



RESEARCH ARTICLE

Efficiency of some local isolates of arbuscular mycorrhizae in the growth and productivity of potatoes (*Solanum tuberosum* L.) in plastic pots

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Abstract

The present study was conducted using plastic pots to investigate the efficiency of 15 local isolates of mycorrhizal fungi in enhancing the growth and productivity of Arizona variety potatoes (*Solanum tuberosum* L.). These isolates were obtained from various wild plants, including the Sweet rush plant, Sudan grass, and Millet, collected from different districts in Diyala Governorate. The isolates were obtained through the single spore cultivation technique in the Department of Agricultural Research in Al-Zafaraniya. The results demonstrated that all 15 fungal isolates had the ability to infect the roots of potato plants. Notably, M11 showed a significant superiority in infection severity (98.3%), the number of spores was 67.67 spores/gm of soil, and the infection rate was 90.0%. The results also highlighted the impact of these isolates on various parameters related to vegetative and root growth as well as yield. Specifically, isolates M1 and M12 were found to be superior in promoting plant height and increasing leaf area, which reached 218.3 cm². Isolate M4 was superior in increasing the chlorophyll content of leaves, reaching a level of 47.4 spad. In the context of vegetative growth, isolate M3 produced a notable fresh weight of 8.236 g, while isolate M8 yielded a dry vegetative weight of 7.533 g. Regarding the root system, isolate M11 displayed superiority in root length, reaching 45.20 cm. Isolate M8 showed a higher number of tubers, amounting to 11.33 g, whereas isolate M7 produced tubers with a weight of 178.5 g.

Keywords

Arbuscular mycorrhizae; *Solanum tuberosum*; yield; infection rate; spores

Introduction

Mycorrhizal fungi are microscopic organisms that engage in a symbiotic relationship with the roots of higher plants, aiding in nutrient absorption while receiving carbon from the host plant in return (1, 2). These fungi are known for their wide distribution across various environments and their association with numerous plant families, including Leguminosae and Gramineae (3). However, certain plant families, such as Chenopodiaceae and Cruciferae, tend not to form such relationships (4). Mycorrhizal fungi are identified by the presence of fungal hyphae that penetrate the root cortex cells. These hyphae form arbuscular structures, facilitating nutrient exchange between the plant host and the fungus. Additionally, at the end of these hyphae, vesicles are formed, serving as storage units for fats and carbohydrates (5). These mycorrhizal fungi play a crucial role as an

environmentally friendly biofertilizer alternative. They contribute to ecosystem maintenance, enhance crop productivity, facilitate nutrient transfer, and increase the rate of carbon metabolism (6).

Solanum tuberosum L. (Solanaceae) is one of the most important vegetable crops globally, ranking fourth after wheat, corn, and rice in terms of cultivation, production, and economic importance. South America is regarded as the original area of planting potatoes, from where it spread to various parts of the world (7). Research has shown that biological fertilization contributes to an increase in the growth and yield of potatoes. Furthermore, it plays a vital role in reducing the excessive use of chemical fertilizers, making it more environmentally friendly and eliminating the pollutants caused by their excessive use (8). Additionally, potato roots form a symbiotic relationship with various types of mycorrhizal fungi (9). Therefore, the use of biofertilizers has become one of the methods employed to enhance the growth and productivity of the plant. The current study hence aims to determine the efficiency of some mycorrhizal isolates in the growth and productivity of potatoes.

Materials and Methods

Samples collections

Five regions of Diyala governorate (Kanaan, Mandali, Khan Bani Saad, Al-Khalis and Baladruz) were selected for the isolation of endophytic mycorrhizal fungi. The process involved collecting random soil samples from three different types of wild plants: sweet rush, Sudan grass, and millet, with 15 isolates obtained for each plant type and replicated three times. The soil samples were collected from a depth of 5-25 cm in the rhizosphere of various soils and subsequently mixed to create a homogeneous composite sample. These composite samples were then placed in clean plastic bags, with detailed information about each sample recorded and stored in the laboratory for later isolation purposes.

Isolation and propagation of mycorrhiza fungi

The isolates were obtained, purified, and cultivated at one of the experimental stations under the Ministry of Science and Technology, following the wet sieving and decanting method (10). The procedure involved mixing the contents from each area thoroughly, taking 250 grams of soil, and adding one liter of tap water. After allowing it to settle for 1-2 minutes, the upper water layer was removed, and the remaining material was passed through sequential sieves with diameters of 0.025, 0.045, 0.075, 0.125, and 0.25 microns, respectively.

