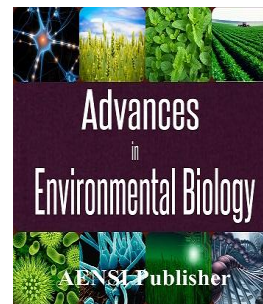




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## Effects of Foliar Application of *Methylobacterium* and Methanol on Growth and Yield of Peanut (*Arachis hypogaea* L. cv.NC2)

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### ABSTRACT

Other than rhizobacteria, some phyllosphere bacterial strains such as *Methylobacterium* spp. alone or along with methanol have been reported to increase plant growth and nutrient uptake of grasses, cereals and legumes. Accordingly, the present study investigated the effects of foliar application of *Methylobacterium* spp. and methanol on growth and yield of peanut under field conditions, in 2010 and 2011. Results showed that application of 1012 CFU/ml of *Methylobacterium* spp. or 20% methanol as foliar spray significantly increased main stem height, leaf area index, number of mature and immature pod per plant, harvest index, leading to an increase of pod and seed yield of peanut over control. Peanut leaf area index (LAI) also affected by *Methylobacterium* and methanol spraying, so that the highest LAI obtained 105 days after planting in 1012 CFU/ml of *Methylobacterium* and 20% methanol. Foliar application of 1012 CFU/ml of *Methylobacterium* middlingly led to a seed yield increase of 12.25% over control in both years.

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### INTRODUCTION

Methylotrophy is defined as the ability to grow at the expense of reduced carbon compounds containing one or more carbon atoms but containing no carbon-carbon bonds [10]. Bacteria of the genus *Methylobacterium* (PPFMs, pink-pigmented facultative methylotrophic bacteria) (fig. 1) are strict aerobic, Gram-negative rods, able to grow on C1 compounds [17 and 47]. They are classified under  $\alpha$ -Proteobacteria and distributed in diverse angiosperms, gymnosperms, and even lower plants [3, 5, 11, 12 and 18]. In recent decades, there has been increasing evidence that besides N<sub>2</sub>-fixation and increased nutrient uptake, the synthesis and export of phytohormones by plant-associated microorganisms may play an important role in plant growth promotion. Phytohormones, believed to assimilate partitioning patterns in plants and affect growth patterns in roots, are also called plant growth regulators because of their regulatory role in plant growth development. There is also evidence that the growth hormones produced by bacteria can in some instances increase growth rates and improve the yield of host plants [4 and 7]. PPFMs influence seed germination and seedling growth by producing plant growth regulators, cytokinins, and auxins [4, 13, 19, 20, 21, 22 and 25] and altering agronomic traits like branching, vigor, rooting, and heat/cold tolerance [15 and 19]. They indirectly reduce or prevent the deleterious effects of pathogenic microorganisms, through induced systemic resistance [32], and their inoculation was found to increase the photosynthetic activity by enhancing the number of stomata, chlorophyll concentration and malic acid content of crops [9]. Also methanol, one of the simplest organic molecules, is a natural product of plant metabolism, and it has now come to light that most plants also emit it from their leaves. All plant tissues emit methanol [14, 16, 23, 29, 36, 38 and 40], especially during early stages of leaf expansion [24]. Nonomura and Benson [37] reported that foliar applied methanol increased the growth and yield of C3 crops. Positive growth

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effects of methanol have been reported earlier in a variety of C3 plants like wheat, barley [1 and 37], mungbean [6], tomato [42] and cotton [33].

