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Isolation, Identification and Evaluation of the Endophytic *Methylobacterium radiotolerans* to Stimulate Growth of *Jatropha curcas* Plants

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Abstract: An endophytic facultative methylotrophic bacterium with pink pigment was isolated from *Jatropha curcas* leaves and *in vitro* tests were used to identify its growth-promoting properties. Using morphological and 16S rRNA sequencing features, the isolate was recognized as *Methylobacterium radiotolerans* strain H-0A1. The identified strain H-0A1 of *M. radiotolerans* produces IAA, fixes nitrogen, forms ammonia, and solubles phosphate, according to the results of an *in vitro* evaluation. Furthermore, the stimulation effect of *M. radiotolerans* strain H-0A1 on the growth of *J. curcas* was investigated in a pot experiment. Plant height, leaves parameters (number, area, and dry weights), as well as stems diameter and dry weight, length, and dry weight of roots, were all greatly enhanced by inoculation and/or foliar spraying treatments of *J. curcas* seedlings. These treatments also had an impact on the amount of chlorophyll and the uptake of nutrients. According to the current research, the bacterium *M. radiotolerans* strain H-0A1 has a lot of potential for use as a biofertilizer in sustainable agriculture.

Key words: Endophytic methylotrophic bacteria, *Methylobacterium radiotolerans*, *Jatropha curcas*,16S rRNA sequencing.

INTRODUCTION

The term "endophyton" comes from the Greek "endon" which means "inside" and "phyton" which means "plant" **(Kobayashi and Palumbo, 2000)**. Endophytic bacteria are known to inhabit the internal tissues of almost all plants. They can establish a variety of relationships, such as symbioses. The majority of endophytes seem to come from the phyllosphere or rhizosphere, though some might spread

via seeds. According to **Ryan *et al.* (2008)**, endophytic bacteria produce a variety of natural products that may be used in industry, medicine, or agriculture. They can also function as biocontrol agents and enhance plant growth and yield. Found in the rhizosphere and on the leaf and seed surfaces of a wide range of plants, pink-pigmented facultative methylotrophic bacteria (PPFM) are members of the genus *Methylobacterium* **(Madhaiyan *et al*., 2005** and **Rekadwad, 2014)**. As energy and carbon sources, PPFM can use a variety of C2, C3, and C4 compounds as well as one-carbon compounds (C1), like methylamine and methanol **(Camargo-Neves and Araújo, 2018)**. **Sy *et al*. (2005)** confirmed that *Methylobacteria* used methanol as their only carbon source after being drawn to the stomata by cell expansion, as evidenced by earlier reports.

Jatropha curcas the woody perennial shrub, also known as "Jatropha" is a member of the Euphorbiaceae family and is widely found in tropical and subtropical areas. Jatropha seeds have a high triacylglyceride content and a fatty makeup that makes them ideal for producing biodiesel. **(Openshaw, 2000)**. In climates and soils unsuitable for the cultivation of food crops, jatropha can flourish on marginal land because it is drought-resistant **(Achten *et al*., 2013)**. Apart from storing carbon dioxide and decreasing global dependency on fossil fuels, Jatropha aids in managing soil erosion **(Reubens *et al.*, 2011)** and purifying contaminated soil **(Becker *et al*., 2013)**.

Methylobacterium species are significant endophytes that contribute positively to the growth and yield of *Jatropha curcas* **(Madhaiyan *et al.*, 2015)**. This current work intends to isolate the endophytic *Methylobacterium* from leaves of Jatropha plants, characterize it through 16S rRNA based molecular technique and study of its plant growth-promoting properties and assessment of the impact of its inoculation on the stimulation of Jatropha plant growth.

MATERIALS AND METHODS

Isolation of endophytic bacteria

The isolation of endophytic bacteria from leaves was described by **Madhaiyan *et al*. (2015)** as follows, three separate plants were chosen to provide healthy, symptom-free leaves, which were then sanitized in two steps: first, they were washed for five minutes in 1% (w/v) sodium hypochlorite solution supplemented with one drop of Tween 80 per 100 ml solution; next, they were rinsed three times in 70% ethanol in sterilized distilled water for one minute. In order to guarantee total surface sterilization, the tissues underwent a second treatment consisting of 15 minutes of washing in 15% H_2O_2, 1 minute in 70% ethanol, and a rinse in sterilized distilled water. The surface-sterilized tissues were crushed by grinding in 50 ml of 10 mM $MgSO_4$, then, streaked on Methanol Mineral Salts agar plates **(Holland and Polacco, 1992)** which contains 0.79 g K_2HPO_4, 0.54 g KH_2PO_4, 1.00 g $MgSO_4.7H_2O$, 0.20 g $CaCl_2.H_2O$, 0.50 g NH_4Cl, 4.00 µg $FeSO_4.7H_2O$, 0.10 µg $ZnSO_4.7H_2O$, 0.04 µg $MnCl_2.4H_2O$, 0.30 µg $H_3BO_4.6H_2O$, 0.20 µg $CoCl_2.6H_2O$, 0.10 µg $CuCl_2.2H_2O$, 0.02 µg $NiCl_2.6H_2O$, 0.60 $Na_2MoO_4.2H_2O$ µg, 20.00g agar and distilled water 1 L. The pH was adjusted to 6.8 before autoclaving at 121 ^oC for 20 min. Filtered methanol was added to cold sterilized medium at volume 5 ml per L and incubated for three to five days at 30 °C. Pink-pigmented single colonies were re-streaked on the same medium and incubated for three days at 30 °C in order to further purify them. The isolate was kept at -20 °C in glycerol (20% v/v).