The contents of each sieve were thoroughly washed with plain water, and the resulting washing water was collected over each of the previous sieves in test tubes. These test tubes were then centrifuged at a speed of 5000 rpm⁻¹ for 5 minutes. After centrifugation, the filtrate was discarded, and a 50% glycerol solution was added to the precipitate (11). The tubes were manually shaken and

then centrifuged at the same speed as before for 10 minutes. Subsequently, the contents were transferred to glass petri dishes for examination purposes. The mycorrhizal fungal inoculum was propagated in plastic pots with a soil capacity of 10 kg, consisting of a mixture of 3 parts organic matter (Peatmoss) and 1 part soil, which had been sterilized using an autoclave at 121°C for one hour on two consecutive days. To initiate the process, surface-sterilized seeds of yellow corn (*Zea mays* L.) were sown at a rate of 25 seeds per pot. After germination, the pollination process was carried out by transferring a single spore or a single spore cluster by means of fine forceps from the suspension with the help of a light microscope and planting near the roots of the yellow corn seedlings, following the method described by Fracchia *et al.* (12). Four months later, the vegetative mass of the plants was removed, and the mycorrhizal soil (spores + fungal hyphae + infected root pieces) was taken, and this soil was placed in sterile plastic bags. It was kept in a cool and dry place until the mixture was used as a mycorrhizal fungal inoculum, after which it was examined under a light microscope to ensure the presence of any mycorrhizal infection of the roots after dyeing the roots with acid fuchsin, according to the method of Phillips and Hayman (13). The isolates were numbered according to Table 1.

Table 1. Isolates of the mycorrhizal fungus from wild plants distributed in the districts of Diyala Governorate

isolation number	isolated area	plant host
M1	Kanaan	Sudan grass
M2	Kanaan	Sweet rush
M3	Kanaan	Millet
M4	Mandalay	Sudan grass
M5	Mandalay	Sweet rush
M6	Mandalay	Millet
M7	Banysaad	Sudan grass
M8	Banysaad	Sweet rush
M9	Banysaad	Millet
M10	Khalis	Sudan grass
M11	Khalis	Sweet rush
M12	Khalis	Millet
M13	Baldroz	Sudan grass
M14	Baldroz	Sweet rush
M15	Baldroz	Millet

Mycorrhizal characteristics

The quantification of mycorrhizal fungal spores in each soil sample was carried out following the procedure outlined in (14). To determine the average number of spores in each sample, the spore count in each sample was multiplied by the product of the spore rate in 1 ml and the dilution factor. The infection rate (%) was calculated through the equation described by (15).

(%) infection with mycorrhizal fungi

$$= \frac{\text{infected root cutting number}}{\text{examined root number of total cutting}} \times 100$$

The severity of mycorrhizal fungal infection was determined using the method outlined by Philips and Hyman (13) and calculated based on the formula provided in (16):

Degree	Percentage of root parts infected (%)
1	0%
2	1-25%
3	26-50%
4	51-75%
5	76-100%

Severity of infection with mycorrhizal fungi

$$= \frac{\text{Total (number of affected pieces} \times \text{degree of endemism)}}{\text{total number of pieces examined} \times \text{highest degree of settlement}} \times 100$$

Experiment to test the effectiveness of isolates on growth and productivity of potato

This experiment was carried out in a nursery in the city of Baquba - Diyala Governorate, under shade conditions on 28/10/2022, as potato seed. Dutch-origin (Arizona variety) Elite rank for the fall harvest was obtained from Al-Nahar Company for Plant Production. The seeds were stored at ± 25 degrees in a growth room illuminated with fluorescent lamps to stimulate the tubers to seed. To ensure uniformity, these tubers were evenly distributed on wooden racks inside the room to provide adequate ventilation and indirect lighting. With 45 pots, 9 pots were allocated for each district, and three replications for each plant, each with a capacity of 20 kg. Sandy soil was used, and then a mycorrhizal inoculum was placed at a rate of 10 g inoculum / pot, which consists of spores, hyphae and infected roots. At that point, one tuber was placed for each pot, and the irrigation process was carried out, then it was distributed according to the Randomized Complete Block Design (RCBD). One month later, urea fertilizer (N 46%) was applied, followed by adding half of the recommended NPK fertilizer to promote plant growth. At the end of the season, which was 90 days after planting, various growth parameters were measured, including plant height, leaf area, chlorophyll index, weight of the dry root system, number of tubers, and weight of tubers.