## MATERIALS AND METHODS

We employed the genus *Methylobacterium* spp. that is composed of a variety of pink-pigmented facultative methylotrophic (PPFM) bacteria, isolated from the phyllosphere of peanut. Isolation and cultivation of *Methylobacterium* strains was carried out on the basis of their growth and biochemical characteristics that were described by Green [17]. These bacteria were grown on methanol mineral salts (MMS) medium supplemented with 0.2% (v/v) methanol as a sole C source and incubated at 28°C [17]. After 7 days Bacterial cells harvested [17] and were used for colonizing the plant. Four concentrations of bacterial colonies (0, 106, 108 and 1012 CFU/ml) were made by using dilution series and spectrophotometer at 600 nm [8]. Also, four aqueous methanol (0, 10, 20 and 30% (v/v)) were used along with *Methylobacterium* for spraying on plants. In 2010 and 2011, a field experiment was conducted in kyashahr port (37°, 26' N and 49°, 57' E), Guilan, north of Iran, to study the effects of foliar application of *Methylobacterium* spp. and methanol on growth and yield of peanut (*Arachis hypogaea* L. cv.NC2). The study carried out as a factorial experiment in a randomized complete block design with 3 replications. Plots were 12m<sup>2</sup> with seven rows spaced at 40 cm×40 cm (square planting pattern). Soil of experimental field had the following properties: pH 7.7, EC 0.3 dS.m<sup>-1</sup>, soil total nitrogen 0.06%, available P<sub>2</sub>O<sub>5</sub> 3.1 mg.kg<sup>-1</sup>, available K<sub>2</sub>O 140 mg.kg<sup>-1</sup> and available Ca 3.2 meq.l<sup>-1</sup>, Zn 2.6 ppm, Fe 31 ppm. The crops were fertilized at sowing with the recommended level of fertilizers (60 kg.ha<sup>-1</sup> urea, 100 kg.ha<sup>-1</sup> triple superphosphate, 30 kg.ha<sup>-1</sup> potassium sulfate and 400 kg.ha<sup>-1</sup> gypsum). Peanut seeds obtained from local farmers and were planted as a rainfed crop. Foliar application of methanol (v/v) and *Methylobacterium* (CFU/ml) were made with a hand-operated natural gas pressurized sprayer between 16:00 and 19:00 pm at the beginning of peanut pod and seed growth stages, in both years. Biometric observations, which included main stem height and yield components, included number of mature and immature pod per plant, harvest index and finally peanut pod and seed yield were made at harvesting stage. The leaf area index (LAI) was measured using a Sun Scan Canopy Analysis System (Delta-T Devices, UK) at noon from 30 days after planting with 15 intervals [28 and 39]. The data were subjected to statistical analysis and significant difference was calculated at  $P \leq 0.05$  using SAS, Version 9.1 [43].

## RESULT AND DISCUSSION

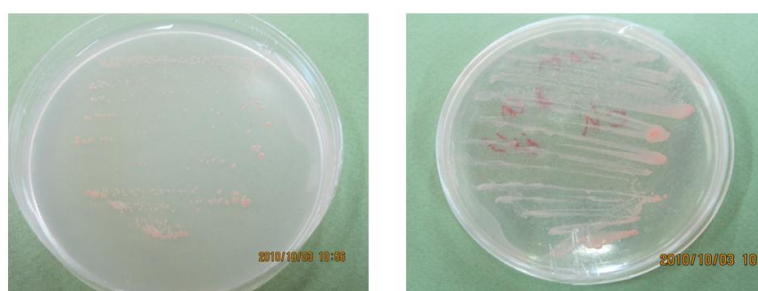
Under field conditions, foliar application of *Methylobacterium* and methanol had significant effect on the main stem height, yield and yield components of peanut in 2010 and 2011. *Methylobacterium* and methanol had positive influence on the main stem height in both years, so that we recorded the highest main stem height in 1012 CFU/ml of *Methylobacterium* and 20% v/v methanol followed by 108 CFU/ml of *Methylobacterium* and 30% v/v methanol (tables 1 and 2). In the present study, increasing the main stem height may due to the coordination between auxin and cytokinins allow a balance of growth in the shoots and root system, when roots become more extensive by the action of auxins, then the cytokinins of the plant signals the shoot system to form more branches. The fact that methylotrophic bacteria promote seed germination and the growth of seedlings suggests that these bacteria may synthesize not only cytokinins but also other phytohormones for instance auxins [27].

