Sys Rev Pharm 2020; 11(4): 476 – 481
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Efficiency of Double Vaccine by *Azotobacter chroococcum & Pseudomonas chlororaphus* on Some Biological characteristics of *Hordeum vulgare*

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Article History: *Submitted: 11.01.2020* *Revised: 10.03.2020* *Accepted: 25.04.2020*

ABSTRACT
This experiment was carried out to investigate the effect of the double agent of *Azotobacter chroococcum* and *Pseudomonas chlororaphus*. Pots were used in the experiment by following the RCBD design with five replicates in green clay mixed soil on the barley plant. The following parameters were studied: germination ratio, root length and leaf area, total plant weight, total root weight, and nitrogen and phosphorus concentration. After 45 days of planting, the results showed a significant increase in germination rate which was 90% in the treatment of the vaccine compared to control, which was 73%. There was also a significant increase in leaf area in addition to vegetative and root weight. The treatment of double vaccine yielded a significant increase in nitrogen and phosphorus rates, as nitrogen reached 2.4 Melgm.gm-1 and phosphorus 1.5 Melgm.gm -1.

Keywords: Double Vaccine, *Azotobacter chroococcum* & *Pseudomonas chlororaphus*, Hordium

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DOI: 10.31838/srp.2020.4.72

INTRODUCTION

Biofertilization is one of the most important biotechnologies. It is carried through the isolation, purification and characterization of different microorganisms added in the form of vital vaccines to the medium in which the plant grows in order to increase nutrient absorption. Its success depends on the efficiency of the organism used, the compatibility between the organism and the vegetarian host, the ability to compete with living organisms already present in the soil as well as the number and survival of living organisms in the area of the Rhizosphere.(1)

The effect of bio-fertilization on the plant host is carried by various mechanisms, such as atmospheric nitrogen stabilization, production of growth promoters, increase the absorption of nutrient uptake and protection of plant host from pathogens. The use of bio-fertilizers is a cheap and reliable method in terms of application. "They reduce the use of chemical fertilizers in about 25% in addition to its role in reducing environmental pollution problems. (2) Microbial Biomass is the living constituent of organic soils in the soil. It is composed mainly from bacteria and fungi, as well as algae, protozoa and nematodes, which constitutes of total organic soil carbon 5-2%, which is 0.02- 1.5 kg of soil.(3) An example of this bacteria is *Pseudomonas* spp which solves Phosphate and biocontrols plant pathogens at the same time . It grows rapidly and benefits from root secretions and multiply rapidly and produce.

(4) *Azotobacter* is one of the nitrogen-fixing bacterium that live free of soil and produces hieroglyphs such as algebra, cytokines, enzymes and vitamins.(5)

Microscopic Rhizosphere can also increase root growth and stimulate the development of root hairs through the production of phytohormones, which can result in increased absorption of phosphorus and other essential elements from the roots of the plant.(6) found that *Rhizobia* and *Pseudomonas* is able to increase vegetative and root weight and chlorophyll substance in Beans plants.(7) demonstrated the ability of *Pseudomonas* and *Azoprium* bacteria to produce phenol compounds, which created an ideal condition for absorbing the elements from the soil surrounding the root hairs and their ability to produce enzymes that convert some elements such as phosphorus into the picture.(8) *Azotobacter* was able to produce the hormone algebraine, which increased the growth properties of wheat plant, such as dry weight, paper area and its component content by 70% and increased germination rate by 74% compared to the treatment of control and this is also supported by (9)

Barley is one of the important grain crops in Iraq for the use as a large-scale animal feed material. The *Hordium vulgare* species is highly productive in irrigation conditions in central and southern Iraq because it tolerates salinity. (10) Due to the importance of the use of bio-fertilizers as an alternative to chemical fertilizers and in order to search for a mixture of great benefit to the plant to stimulate its growth and protection from fungal pathogens and bacteria, our study aimed at:

1. Isolation of two types of bacteria, each working on the installation and processing of an element: the first *Aztobacter* nitrogen stabilizing, and the second element of phosphorus equipped with bacteria *Pseudomonas chlororaphus*.

2 - the role of vaccination combined for both types isolated in the growth characteristics of barley plant and the processing of the necessary elements.