Studied characteristics

1. Plant height (cm)

The height of the plant was measured by a metric ruler from the base of the stem to the top of the plant.

2. Leaf area ($\text{cm}^2 \cdot \text{plant}^{-1}$)

The leaf area was measured in the Plant Physiology Laboratory - Department of Horticulture and Landscape Engineering - College of Agriculture - University of Diyala by taking three leaves from each experimental unit using the CI202 - LASER AREA METER, then the mean was calculated.

3- Chlorophyll index in the leaves (SPAD unit)

The chlorophyll index in the leaves was estimated by selecting the most recent fully developed leaf. Also, chlorophyll was measured directly in the field by a SPAD-502 chlorophyll meter, and the mean was taken for three readings per plant and for three plants per experimental unit (17).

4- Fresh and dry vegetable weight (gm)

The fresh weight of the shoot was taken after removing the plants from the soil and separating the shoot from the root system at the base of the stem. The fresh weight was measured using an electric balance.

5- Root length (cm)

Root length was measured with a measuring ruler.

6- Fresh and dry weight of the root system (g. plant^{-1})

The fresh weight of the root system was carried out after separating it from the shoot from the base of the stem, and the roots were washed well to remove soil from them, and the dry weight was taken by placing the roots in perforated paper bags in an electric oven at a temperature of 70 °C until the weight stabilized (18).

7- Number of tubers (tuber pot^{-1})

8- Tuber weight (gm)

The weight of the tubers was measured using an electric balance.

Statistical analysis

The data collected from the experiment were subjected to statistical analysis using the Genstat statistical program. The study utilized a Randomized Complete Block Design (RCBD) with three replications. To compare the differences between the means, the Least Significant Difference (LSD) test was employed at a significance level of 0.05.

Results and discussion

The results in Table 2 show that all isolates have the ability to colonize the roots of the potato crop. The highest value for the number of spores reached 3383 spores per gm soil in M3, M8, and M14, while M6 recorded the lowest value for the number of spores, amounting to 36.67 spores. $\text{gm}^{-1} \cdot \text{soil}$. On the other hand, isolates M3, M8, and M14, which amounted to 83.33 spores. $\text{gm}^{-1} \cdot \text{soil}$, were significantly superior to M1, M4, M6, M9, M10, and M13, which amounted to 73.33, 43.33, 36.67, 66.67, 66.67, and 63.33 spore. $\text{gm}^{-1} \cdot \text{soil}$, respectively. Regarding the severity of the injury, it reached the highest rate of 98.3% in the isolation M11, while the isolation M5 recorded the lowest percentage of 58.3%, and the isolation M11 was morally exceeded, amounting to 98.3% on the insulation M3, M4, M5, M6, and M13, which is 73.3, 71.7, 58.3, 75.0, and 61.7%, respectively. With respect to the infection rate, the highest infection rate value was 90.0% in isolate M11, while isolate M5 recorded the lowest value, amounting to 53.3%. M11 was significantly superior to isolates M3, M4, M5, M6, and M13, amounting to 66.7, 66.7, 53.3, 70.0, and 56.7%,

respectively. The reason for this is attributed to the efficiency of the vaccine used and the host's response to inoculation with mycorrhizae, which leads to an increase in the plant's supply of phosphorus. Therefore, the potato plant forms a symbiotic relationship with mycorrhizal fungi (19). Additionally, this can be attributed to the chemical secretions of the roots in the rhizosphere, which have a role in enhancing the symbiotic relationship between mycorrhizal fungi and the roots because the secretions contain compounds that encourage the germination of fungal spores and thus the occurrence of infection.