DNA isolation and 16S rRNA amplification

DNA was isolated and purified utilizing the procedure outlined in **(Sambrook and Russell, 2001)**. The whole genomic DNA of bacterial species was extracted; its quality was assessed on a 1% agarose gel. Table (1) illustrates the primers sequences of PCR amplification of isolated DNA using the 16S rRNA gene, which was done in a 20 µl reaction solution with universal primers 27F/1492R 3 **(Yang *et al*., 2010)**. The amplification process involved five cycles of denaturation at 95^oC for five minutes, annealing at 55 ^oC for sixty minutes, extension at 72 ^oC for sixty minutes, and final extension at 72^o C

for thirty minutes. A positive control (the genomic DNA of *E. coli*) and a negative control were included in the PCR. By electrophoresis in 1.5% agarose gel, the PCR products were resolved**.**

DNA Sequencing and Phylogenetic analysis

Using the Montage PCR Clean-up kit (Macro gen), a column-based technique was used to purify the PCR amplicon. Two primers were used to sequence the purified PCR products, as indicated in Table (1). The Big Dye terminator cycle sequencing kit was used for the sequencing process, and an automated DNA sequencing system, the Applied Biosystems model 3730XL (Applied Bio Systems, USA), was used to resolve the results. Gen Bank used the Basic Local Alignment Search Tool (BLAST) to perform identification. Using MEGA X, phylogenetic analysis was carried out **(Kumar *et al.*, 2016).**

Table (1). The primer sequences used for 16S rRNA gene amplification

Primers	Sequences
16S rRNA	27F 3′ AGA GTT TGA TCM TGG CTC AG 5′
	1492R 3′ TAC GGY TAC CTT GTT ACG ACT T 5′
Sequencing	518F 3′ CCA GCA GCC GCG GTA ATA CG 5′
	800R 3′ TAC CAG GGT ATC TAA TCC 5′

Plant growth promoting attributes

Production of Indole Acetic Acid (IAA)

Using the procedure outlined by **Zahroya *et al.* (2020)**, a qualitative analysis of indole-3-acetic acid (IAA) was conducted. After being grown on Luria-Bertani agar medium supplemented with L-tryptophan, the tested endophytic isolate was incubated at 28 °C. IAA production of was detected and checked after 48 hrs of inoculation by observing color changes in a filter paper which was saturated with 2 ml of Salkowski reagent (2% 0.5M $FeCl_3$ in 35% perchloric acid) and placed on the tested bacterial growth then left in darkness for one to two hours. A change from yellow to red color was recorded as IAA production.

Nitrogen fixing ability

Nitrogen fixing ability was examined using Jensen's medium and Bromothymol Blue (BTB) as a color indicator according to **Sulistiyani and Meliah (2017).** After 3 days of incubation the color of medium surrounded growth will change from greenish blue to dark blue.

Ammonia formation

The ability of the endophytic isolate to produce ammonia in peptone water was tested. Nessler's reagent (0.5 ml) was added after the freshly grown culture was inoculated into 10 ml peptone water in each tube and incubated for 48 hours at 30 °C. A positive test result for ammonia production was the development of a brown to yellow color **(Lata and Saxena, 2003)**.

Examination of phosphate solubilization

Using the PVK (Pikovskaya's agar plate) medium, the qualitative determination of phosphate solubilization was carried out **(Pohjanen *et al.*, 2014)**. The isolate was incubated at 28 ± 2 °C after being spot-inoculated. After three to seven days of incubation, the size of the halo corresponding to phosphate solubilization was measured. Phosphate solubilization was expressed as solubilizing efficiency (SE%) as following equation:

Phosphate solubilization efficiency = (Solubilization diameter (S) x 100) / (growth diameter).

Pot experiment

A pot experiment was done under shade net in the open field of Biofertilizer Production Unit (BPU), Agricultural Microbiology Res. Dept. (AMRD), Soils, Water and Environ. Res. Inst. (SWERI), ARC, Giza, Egypt during the 2021 season to assess the effectiveness of using identified endophytic *Methylobacterium* strain as a nitrogen fixer and plant growth promoter of *Jatropha curcas* plants as compared to the control, chemical fertilization and *Azospirillium brasilense* (a PGPR and nitrogen fixer for non-legumes). *A. brasilense* was obtained from BPU, AMRD, SWERI, ARC, Giza, Egypt. Seedlings of 50 to 60 cm height ,10-12 cm stem diameter, jatropha (*Jatropha curcas)* were planted on 27th February 2021 in 30 cm diameter plastic pots filled with 5 kg mixture of washed and sterilized sandy soil and cocopeat (1:1, v/v). The characteristics of the used cocopeat were: pH = 6.02, E.C. = 0.23 dSm^{-1}, bulk density = 0.07 gcm^{-3} and air field porosity = 33.2%, while for the used sandy soil were: pH = 7.43, E.C. = 0.41 dSm^{-1}, organic matter = 0.29% and total nitrogen = 0.011%.

