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INOCULATION ROLE OF *PENICILLIUM PINOPHILUM* TO DIVERSE SEED SIZES OF BROAD BEANS (*VICIA FABA*)

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Abstract

Penicillium pinophilum and nitrogen fertilization affect some growth traits of *Vicia faba*, which were resulted from different sized seeds (small, medium and large). These categorized seeds were planted in pots. Least Significant Difference (5%) to figure out the differences between the studied traits were used**.** The results showed a significant increase in the ratio of the vegetative part as compared to the root system of plants that were inoculated with fungi and fertilized**.** The number of root forks reflects the dry weight of the root that was significantly increased with these treatments. The results also indicated that the inoculation of large and medium-sized broad bean seeds led to significant increases in agronomic traits compared to the small sizes of broad bean plants**.** The highest values of the root to shoot ratio, the number of branches, and the dry weight of root were obtained by using large and medium seeds for the broad bean in the absence of nitrogen fertilization.

***Keywords*:** Broad bean, fungal inoculation, *Penicillium pinophilum.*

Introduction

Broad bean is a very important crop because of its economic importance as a nutrient for humans and animals, Duc (1997); and Khaeim *et al.* (2019). The broad or faba beans are important in improving the properties of different soils through their ability to stabilize the atmospheric nitrogen by bacterial nodes located in the roots of the plant Li *et al.* (2010). Many fungi are transmitted by the seeds of the broad beans, causing them to lose their vitality and producing weak and deformed crops, in addition to causing many plant diseases, Wood *et al.* (1989); and Hussein *et al.* (2019). Wilt and root rot are important diseases of the crop, Baker *et al.* (2020).

Fungi are microscopic organisms that have a direct relationship with the soil and its direct relationship with the plant. Fungi are of great importance in the analysis of the organic matter, and the changes of the organic matter in well-ventilated soils are more pronounced than the changes caused by bacteria in addition to their ability to analyze nitrogenous materials and result in ammonia and simple nitrogenous compounds, Wang *et al.* (2013). Living organisms benefit from the analysis process because they supply the organisms with the necessary nitrogen for growth and carbon under special conditions, Al-Baldawy (2019). Fungi may compete with plants to obtain nitrates and ammonium, and these lead to a decrease in these materials from the soil, and thus their harmful effect is demonstrated here. Researchers are aware of the importance of nitrogen fertilization of the plant because it is an important element in the formation and construction of the living cell, Rando *et al.* (1997); and Jeber *et al* (2019). As for the leguminous plant, this importance manifests itself under a certain concentration because it is excessive by increasing the nitrogen fertilizer concentration of the plant leading to harmful effects on the legume plant. Because it blocks the action of the gene responsible for forming the nitrogen enzyme, thereby nullifying the action of the root ganglia in fixing the nitrogen, Brown *et al.* (1987).

The application of nitrogen fertilizers more than the recommended quantity, especially ammonium would negatively affect leguminous plants. Consequently, the excessive nitrogen application leads to restricting the function of genes that are responsible for forming the nitrogen enzyme, thereby nullifying the effect of the root nodes in nitrogen fixation and becoming ineffective. Ammonium salts application to soil containing fungi indirectly affects the growth and formation of the bacteria of *Rhizobium sp*, Rashid *et al.* (2016).

Results of some research present that inoculation of small and medium-sized broad bean seeds significantly increased the dry weight of the root compared to unfertilized seeds, Yassen *et al.* (1995). The inoculation did not show any increase in the other characteristics. Using seeds of large size, led to an approximate increase in almost all vegetative traits of the plant, Cai, *et al.* (2011). The present research aims to study the effect of inoculation with *Penicillium pinophilum* and nitrogen fertilization on the different seed size of a broad bean. The above factors were studied individually in previous studies.

Methods and Materials

Pots study was conducted in the Department of Horticulture and Gardening Engineering / Faculty of the Agriculture / University of Al-Qadisiyah using mix soil with pH of (7.5) The soil was placed in plastic pots at a rate of (3) kg for each. In this experiment, (3) different sizes of broad bean seeds (large, medium and small) were used. Three seeds were placed in each pot after some of them were inoculated with a fungal suspension and about (1.5 * 1-6) spore/cm^3. Nitrogen fertilizer (40 kg.N.ha) was applied after (14) days of the planting date in the form of urea (47% N) and different plant traits were measured. The following vegetative growth traits were measured to find out the extent to which each of them was affected by the factors: the number of plant branches, the number of branching roots, the ratio of the vegetative system to the root system, and the dry weight of the root.

The experiment was carried out according to the Complete Randomized Block Design (C.R.B.D). Least significant difference at the level of 5% to investigate the differences between means were used. The number of treatments was (12) and three replicates. The roots of the plants were dried at the end of the experiment in ovens under a temperature of (70)C for (48) hours.

Isolation and identification

The seeds were sterilized superficially for one minute using (1)% sodium hypochlorite. They were planted in Petri dishes (9) cm in diameter) containing agar culture, dextrose and potatoes/potatoes (PDA) (10 seeds/plate), Castanares, A., McCrae, S. I., & Wood, T. M. (1992). They were incubated at (25)C. After 5 days, a small portion of the edge of the outer fungus growths of the evolving colony is transferred to dishes containing agar, dextrose and potatoes/potatoes for examination and identification. Then the percentage of infected seeds was calculated, De Stefano, S., *et al* (1999).