Table 2. The effect of mycorrhizal fungus isolates on some parameters of mycorrhizal characteristics of potato crop (Arizona variety)

Isolation name	Number of spores	injury Severity (%)	Incidence rate (%)
M1	73.33	95.0	86.7
M2	80.00	90.0	81.7
M3	83.33	73.3	66.7
M4	43.33	71.7	66.7
M5	76.67	58.3	53.3
M6	36.67	75.0	70.0
M7	76.67	95.0	86.7
M8	83.33	95.0	86.7
M9	66.67	86.7	80.0
M10	66.67	81.7	76.7
M11	76.67	98.3	90.0
M12	76.67	83.3	76.7
M13	63.33	61.7	56.7
M14	83.33	93.3	86.7
M15	73.33	86.7	80.0
L.S.D 0.05	9.447	17.58	15.40

The results in Table 3 revealed the impact of isolates of the shrub mycorrhizal fungus on various vegetative growth parameters of the potato crop. The highest recorded value for plant height was 67.0 cm in M1, while M5 has the lowest value of 27.3 cm. Isolate M11 was significantly superior to isolates M3, M4, M5, M6, M9, M10, M11, M12, M13, M14, and M15, reaching 52.2, 52.2, 27.3, 41.3, 52.3, 50.7, 45.0, 42.3, 45.3, 42.3, and 41.3, respectively.

Regarding leaf area, the highest value was 218.3 cm² per plant in M12, while isolate M8 recorded the lowest value of 167.9 cm² per plant. Isolate M12 significantly surpassed M1, M3, M6, and M8, with values of 218.3, 186.2, 95.8, 182.4, and 176.9 cm² per plant, respectively.

In terms of chlorophyll pigment content in the leaves, the results from Table (3) showed that the highest SPAD value was 47.4 in M4, while M5 had the lowest value of 28.8 SPAD. The M4 isolate was significantly superior to the M5 of 28.8 SPAD, with a value of 47.4.

The table also indicated that the highest value for vegetative wet weight was 8.236 g per plant in M3, whereas the lowest value was recorded in M6 at 2.499 g per plant. M3 significantly outperformed the other isolates, including M2, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, and M15, which had respective weights of 7.149, 6.234, 4.154, 2.499, 7.199, 7.149, 7.169, 5.177, 6.155, 4.188, 5.455, 5.154, and 3.148 g per plant.

Regarding the dry weight of the shoots, the results indicated that the highest value was in M3, reaching 4.160 g per plant, while the lowest value was recorded in M6 at 1,241 g per plant. M3 significantly outperformed M1, M2, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, and M15, with respective weights of 3.791, 3.174, 3.075, 2.238, 1.241, 3.206, 3.194, 3.119, 2.576, 3.189, 2.221, 2.274, 2.390, and 1.338 g per plant.

Table 3. The effect of isolates of the mycorrhizal fungus on some parameters of vegetative growth of potato crop (Arizona variety)

Isolation name	Plant height	Leaf area	Chlorophyll Index	Fresh weight of shoots	Dry weight of shoots
M1	67.0	186.2	43.0	7.887	3.791
M2	64.7	208	44.5	7.149	3.174
M3	52.2	95.8	43.2	8.236	4.160
M4	52.2	200.6	47.4	6.234	3.075
M5	27.3	202.2	28.8	4.154	2.238
M6	41.3	182.4	46.7	2.499	1.241
M7	56.8	195.3	44.9	7.199	3.206
M8	56.8	176.9	46.1	7.149	3.194
M9	52.3	190.6	43.8	7.169	3.119
M10	50.7	190.6	44.0	5.177	2.576
M11	45.0	203.2	45.5	6.155	3.189
M12	42.3	218.3	45.1	4.188	2.221
M13	45.3	213.9	43.4	5.455	2.274
M14	42.3	212.5	43.2	5.154	2.390
M15	41.3	206.3	41.3	3.148	1.338
LSD0.05	14.23	28.01	11.45	0.4077	0.3696

The results presented in Table 3 indicate that the inoculation with mycorrhizal fungi had a significant impact on all aspects of vegetative growth in the potato crop. This effect can be attributed to the positive influence of mycorrhizal fungi on plant growth, as they produce several plant growth regulators, including auxins, cytokinins, and gibberellins. Moreover, mycorrhizal fungi enhance the availability of essential nutrients for the plant, such as nitrogen, phosphorus, potassium, and other vital elements required for plant vitality. This impact is evident in the growth parameters discussed above, and it aligns with findings from various studies (20-22).