Also, in our study, yield and yield components affected by foliar application of *Methylobacterium* and methanol in both years (tables 1 and 2). The yield enhancement through foliar *Methylobacterium* and methanol applications was associated with increased number of mature pod per plant, harvest index and reduced number of immature pod per plant. The yield parameters such as number of mature and immature pods per plant, harvest index, pod and seed yield were recorded highest in 1012 CFU/ml of *Methylobacterium* and 20% v/v methanol followed by 108 CFU/ml of *Methylobacterium* and 30% v/v methanol. Under field experiment, the application of PPFMs as a foliar spray increased the growth and yield attributes in peanut which are equivalent to the effect of 20% foliar applied methanol (tables 1 and 2).

The increase in the yield and yield parameters is because of the several factors such as release of growth promoting substances like IAA and GA, proliferation of beneficial organisms in the phyllosphere, control of plant pathogens in addition to growth promotion. The response might be due to of beneficial of microorganisms [26]. Similarly, the production of the plant growth regulators like auxins, particularly indole-3-acetic acid (IAA) and indole-3-pyruvic acid [22], zeatin, zeatin riboside and reacted cytokinins by *Methylotrophs* [21] has been reported as the factors that enhances plant growth of crops, the increase in the vegetative growth of the plant attributed to the increase in the yield of a crop. This results are similar with the various literatures on the beneficial aspects of pink pigmented *Methylobacterium* sp. as a potent biofertilizer for increasing crop production in soybean [27], maize, blackgram, groundnut, sugarcane, rice, cotton, sunhemp [30], tomato [2 and 46]. Similarly, Suresh Reddy *et al.* [44] worked on the effect of combined inoculation of PPFMs and *Rhizobium* on groundnut cultivar Co(Gn)4 and observed that there was significantly increase in plant growth, biomass

production and yield parameters of groundnut. Munsanje [35] demonstrated that foliar applications of PPFMs during seed set in soybean resulted in higher yields in field trials. In sugarcane application of PPFMs, increased the cane yield and the sugar quality [31]. Nonomura and Benson [37] reported that sprays of aqueous 10–50% methanol increased plant height and growth by 50% in various C3 crops and inferred that crop water use declined due to accelerated phenology, which led to fewer irrigation events. Since then, similar positive effects on the water use efficiency and water relations and growth have been reported in crops like tomato [42] and cotton [33]. No significant effects of foliar sprays of methanol alone or in combination with nutrients could be observed under greenhouse conditions in roses, tomato and cucumber. So applications of methanol to plants under greenhouse conditions could be of interest only under sunny weather conditions [34]. No effects could be found on the CO<sub>2</sub> compensation point of field grown cotton when applied with foliar methanol, indicating common rates of photorespiration [48].

Leaf area index (LAI) in peanut affected by *Methylobacterium* and methanol spraying in both years. Trend analysis of leaf area index showed an increasing during leaf area measurements until 105 DAP. While after that, the leaf area trend was descending (Figs. 2 and 3). The highest LAI obtained at 105 DAP by spraying 10<sup>12</sup> CFU/ml of *Methylobacterium* (Figs. 2A and 3A) and 20% v/v methanol (Figs. 2B and 3B) that had perceptible differ compared with control in both years. Because increasing of methanol can influence of methylotrophs bacteria on the phyllosphere and these bacterial accumulation is caused plant regulators production like auxins that led to develop of leaf area [49 and 50]. Taiz and Zeiger [45] reported that production of auxins can effect positively on leaf cells wall to become softness, so that leaf area be expanded. It is noticed that in all of measured attributes, we recorded the higher data in first year than the second year, so that it was significantly superior over the second year because of suitable of weather conditions in 2010. The suitable conditions like temperature and sunlight duration (Data not shown) led to better influence of *Methylobacterium* and methanol on the peanut metabolism, growth and yield.



**Fig. 1:** The genus of *Methylobacterium* spp. isolated from peanut leaves.

**Table 1:** Effect of foliar application of *Methylobacterium* and metanol the main stem height, yield and yield attributes of rainfed peanut under field conditions in 2010.

