similar letters in each column hadn't any significant statistical difference.		

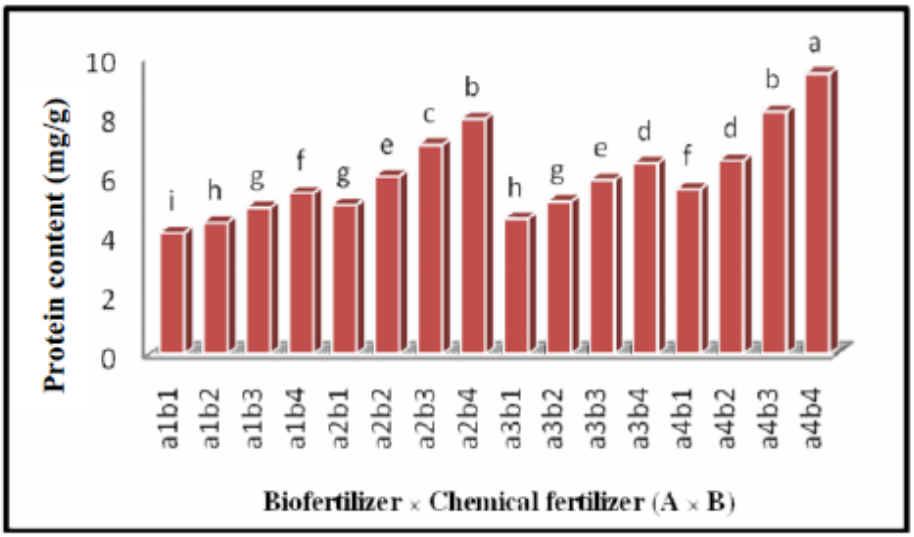
The effect of chemical, biological fertilizers and interactions on artemisinin content

Results showed that chemical and biological fertilizers impact in 1% level and their interaction in 5% level on protein which exists in *Artemisia annua L.* was significant (table 1).

According to means comparison (table 3) which shows biological and chemical fertilizers interaction on protein content, we can observe that by increasing N and P fertilizer along with biological fertilizers especially Vermicompost, a significant ascending on this material appears (Figure 1).

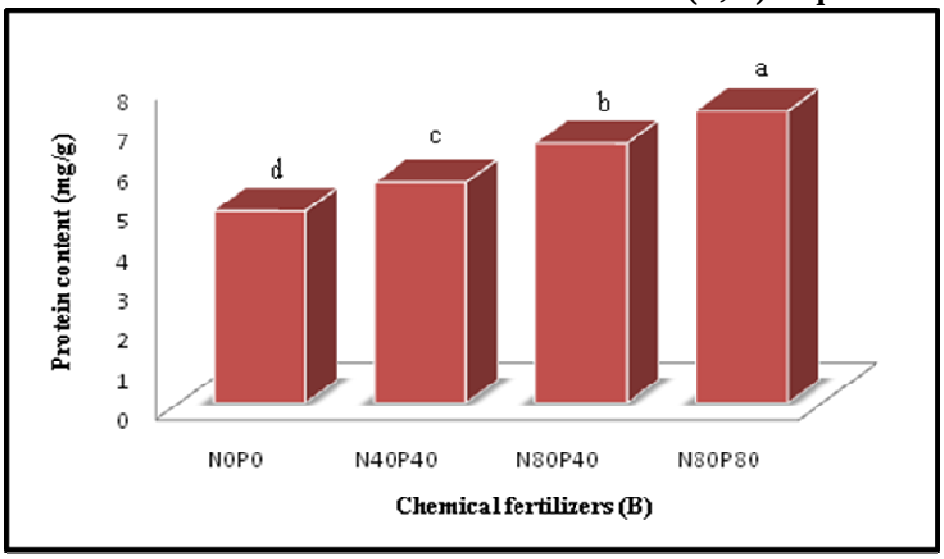
The most enhancement of protein content (9.46 mg/g) is for applying chemical fertilizers (B_4) plus 10 tons Vermicompost which in comparison with control treatment (without biological and chemical fertilizers) has 5.38 mg/g enhancement.

Figure 1: Effect of biological and chemical fertilizers on protein content



Comparison means of chemical fertilizers impact on protein content shows that by increasing N and P, protein content will increase also (Figure 2). According to this result the most protein content relates to (B_4) which are about (7.312 mg/g) and have 52.04% enhancement.

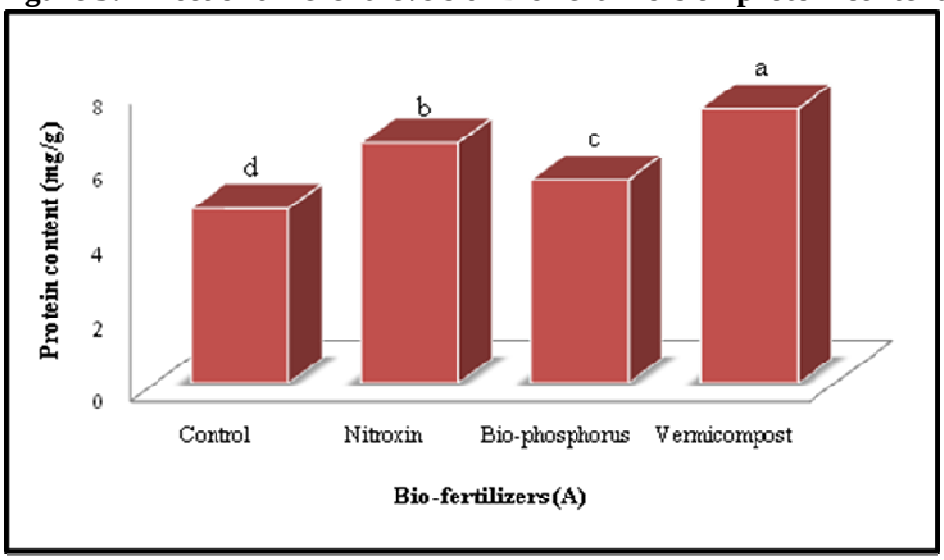
Figure 2: Effect of different levels of chemical fertilizers (N, P) on protein content



About applying biological fertilizers, by considering means comparison (table 2) web find that, using bio-fertilizers leads to protein content enhancement which among them, Vermincompost (A_4) have better function cause, in increase protein content to (7.431 mg/g) (Figure 3).

Studies about using N and P fertilizers in crops show that crop index; protein concentration, enhancement of N and Nitrate concentration related to dependent fertilizers [16, 17]. According to this fact that N is the base of Nocloeick acid and protein buildings, and as the Vermicompost fertilizer has all types the nutrition materials for crops included N, this fertilizer application in present article shows significant increasing on protein solvent amount in *Artemisia annua L.* leaves.

Figure 3: Effect of different levels of Bio-fertilizers on protein content



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