Regarding the fresh weight of the roots, the results in Table 4 illustrate the impact of mycorrhizal fungus isolates on certain parameters of root growth in the potato crop. The highest recorded value was in M8, reaching 13.50 g per plant, while the lowest value was observed in M6 at 3.46 g per plant. M8 significantly outperformed all other isolates. Concerning the dry weight of the roots, the highest value was recorded in M8 at 7.533 g per plant, whereas the lowest value was found in M5, which reached 1.533 g per plant. Once again, M8 displayed significant superiority over the other isolates. In terms of root length, the highest measurement was in M11, reaching 45.20 cm,



Table 4. The effect of isolates of the mycorrhizal fungus on some parameters of root growth of potato crop (Arizona variety)

Isolation name	Fresh weight of root (gm.plant ⁻¹)	Dry weight of root (gm.plant ⁻¹)	Root length (cm)
M1	8.500	4.533	31.63
M2	9.467	3.600	30.07
M3	7.567	3.233	30.10
M4	6.533	2.633	21.67
M5	5.533	1.533	24.70
M6	3.467	2.300	21.80
M7	6.367	3.667	25.50
M8	13.500	7.533	41.43
M9	6.533	2.567	23.80
M10	7.400	4.267	27.93
M11	12.500	6.567	45.20
M12	9.500	4.233	39.93
M13	9.400	5.533	33.90
M14	11.567	5.467	35.47
M15	8.267	3.433	32.53
L.S.D 0.05	0.8217	0.3389	2.900

while the lowest value was recorded in M4 at 21.67 cm. M11 outperformed all the isolates, and this can be attributed to the role of these organisms in enhancing the availability of essential nutrients and activating growth regulators, ultimately promoting root branching (23). This is also attributed to mycorrhizal fungi's ability to supply the plant with critical nutrients like NPK and various other elements, thereby improving overall plant growth and obtaining larger amounts of organic carbon (24). These findings align with previous studies conducted by Al-Umrani (25) and AbdEl-Baky et al. (26).

Concerning the effect of mycorrhizal fungus isolates on some yield standards for the potato crop, the results of Table 5 showed that the highest value for the number of tubers was in M8, reaching 11.33 g.pot⁻¹, while the lowest value was recorded in M6, reaching 5.0 g.pot⁻¹. M8 was significantly superior, amounting to 11.33 g.pot⁻¹ on M1, M2, M3, M4, M5, M6, M7, M9, M10, M11, M12, M13, and M15, reaching 8.33, 7.00, 7.67, 6.00, 5.00, 5.67, 5.33, 7.67, 7.33, 6.33, 5.67, and 7.33. With respect to the tuber weight, it reached the highest value in the M7, which amounted to 178.5 g.pot⁻¹, while the lowest value was recorded in M12, which reached 92.6 g.pot⁻¹, and M7, which was 178.5 g.pot⁻¹. The increase in the number of tubers can be attributed to

Table 5. The effect of isolates of the shrub mycorrhizal fungus on some yield parameters of potato crop (Arizona variety)

The name of isolation	Number of tubers (gm.pot ⁻¹)	Weight of tubers (gm.pot ⁻¹)
M1	8.33	130.8
M2	7.00	102.1
M3	7.67	118.9
M4	6.67	121.6
M5	6.00	101.1
M6	5.00	63.8
M7	5.67	178.5
M8	11.33	136.5
M9	5.33	140.7
M10	7.67	140.6
M11	7.33	130.1
M12	6.33	92.6
M13	5.67	108.2
M14	11.00	131.1
M15	7.33	114.5
L.S.D 0.05	3.077	34.79

the role of mycorrhizal fungi in secreting growth hormones, as these hormones work to increase vegetative and root growth as a result of increased division and expansion of cells and tissues, and this in turn leads to an increase in the number of tubers (27). The reason for the superior tuber weight is attributed to cell expansion due to the accumulation of water, mineral elements, carbohydrates resulting from the process of photosynthesis, and proteins resulting from the process of nitrogen assimilation (28). This is consistent with Liu *et al.* (29) on the potato plant, indicating the connection between mycorrhizae and this plant increases crop production (28).

Conclusion

The study shows that all 15 fungal isolates had the ability to infect the roots of potato plants. Satisfactory results were obtained for the characteristics of vegetative and root growth, yield, and chemical content characteristics in the leaves of potato plants inoculated with the endomycorrhizal fungus.

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Authors contributions

All authors contributed equally to the preparation of the manuscript in terms of developing the research plan, implementing it, conducting examinations, and finally pro-

ducing the manuscript in its final form.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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