Isolation and identification of roots and bases of leguminous stem

Bean plants were collected from farmers' fields. Small bases of infected plants have been cut into small pieces. Surface sterilized with (1)% sodium hypochlorite solution for three minutes. They were washed with sterile distilled water, dried with filter paper and planted in a petri dish containing agar, dextrose and potatoes/potatoes. Incubated at a temperature of (25)C, and after 4 days the isolates were purified in the same way as above. The isolated fungi were determined according to the colony shape, spores, and structures. The types of *Penicillium pinophilum* have been determined according to Booth according to the approved classification keys.

Penicillium pinophilum was multiplied on moist and millet as part of (500) mm flasks, then used to prepare the vaccine by (10) g of infectious vaccine per mushroom / (1) kg of soil. Sterile soil was prepared with methyl bromide. Then it was placed in plastic pots with a diameter of (9) cm, with an average of three replications/treatment. The pots were watered with light water and covered with perforated polyethylene bags. The readings were taken one week after planting. The percentage of germination was calculated.

Results and Discussion

Table (1) indicates a significant increase in the percentage of the vegetative group to the root system between the pollinated plants *Penicillus pinophalum* and the unvaccinated plants when adding fertilizer to them. This is due to the possibility of the fungal contributing to the analysis of organic matter in the soil and its preparation for the plant.

The number of root tillers and dry weight of the root were significantly reduced in fungi and unvaccinated plants when fertilizer was applied. This may be due to the application of nitrogen fertilizer as a reason for limiting the activity and growth of root ganglia bacteria.

Table (2) shows that pollination of seeds with large and medium-sized fries increased significantly the number of plant branches, as well as the branches of the root resulting from large or medium seeds compared to the small seeds and for the same treatment. This may be because large and medium seeds have large flakes compared with small seeds. Considering that cotyledons are a storehouse of nutrients in seeds, they appear to give strong and large plants compared to plants produced from small seeds. This is in application to the role that fungal mycelium plays, which appear to contribute to the absorption of nutrients from the soil.

The results in Table (3) indicate that the percentage of the vegetative system to the root, the number of branches in the pea plant and the weight of the roots were negatively affected by the fertilization treatment applied in the experiment. The values recorded in plants resulting from large seeds are unfertilized (1004, 3, and 0.658), respectively, concerning the traits indicated above. The lowest values obtained in plants resulting from the seeds of small fertilized (0.925, 1.83, and 0.478), respectively for the same traits studied.

Table 1 : The effect of inoculation with *Penicillus pinophalum* and application of nitrogen fertilizer on the ratio of the vegetative growth to the root growth, the number of branching roots and the dry weight of the roots of the broad beans.

Fungal Inoculation	Root to Shoot Ratio		# of Roots Branches/Plant		The dry weight of roots (g)	
	Yes	No	Yes	No	Yes	No
Yes	1.021	1.048	20.55	26	0.360	0.400
No	0.997	0.948	30.77	41.88	0.654	0.881
L.S.D (0.5)	0.026		1.24		0.036	

Table 2 : The effect of inoculation with *Penicillus pinophalum* fungus and seed size on the number of plant branches and the roots of broad bean roots

Fungal Vaccination	Number of branches-per plant		Number of roots branches per plant	
	Yes	No	Yes	No
Small	2.16	1.83	22	36.0
Medium	2.16	2.16	24.3	36.16
Large	3.00	2.50	25.5	36.83
L.S.D (0.5)	0.255		1.28	

Table 3 : The effect of seed size and nitrogen application on the ratio of the vegetative to the root system, dry weight, and the number of branching roots of the broad beans.

Fungal Vaccination	Vegetative to root parts percentages		Pant Branches		The dry weight of roots (g)	
	Yes	No	Yes	No	Yes	No
Small	0.925	1.0	1.83	2.33	0.478	0.60
Medium	0.945	1.0	2.0	2.66	0.488	0.640
Large	1.0	1.04	2.0	3.0	0.556	0.658
L.S.D (0.5)	0.045		0.225		0.0098	

These results are reasonable, as the application of nitrogen fertilizer directly affected the effectiveness and growth of root ganglia bacteria, which reflected negatively on the attributes studied. As the topic needs future studies, especially about the new relationship between *Pinophilum penicillium* and root bacteria in the legumes. Likewise, to confirm the possibility of using the fungus for biological control through its effect on the rest of the microorganisms in the soil.

Conclusion

The inoculated plants with *Pinophilum penicillium* and fertilized resulted in a significant increase in the shoot to the root ratio**.** The number of root forks reflects the dry weight of the root that was significantly increased with these treatments. The inoculation of large and medium-sized broad bean seeds led to significant increases in agronomic traits compared to the small sizes of broad bean plants**.** The height values of agronomic traits resulted in using large and medium seeds in the absence of nitrogen fertilization.

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