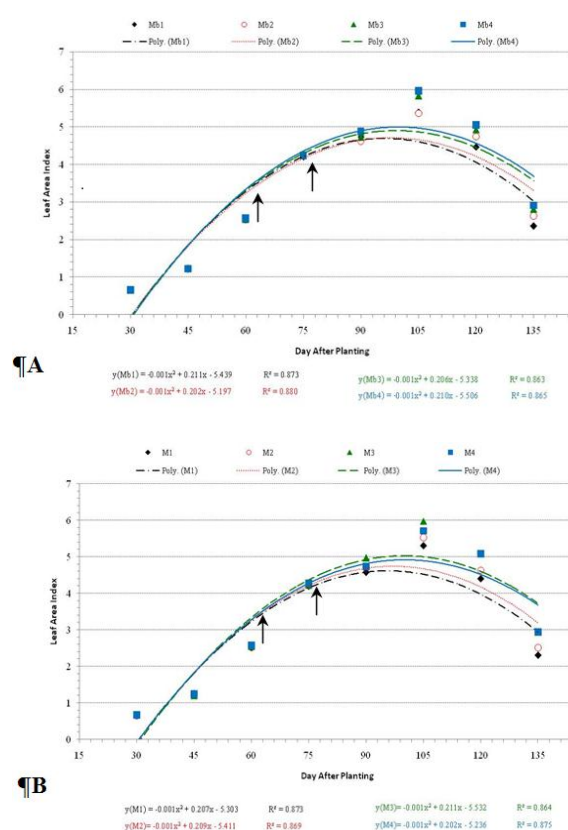
	Main stem height (cm)	Mature pod number per plant	Immature pod number per plant	Harvest index (%)	Pod yield (kg.ha <sup>-1</sup> )	Seed yield (kg.ha <sup>-1</sup> )
<i>Methylobacterium</i> concentration (CFU/ml)						
0	52.25 b	33.41 b	21.25 a	44.15 a	4936.8 b	3477.2 b
10 <sup>6</sup>	54 b	33 b	21.5 a	44.08 a	5016.5 b	3528.9 b
10 <sup>8</sup>	53.75 b	35.33 b	19.75 ab	44.4 a	5180 ab	3724.4 ab
10 <sup>12</sup>	58.25 a	40.58 a	16.75 b	44.45 a	5421.3 a	3969 a
Methanol rates (% v/v)						
0	50.75 c	32.33 c	22 a	42.78 c	4550.8 b	3170.1 b
10	54 b	34.75 bc	21 a	43.12 bc	4753 b	3403.2 b
20	55.75 ab	39.08 a	17 b	45.65 a	5755 a	4194.8 a
30	57.75 a	36.16 b	19.25 ab	45.52 ab	5495.8 a	3931.4 a
H.S.D ( $P \leq 0.05$ )	±3.22	±2.89	±3.06	±2.52	±330.91	±396.96

Each value represents mean of three replicates per treatment. In the same column, significant differences at  $P \leq 0.05$  levels are indicated by different superscript letters (a–c). Data followed by same superscript letter in the same column are not significantly different from each other. Foliar application of *Methylobacterium* and methanol were made at 63 and 77 day after planting (DAP) or beginning of peanut pod and seed growth stages.

Each value represents mean of three replicates per treatment. In the same column, significant differences at  $P \leq 0.05$  levels are indicated by different superscript letters (a–c). Data followed by same superscript letter in the same column are not significantly different from each other. Foliar application of *Methylobacterium* and methanol were made at 56 and 70 day after planting (DAP) or beginning of peanut pod and seed growth stages.