Twelve treatments were set up in a randomized complete block design with three replicates by the following:

T1: Without any addition (untreated, Control).

T2: Recommended full dose of N fertilization (100% N).

T3: Recommended half dose of N fertilization (50% N).

T4: Inoculation with *Azospirillum* (Az. Inoc.) + foliar spraying with *M. radiotolerans* (Me. F.S.)

T5: Az. Inoc. + 50% N.

T6: Inoculation with *Methylobacterium* (Me. Inoc.) + Me. F.S.

T7: Me. Inoc. + 50% N.

T8: Az. Inoc. + Me. Inoc. + Me. F.S.

T9: Az. Inoc. + Me. Inoc. + 50% N.

T10: Az. Inoc. + Me. F.S.+ 50% N.

T11: Me. Inoc. + Me. F.S. + 50% N.

T12: Az. Inoc. + Me. Inoc. + Me. F.S. + 50% N.

These seedlings were inoculated twice, after seven days from planting and then after fourteen days from planting by adding 10 ml (containing 3 x 10^9 cells ml^{-1}) of broth cultures of *Azospirillium brasilense* and *M. radiotolerans* strain H-0A1 separately or mixed for each pot. Foliar spraying with *M. radiotolerans* strain H-0A1 at the rate of 5.0 L fed^{-1} was applied after 45 days from planting.

After 4 weeks from planting, nitrogen fertilization treatments were applied to the soil as ammonium sulphate (20.5 % N) at the rates of 5.0 and 10.0 g pot^{-1} (for half and full recommended doses, respectively). To supply the growing medium with both P and K fertilizers, calcium super phosphate (15.5 % P_2O_5) was added before planting by the rate of 12.0 g pot^{-1}, while potassium sulphate fertilizer (48 % K_2O) was supplemented at the rate of 5.0 g pot^{-1} after two weeks from planting.

Growth parameters determinations

After 180 days of planting, samples were collected to ascertain plant height (cm), number of leaves, leaf area (cm^2), leaves dry weight (g), stem diameter (mm), stem dry weight (g), root length (cm) and roots dry weight (g). To calculate leaf area (cm^2), fresh leaf samples were gathered, digitally scanned at 300 dpi, and the leaf area was computed using ImageJ software in accordance with the instructions of **Ferreira and Rasband (2012)**.

Plant chemical determinations

Photosynthetic pigments content

According to **Wellburn and Lichtenthaler (1984)**, the pigment content (mg/g f.w.) of fresh leaf samples was measured as well as the levels of chlorophylls a, b, and carotenoids.

The percentage of total carbohydrates

Total carbohydrates percentage was calculated in dry leaf samples using the **Herbert *et al.* (1971)** method.

Nitrogen Percentage (N %)

Nitrogen (N %) was evaluated using the modified Kjeldahl technique, which was established by **Cottenie *et al.* (1982).**

Phosphorus (P %) and potassium (K %) Percentage

Phosphorus (P %) and were determined in the dry leaves as mentioned by **Chapman and Pratt (1961).**

Statistical analysis

The MSTAT Computer Program **(MSTAT Development Team, 1989)** was used to statistically analyze the data obtained by applying the analysis of variance (ANOVA) test for a complete randomized block design module **(Gomez and Gomez, 1984)**. Duncan's multiple range test was used to compare the mean differences **(Duncan, 1955)**.

RESULTS AND DISCUSSION

The bacterial isolate's appearance

One shaped endophytic Gram-negative, rod-bacterial isolate recovered from leaf tissue of jatropha plants. When cultivated on MMS agar medium, pink pigmented colonies were appeared.

Molecular characterization of the isolate by 16S rRNA gene

Molecular characterization of the isolate *Methylobacterium radiotolerans* strain GU294321. was achieved using the 16S rRNA gene. The amplified size designed for the 16S rRNA gene, about 1500 bp, was used (Table 1). The PCR products were separated in gel, and the DNA bands conforming to the expected size of the gene were purified from the gel for sequencing. With the use of the forward and reverse primers, the product was sequenced, resulting in a 1404 bp long sequence. This isolate's partial sequence was aligned with the partial sequences of the neighbor-joining sequences in Gen Bank and then deposited under accession number OR185547.1 and described by the phylogentic tree as a novel strain (strain H-0A1) of *Methylobacterium radiotolerans* as demonestrated in Fig. (1).