MATERIALS AND METHODS

The experiment was carried out in pots containing 5 kg of sterile alluvial clay soil using a randomized design and five replicates. Azotobacter was isolated from a field planted with algal plants in Al Habbaniyah area on Jensen agar media in accordance with the procedures(11) As for Pseudomonas bacteria it was isolated from the soil of the rhizosphere of field plants in the same area on the center of Crystal violet agar .(12) Morphological, agricultural and bio-

chemical tests were performed using the taxonomic keys mentioned . [13] after comparison and verification against [14] We studied the effect of *Azotobacter* and Pseudomonas (RP) to the rate of germination of barley plant for 25 seeds per pot - 1 after sterilized with 1% solution of sodium hypochlorides for 3 minutes and then washed with sterile water. The double vaccine was synthesized by mixing the two bacteria types after the activation of each type separately for 48 hours at a temperature of 25 m and counted. The results were: zucchini bacteria 3.6x 10 8 cfu ml on Jensen medium and *Pseudomonus* 2.4 x 10 7 cfu \ ml on central King B. Barley seeds were immersed in a 20% glycoprotein solution to ensure adhesion of the vaccine to the seed surface. The seeds were then treated with a double bacterial vaccine for both isolates together, after 3-7 days results were recorded. [15]

Some chemical, physical and biological characteristics of the soil were estimated in the advisory laboratories of the College of Agriculture, University of Baghdad, as shown in Table (1). The study used half of the fertilizer recommended for the barley plant which is 75 kg. 1 - N (urea fertilizer) and 30 kg. P (superphosphate triphosphate) and 50 kg.e-1 K (potassium sulphate). After 45 days of biological experimentation, the length of the root and area of the leaf was measured in centimeters . The dry weight of the vegetative and root total [16] and nitrogen and phosphorus concentrations were also measured. Complete operationality experiment was performed using randomized design C.R.D Gen at significant difference of 0.05. [17]

Table 1: Some Bio-chemical and physical Properties of Soil used in the Experiment

Property	Value	Unit
Electric Conduction	5	Dc\m
Hydrogen H	8.1	
Available Nitrogen	60	Mg\kg
Available Phosphorus	5.11	-
Organic Matter	11.9	g\ kg
Soil Composition	l Clay Mix	
Bacteria *Azotobacter*	3.6x 10 8	c.f.u \ g of soil
P. chlororaphus	2.4 x 10 7	c.f.u \ g of soil

RESULTS AND DISCUSSION

The results of the study showed that colonies of Sodomas bacteria appeared on the agricultural mediums asthe small, circular, smooth, and full-fledged areas in smell and regular circular shape with smooth surface, complete edge, and smell similar to rotten apples." While the Azotobacter bacteria showed a convex circular with a reddish color Figure 1 shows the results of the double-bacterial RP test on the rate of barley germination. The highest germination rate was 90% as compared to the control coefficients that did not exceed 74%. This may be due to the production of isolates of the germination stimulants compounds.[18] A study also showed the ability of Sodomas bacteria to increase plant germination by 38% compared to control.[19]

A study by[20] showed an increase in the percentage of wheat germination when treated with *Azotobacter* and *pseudomonas* and gave protection of seeds from fungal infections[21] studied the increase in the germination rate of a group of field plants such as cucumber and tomato through the production of the Indol 3-acetic acid compound and cytokine in the dishes that were planted with the *Azotobacter* bacteria.

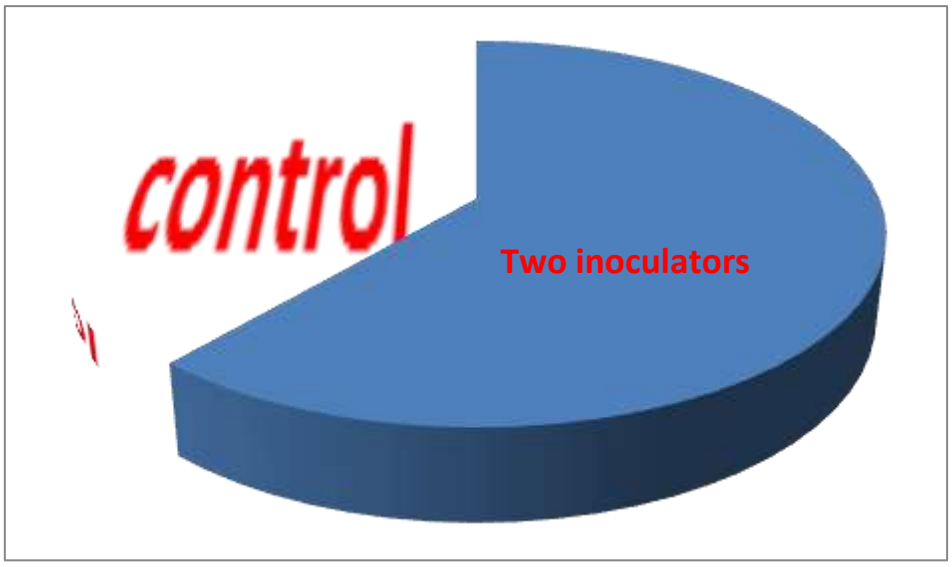

Figure 1: Effect of double vaccine on germination rate within 2-7 days

Table 1 shows some chemical and physical properties of the soil of the experiment that were sterilized by autoclave and were suitable for the growth of the bacterial isolates and the barley plant, and it is similar to the soil tissue from which it was isolated.