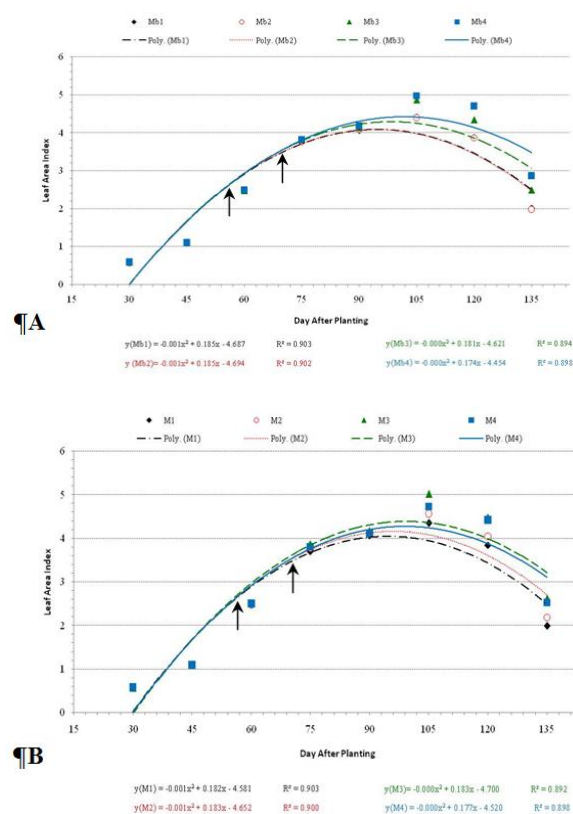
**Table 2:** Effect of foliar application of Methylobacterium and metanol the main stem height, yield and yield attributes of rainfed peanut under field conditions in 2011.

	Main stem height (cm)	Mature pod number per plant	Immature pod number per plant	Harvest index (%)	Pod yield (kg.ha <sup>-1</sup> )		Seed yield (kg.ha <sup>-1</sup> )
<i>Methylobacterium</i> concentration (CFU/ml)							
0		51.5 b	29.66 b	25.5 a	43.02 a	4457.8 b	3080.8 b
10 <sup>6</sup>		52.25 b	30.41 b	26 a	42.85 a	4518.8 ab	3140.5 b
10 <sup>8</sup>		52.5 b	32.08 b	24.5 a	42.95 a	4625.5 ab	3284 ab
10 <sup>12</sup>		57.75 a	36.25 a	19.75 b	42.85 a	4816.3 a	3504.8 a
Methanol rates (% v/v)							
0		50 c	29.83 b	27 a	41.27 b	4036 b	2776.3 b
10		52.5 bc	31.66 ab	24.25 ab	41.65 b	4250 b	2967.4 b
20		55 ab	34.75 a	20.5 b	44.6 a	5206.3 a	3766.3 a
30		56.5 a	32.16 ab	24 ab	44.15 ab	4926 a	3500.1 a
H.S.D ( $P \leq 0.05$ )		±3.49	±3.21	±3.93	±2.94	±317.9	±331.71

**Fig. 2:** Effect of foliar application of Methylobacterium (A) methanol (B) on peanut LAI in 2010 Foliar application of Methylobacterium and methanol were made at 63 and 77 day after planting (DAP) or beginning of peanut pod and seed growth stages (arrows).

Mb1, Mb2, Mb3 and Mb4 represent Methylobacterium population at 0, 10<sup>6</sup>, 10<sup>8</sup> and 10<sup>12</sup> CFU/ml respectively.

M1, M2, M3 and M4 represent methanol rates at 0, 10, 20 and 30% v/v respectively.



**Fig. 3:** Effect of foliar application of Methylobacterium (A) methanol (B) on peanut LAI in 2011 Foliar application of Methylobacterium and methanol were made at 56 and 70 day after planting (DAP) or beginning of peanut pod and seed growth stages (arrows).

Mb1, Mb2, Mb3 and Mb4 represent Methylobacterium population at  $0$ ,  $10^6$ ,  $10^8$  and  $10^{12}$  CFU/ml respectively.

M1, M2, M3 and M4 represent methanol rates at 0, 10, 20 and 30% v/v respectively.

#### Conclusion:

According to the results of the present experiment, it could be demonstrated that foliar application of *Methylobacterium* and methanol may considerably increase peanut grain yield and its attributes. So it could be expected that spraying of  $10^{12}$  CFU/ml of *Methylobacterium* or 20% methanol may result better physiological and qualitative characteristics, higher pod and seed yield of peanut. The ability of methylobacteria to stimulate the growth and yield of plants indicates their promise in experimental physiology and biotechnology. This non-infecting, plant-associated bacterium has attracted increased attention for its plant growth stimulation and environmental friendly plant protection.

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