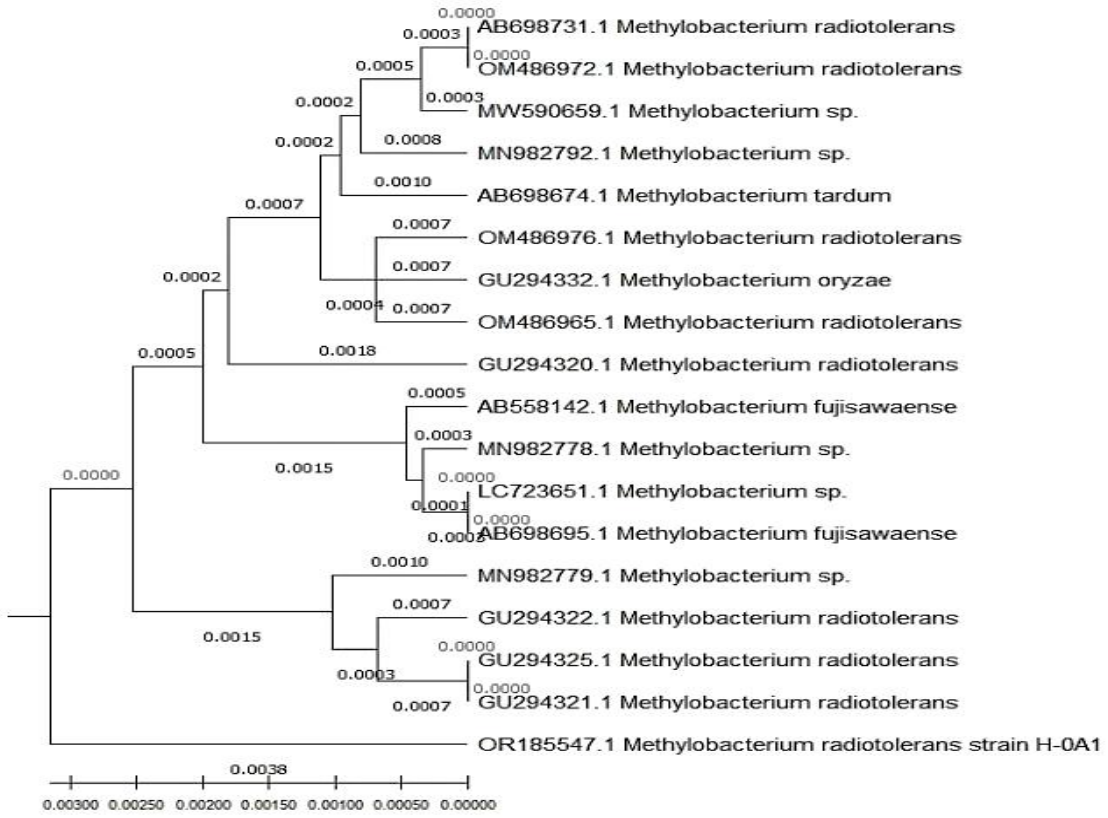

Fig. (1). Phylogenetic tree based on 16S rDNA sequence analysis indicates the phylogenetic relationship of the bacterial isolate.

Assessment of plant-growth-promoting activities *in vitro*

Bacterial strain of *Methylobacterium radiotolerans* strain H-0A1 was examined for its attributes that promote plant growth regarding the synthesis of indole-3-acetic acid (IAA), nitrogen fixation, ammonia production and phosphate (P) solubilization (Fig. 2). The results indicated that the strain H-0A1 was able to generate indole acetic acid within the culture medium (Fig. 2b).

This finding is consistent with studies by **Ivanova *et al.* (2001)** and **Madhaiyan *et al.* (2006)**, which demonstrated the ability of *Methylobacterium* to produce IAA. This implies that *methylobacterium* can be inoculated to increase IAA accumulation in plants, which can stimulate plant growth and development. Also, **Priya *et al.* (2019)** confirmed that, *Methylobacterium radiotolerans* is capable of producing IAA, which increases the IAA concentrations in plants and promotes their growth.

Jensen's medium, which is nitrogen-free, was created to find and grow bacteria that fix nitrogen. The *in vitro* test on Jensen's nitrogen free media showed that the new bacterial strain could grow on Jensen's medium (Fig. 2c). This agrees with **Zhang *et al*. (2021)** who revealed that, *Methylobacterium* sp. 4-46 and L2-4, both of whom are strongly related to *M. radiotolerans*, are involved in nitrogen fixation.

The ability of Methylobacteria to produce ammonia is another significant characteristic that may have an indirect impact on plant growth. It helps in fulfilling the requirement of nitrogen for the

plants. Ammonia production is probably acting as nitrogen source. As shown in (Fig. 2d) the results revealed that the *M. radiotolerans* strain H-0A1 was able to grow on peptone water and produce ammonia. In this respect, **Marques *et al.* (2010)** found that, one of the key characteristics associated with promoting plant growth is the production of ammonia by PGPR. It has generally been demonstrated to provide nitrogen to their host plants, which in turn encourages the elongation of their roots and shoots as well as their biomass.