The results of the study showed a significant increase in the length of the root and the leaf area of the barley plant. The length of the root in the plant treated with the double bacterial vaccine was 15 cm while the control treatment was 9 cm. The leaf area also had a significant increase after the treatment with the bacterial vaccine. It reached 35 cm 2 as compared to 12 cm 2 in the control . The increase in these traits indicates the ability of these isolates to provide the plant with the necessary nutrients Rayswim provides ready nitrogen while Pseudomonas provides the phosphorus accompanying its production of some semi-hormones and growth stimulants.

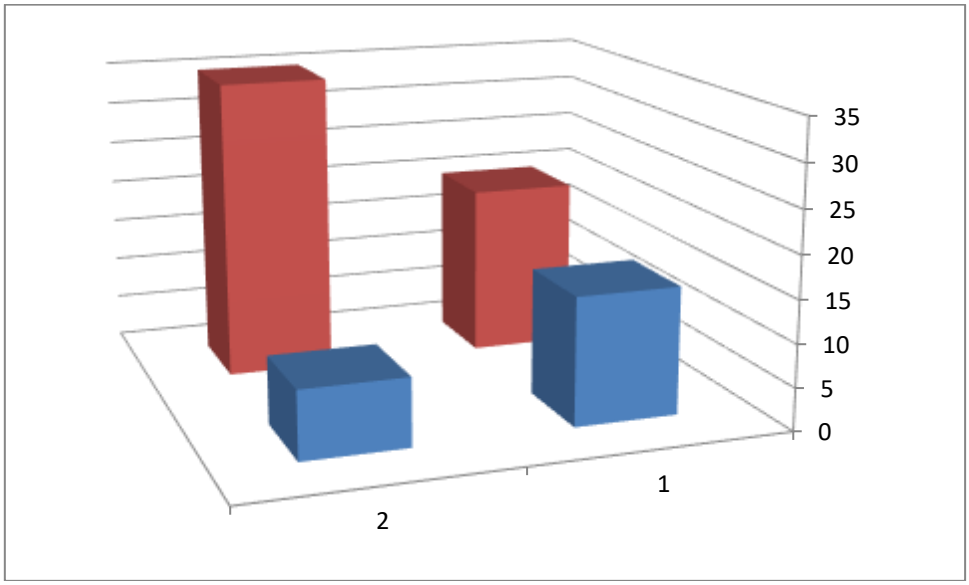

Figure 2: Effects of *Azotobacter* on length of root and leaf area of barley as measured by cm.

Double Vaccine Control (LSD 0.05= 1.77)

The observation of the results of the study shown in Figure 2, makes it clear that there is a significant difference between the treatments using the double vaccine and those using the control treatment. This may be due to the production of fluoridated *Pseudomonas* of growth regulators and growth stimulants, including cytokines and Indol acetic acid, which increase vegetative growth and Azotobacter's ability to provide certain amino acids essentials for plant growth. Moreover, increase in the root mass may also be due to the serious uptake of nitrogen and nutrients near the soil's rhizosphere and the efficiency of the two isolates to provide nitrogen and phosphorus when they are one vaccine. In this case the plant is completely provided with all the necessary elements. There is also a direct relationship between the absorption of nutrients and the dry weight of the plant.

Inoculation of barley seeds with a double bacterial vaccine resulted in an increase in the germination rate and some biological growth properties such as root length and leaf area. This may be due to the the fact that the two isolates simultaneously work to provide the barley root with the largest quantity of plant agents.