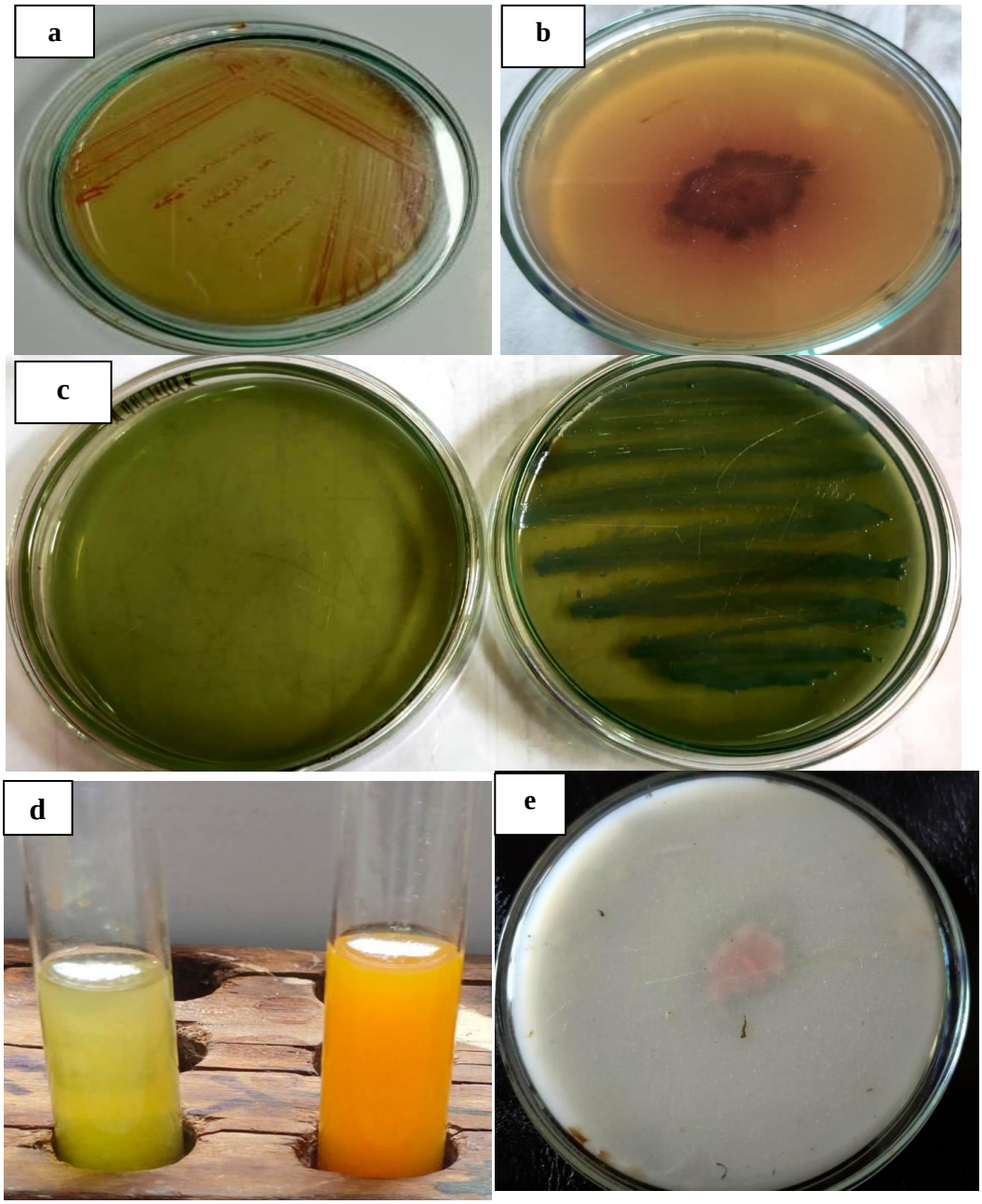

Fig. (2). Assessment of plant-growth-promoting activities *in vitro* by *Methylobacterium radiotolerans* strain H-0A1.

(a) Pink colonies of isolate grown on MMS agar medium, (b) IAA production,

(c) Growth on Jensen's medium, (d) Ammonia formation, (e) Phosphate solubilization

Despite the high total phosphorus concentrations found in soils, it cannot be utilized by plants because the majority of inorganic phosphorus is bonded to calcium, iron, and aluminum, and cannot be utilized by plants **(Peyraud *et al.*, 2011)**. **Lim *et al.* (2007)** stated that, phosphate-solubilizing bacteria are required to solubilize applied phosphates and available phosphorus in soil in order to optimize crop yield. It is well known that microorganisms are capable of dissolving phosphate, increasing plant accessibility and decreasing the need for phosphate fertilizers **(Lavakusha *et al.*, 2014)**. In this investigation, the *M. radiotolerans* strain H-0A1 demonstrated a halo zone on the Pikovskaya agar medium with a solubilization efficiency of 100%, demonstrating that it is able to dissolve tricalcium phosphate **(Fig. 2e**). This agree with **Rodríguez *et al*. (2006)** who reported that, the capacity of *Methylobacterium* species to dissolve inorganic phosphates facilitates phosphate metabolism in microbes and plants.

Impact of bio-fertilization with *Methylobacterium radiotolerans* strain H-0A1 on vegetative plant growth of *Jatropha curcas* plants

1. Plant morphological traits

The impact of inoculation with new bacterial strain on morphological traits of *Jatropha curcas* seedlings have been condensed into Table (2). It is cleared from the data presented that, the combined inoculation of *Azospirillium brasilense* + *Methylobacterium radiotolerans* strain H-0A1 and foliar spraying with the same strain with presence of half dose of nitrogen fertilization significantly accelerated plant growth when compared to the other treatments. This combined treatment (T12) recorded maximum values of plant height (93.00 cm), number of leaves (68.00 plant $^{-1}$), leaf area (218.65 cm^2), leaves dry weight (42.97 g), stem diameter (22.37 mm), root length (84.67 cm) and root dry weight (43.66g). Whereas, (T11) resulted in the greatest amount of stem dry weight (106.875 g). It could be also observed that separately inoculation of *Methylobacterium radiotolerans* strain H-0A1 and foliar spraying with the same strain in the presence of half dose of nitrogen fertilization. (T11) shared the previous treatment (T12) in its effect on plant height, stem diameter and stem dry weight without significant differences between them.

Results agree with **Madhaiyan *et al.* (2010)** who suggested that, methylotrophs were found to improve plant growth as measured by longer shoots or roots when they were applied as seed inoculants, foliar sprays, or in combination with a nitrogen-fixing bacterium (*Azospirillum brasilense* CW903) and demonstrated that, *M. oryzae* can also be used as a single inoculant or as a co-inoculant with other rhizobacteria to boost plant growth, productivity, and yields in a sustainable agriculture system. Several previous studies shown that IAA mainly improves the size, distribution, and quantity of root hairs, which increases the ability to absorb nutrients from soil as indicated by **Mohite (2013).**

These findings may be due to the role that methylotrophs play in the acquisition of phosphorus, nitrogen fixation, production of phytohormones, and promotion of plant growth, as reported by **Kumar *et al.* (2016)**, where, Indole-3-acetic acid (IAA) is the primary auxin in plants and is crucial for root development as illusrtated by **Kousar *et al.* (2020)**. This confirmed by **Chanratana, *et al.* (2018)** who stated that, several species of *Methylobacterium* genus members, such as *M. oryzae*, *M. nodulans*, *M. radiotolerans*, and certain unclassified species, greatly enhance plant growth.

Table (2). Effect of inoculation and/or foliar spraying with *Methylobacterium radiotolerans* strain H-0A1 on morphological traits of *Jatropha curcas* as compared to inoculation with *Azospirillum* and chemical N- fertilization

Treatments	Plant height (cm)	Leaves		
		Number	Area (cm^2)	Dry weight (g)
T1: Control	68.67 ^{f}	16.00 ^{j}	102.28 ^{h}	7.38 ^{f}
T2: 100% N	83.00 ^{c}	40.67 ^{e}	170.48 ^{f}	21.16 ^{e}
T3: 50% N	77.67 ^{d}	39.33 ^{f}	168.92 ^{f}	21.50 ^{e}
T4: Az. Inoc. + Me. F.S.	70.33 ^{f}	18.33 ^{i}	160.93 ^{g}	6.82 ^{f}
T5: Az. Inoc. + 50% N	83.33 ^{c}	41.33 ^{e}	177.81 ^{e}	26.01 ^{d}
T6: Me. Inoc. + Me. F.S.	74.00 ^{e}	25.33 ^{h}	168.31 ^{f}	6.76 ^{f}
T7: Me. Inoc. + 50% N	87.00 ^{b}	41.67 ^{e}	184.45 ^{d}	25.63 ^{d}
T8: Az. Inoc. + Me. Inoc. + Me. F.S.	74.33 ^{e}	37.67 ^{g}	168.43 ^{f}	21.86 ^{e}
T9: Az. Inoc. + Me. Inoc. + 50% N	88.67 ^{b}	53.67 ^{c}	198.36 ^{c}	28.51 ^{c}
T10: Az. Inoc. + Me. F.S.+ 50% N	87.67 ^{b}	46.33 ^{d}	198.32 ^{c}	29.70 ^{c}
T11: Me. Inoc. + Me. F.S. + 50% N	91.33 ^{a}	55.67 ^{b}	209.74 ^{b}	38.87 ^{b}
T12: Az. Inoc. + Me. Inoc. + Me. F.S. + 50% N	93.00 ^{a}	68.00 ^{a}	218.65 ^{a}	42.97 ^{a}
	Stems		**Roots**	
	Diameter (mm)	**Dry weight (g)**	**Length (cm)**	**Dry weight (g)**
T1: Control	18.17 ^{g}	70.40 ^{f}	26.00 ^{h}	17.31 ^{i}
T2: 100% N	21.00 ^{de}	91.84 ^{c}	34.00 ^{e}	30.03 ^{e}
T3: 50% N	21.00 ^{de}	88.24 ^{d}	33.67 ^{e}	28.53 ^{f}
T4: Az. Inoc. + Me. F.S.	20.27 ^{f}	85.22 ^{e}	27.00 ^{gh}	23.43 ^{h}
T5: Az. Inoc. + 50% N	21.32 ^{cd}	91.53 ^{c}	34.33 ^{e}	30.94 ^{e}
T6: Me. Inoc. + Me. F.S.	20.27 ^{f}	84.55 ^{e}	27.33 ^{g}	24.64 ^{g}
T7: Me. Inoc. + 50% N	21.47 $^{b-d}$	90.65 ^{c}	37.67 ^{d}	32.75 ^{d}
T8: Az. Inoc. + Me. Inoc. + Me. F.S.	20.70 ^{ef}	86.58 ^{de}	29.00 ^{f}	28.32 ^{f}
T9: Az. Inoc. + Me. Inoc. + 50% N	21.90 ^{ab}	104.56 ^{a}	41.33 ^{c}	36.00 ^{c}
T10: Az. Inoc. + Me. F.S.+ 50% N	21.77 ^{bc}	94.64 ^{b}	37.67 ^{d}	35.71 ^{c}
T11: Me. Inoc. + Me. F.S. + 50% N	22.36 ^{a}	106.87 ^{a}	68.00 ^{b}	38.72 ^{b}
T12: Az. Inoc. + Me. Inoc. + Me. F.S. + 50% N	22.37 ^{a}	105.55 ^{a}	84.67 ^{a}	43.66 ^{a}

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5% confidence level.