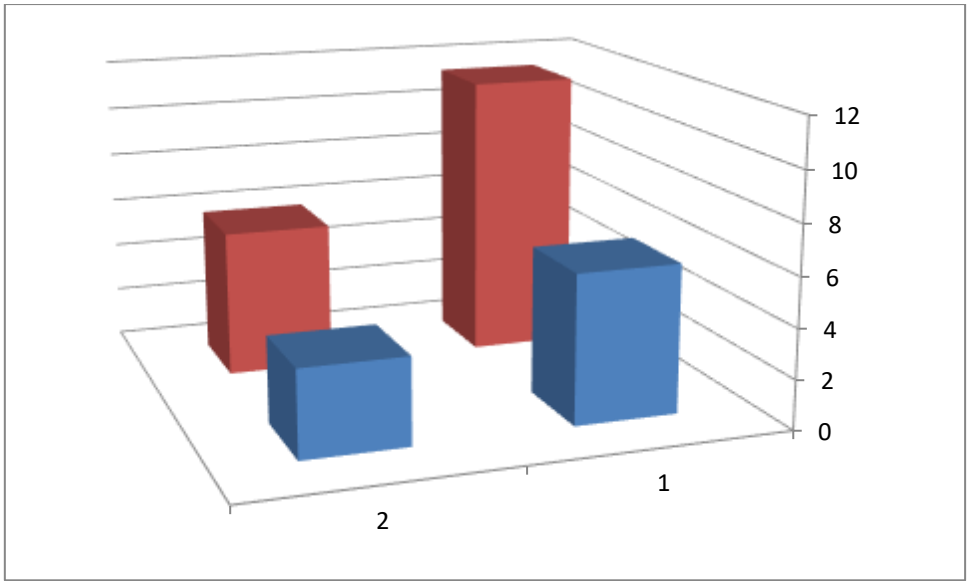

Figure 3: Effects of double vaccine on weight of vegetative and root mass after 45 days of cultivation

Figure (4) shows significant effect of nitrogen and phosphorus concentrations on the vegetative mass expressed in mg per gram of plant and it amounted to 2.4 mg of plant -1 for nitrogen and 1.5 mg of plant -1 for phosphorus in treatments with double bio-vaccine RP after 45 days of cultivation. This increase in nitrogen concentration may be attributed to the ability of *Azotobacter* to process and stabilize atmospheric nitrogen in the soil of the rhizosphere, in addition to the ability of *Pseudomonas* to secrete growth regulators that improve plant growth, increase its absorption efficiency and optimize an excellent root growth that helps increase absorption area. While the significant increase in phosphorus concentration, according to Figure (4), as compared with control coefficients, can be attributed to the production of hormones and growth regulators for the two isolates and the secretion of bacteria to Phosphatase enzymes by Pseudomonas bacteria that dissolve phosphorus and increase its readiness in areas near the roots .

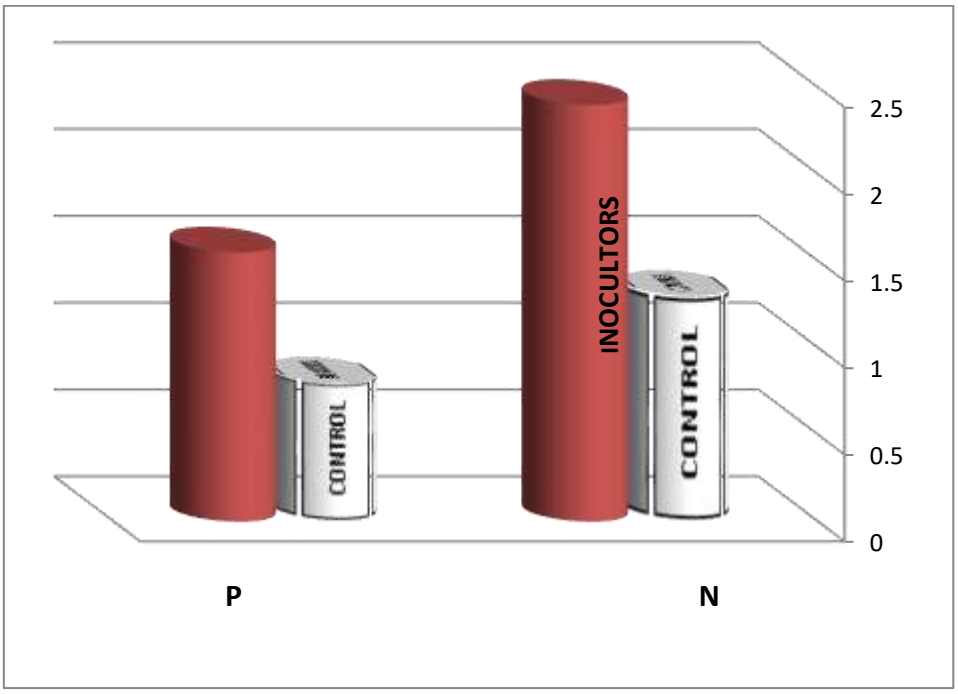

Figure 4: Effects of double vaccine RP on concentration of Nitrogen and Phosphorus in barley after 45 days of cultivation (LSD 0.05=0.0439)

Figure 5: Field photos of barley after 45 days of cultivation (Right: Control Treatment – left: Double Vaccine Treatment).

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