2. Photosynthetic pigments

An essential pigment for photosynthetic processes, chlorophyll (Chl) plays a major role in a plant's ability to photosynthesize and, consequently, in its ability to grow. Results in Fig. (3) showed that, maximum content of Chl.a (2.985 mg/g f.w.), Chl.b (1.588 mg/g f.w.) and Carotenoid (0.368 mg/g f.w.) in plants was acquired using the combined inoculation of *Azospirillium brasilense* + *Methylobacterium radiotolerans* strain H-0A1 and foliar spraying with the same strain in the presence of 50 % N-fertilizer (T12) followed by separately inoculation *with A. brasilense or M. radiotolerans* strain H-0A1 + foliar spraying+50 % N-fertilizer. Same trend was observed by treating with (T11) which shared the previous treatment (T12) in its effect on Chl.b and carotenoid values without significant difference between them.

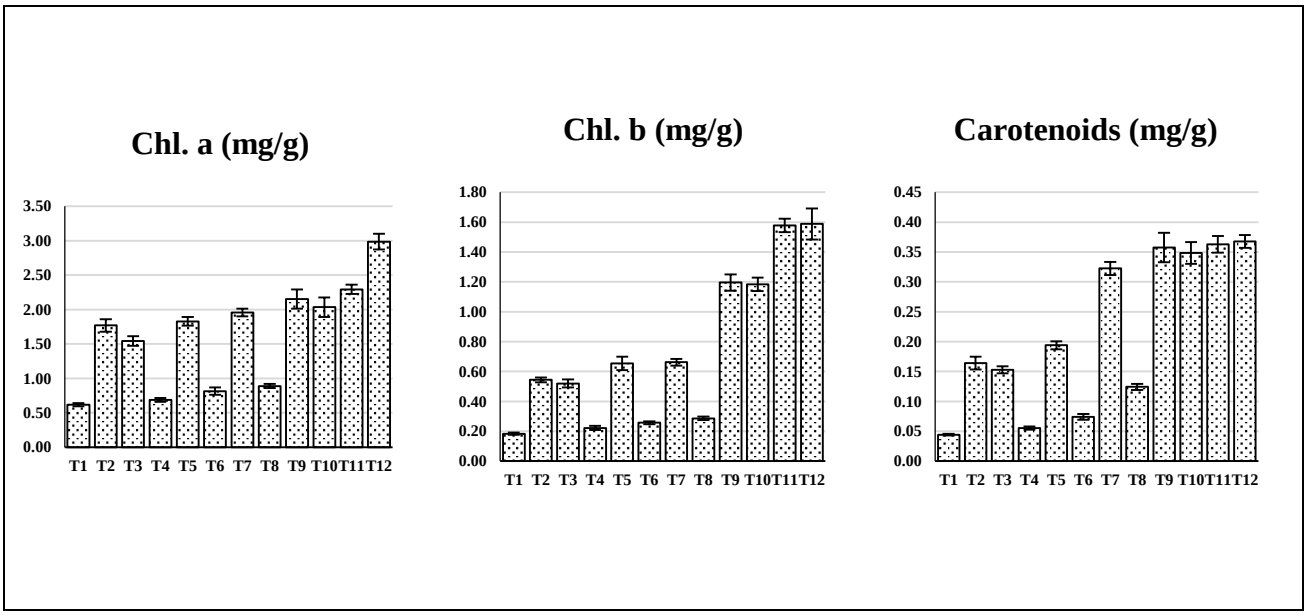

Fig. (3). Effect of inoculation and/or foliar spraying with *Methylobacterium radiotolerans* strain H-0A1 on *Jatropha curcas* leaf pigments as compared to inoculation with *Azospirillum* and chemical N-fertilization.

In this respect, **Chanratana *et al.* (2018)** confirmed that the *Methylobacterium* sp. improved the cytokinin levels in plants, enhanced photosynthetic ability, and promoted plant growth. Moreover, **Mehrvarz *et al*. (2008)** demonstrated that, phosphate solubilizing bacteria inoculation increased the chlorophyll content and photosynthesis rates of barely plants.

3. Nutrients uptake and carbohydrates

Results in Fig. (4) revealed that the combined inoculation of *A. brasilense* + *M. radiotolerans* inoculation and applying with *Methylobacterium* foliar spraying (T12) caused significant increase in the content of carbohydrates (12.9 %) and emphasized the superiority among other treatments in the concentration percentage (%) of N (2.80%), P (0.31%) and K (1.8%) followed by (T11) separately inoculation of *M. radiotolerans* strain H-0A1 +foliar spraying+ 50% N-fertilization. This could be due to the ability of new strain *M. radiotolerans* H-0A1 for nitrogen fixation, phosphate solubilization and indole production.

Nalayani *et al.* (2014) demonstrated that PPFMs can be used as a powerful bioinoculant to boost cotton plant yield by applying as foliar spray or soil inoculation with various strains of *Bacillus*, *Pseudomonas*, and *Azospirillum* with recommended N and P fertilizers. *Bradyrhizobium japonicum* strain SB120 in conjunction with other methylotrophs, such as *Methylobacterium* isolates, shown a positive effect on nutrient uptake, plant development parameters, and soybean (*Glycine max* (L.) *Merrill*) yields **(Madhaiyan *et al.*, 2009)**. Endophytic *Methylobacterium* can be used in the construction of complex biofertilizers as described by **(Ardanov *et al.*, 2013)**.

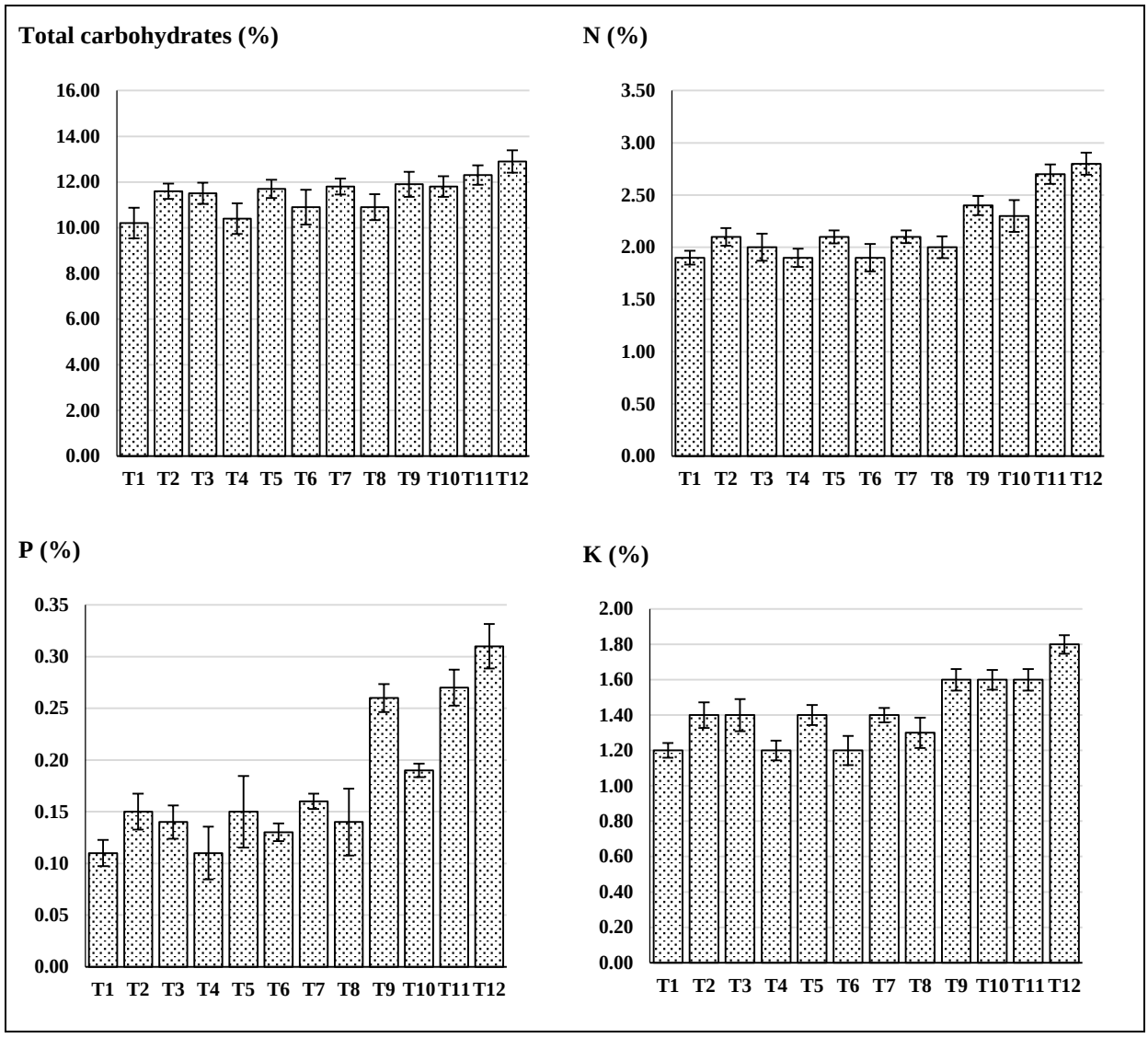

Fig. (4). Effect of inoculation and/or foliar spraying with *Methylobacterium radiotolerans* strain H-0A1 on nutrients uptake and carbohydrates of *Jatropha curcas* leaves as compared to inoculation with *Azospirillum* and chemical N-fertilization.

CONCLUSION

It can be concluded that the application of endophytic *Methylobacterium radiotolerans* strain H-0A1 as bioinoculant and in microbial sprays to Jatropha plants stimulates plant growth by production of phytohormone, nitrogen fixation, ammonia formation as well as phosphate solubilization that is why it a viable and promising option for application in sustainable agriculture., and could be utilized as a substitute for synthetic fertilizers.

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