57
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PRELIMINARY STUDIES ON THE EFFECT OF FOLIAR APPLICATION OF METHANOL AND PINK PIGMENTED FACULTATIVE METHELOTROPHIC BACTERIA ON COTTON PLANT

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ABSTRACT

A pot experiment was carried out in summer season of 2006 to evaluate the different concentrations effect of foliar application of methanol and pink pigmented facultative methelotrophic bacteria (PPFM) on some growth parameters and yield of cotton plant. Data showed that, the highest growth rate value was obtained with PPFM isolates from wheat with 1% methanol. The growth rate of PPFM isolates decreased with increasing the methanol concentration. Foliar applications with 20% methanol with PPFM 3 or 4 spraying times gave the significantly highest values of cotton growth and yield parameters. Such two potent treatments increased leaf area index (LAI) by 51.4 and 55.8 %; number of fruiting branches / plant by 53.1 and 58.0 %; number of total bolls / plant by 38.1 and 43.0 %; seed cotton weight / boll by 37.1 and 48.2 % and seed cotton yield / plant by 46.1 and 50.8, respectively than the control.

INTRODUCTION

There is a great concern that the present knowledge, resources and technologies will not adequate to meet the demands. Once there are 8 billion people on this planet by about 2020, challenges needs of a growing population must depend on a sustainable new ways. This requires a better and more comprehensive insight into ecologically sound group production processes, especially in fragile environments and resources-poor countries. All crop production practices and principles hinge around the fact that the yield of agricultural crops ultimately depends on the ability of plants to carry on photosynthesis. Meanwhile, photosynthesis is dependent on carbon dioxide in atmosphere. The, CO_2 constitutes about 0.03% or 300 ppm of the atmosphere. Unfortunately under certain environmental conditions especially in worm, high solar radiation and quiet air conditions, CO_2 levels in the air may limit photosynthesis and yield. Hence, it is a well known fact that if CO_2 concentrations were increased, many plants would photosynthesize at higher rate.

Foliar application of methanol was used as a precursor of CO_2 on plant in many countries to enhance yield. Moreover, methanol foliar application was recommended to the farmers for crop production in USA **(Arizona Department of Agriculture, 1993)**. Meanwhile, many microbes live on phylloplane and feed on materials leached from the leaf. Phylloplane bacteria produce B-vitamins, auxins and cytokinens among other products. The term methylotrophic is used to describe a wide variety of bacteria which can utilize single carbon compounds more reduced of carbon dioxide as sole carbon source **(Bergey's Manual of Systematic Bacteriology, 2001).**

The most abundant green of methylotrophic isolated from surface of green plant were pink pigmented facultative methylotrophic (PPFMs) **(Holland, 1997).**

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Foliar application of methanol has been reported to increase growth and yield of C_3 crops in warm, high radiation arid climate **(Nonomura and Benson, 1992).** Pink pigmented facultative methylotrophic bacteria (PPFM), plays a significant role in the mechanism of foliar applied methanol effects on crops **Munsanje *et al* (1996)** observed that population of the bacteria on foliar methanol sprayed increased on soybean leaves, and they further correlated PPFM bacterial increases on the leaves with seed yield. These bacteria (PPFM) are ubiquitous in nature, living on plant leaves and use methanol as sole source of carbon, in turn secrete cytokinins. plant growth hormones and urease, which breaks down urea to NH_3 and CO_2 **(Holland and Polacco, 1994).**

Therefore, this work was assigned to determined the influence of foliar applications of methanol and PPFMs applied at different concentrations and spraying times on growth and yield of cotton plant.

MATERIALS AND METHODS

Cultivar used: Seeds of cotton (*Gossypium barbadense* L.) Giza 85 variety were provided from Cotton Research Institute ARC. Agriculture Research Center. Giza – Egypt.

Isolation of pink pigmented facultative methelotrophic Bacteria (PPFM): Isolates were isolated from green leaves of seven different species plants (cotton- barley- wheat- tomato- corn- soybean- sunflower) as following:

1- Green leaves obtained from the seven crops were collected from different locations (Shobra and Shalkan, Kalubia).

2- The leaves were handled and were either used directly and rinsed with sterile water. Then were pressed firmly to surface of specific solid medium of methanol ammonium mineral salts (Met-AMS) agar medium, then discarded and plates were closed, sealed with para film and incubated at 28° C for 3-5 days. **(Holland and Polacco, 1994).**

3- Small pink pigmented separated colonies were selected and successively subcultured on the same specific medium several times.

Then, a well defined pure colonies were subcultured on slants of the Met-AMS medium and incubated at 28° C for 3-5 days.

The growing cultures were kept at 4°C.

Experimental techniques

a) **Effect of methanol concentration on the growth of PPFM:** For this purpose, 8 different concentrations of methanol (0 , 0.5 , 0.75 , 1 , 5 , 10 , 20 , 30 %) were prepared as a final concentrations in conical flasks (25 ml in volume) containing a100 ml of Met-AMS liquid medium **(Holland and Polacco, 1994)**. Each flask was inoculated with 1 ml standard inoculum and shaken on rotary shaker (150 rpm) for 5 days at 28°C. Growth densities as optical density were determined at 600 nm.

b) **Greenhouse experiment:** A pot experiment was carried out during the summer season of 2006 at the experimental farm of Faculty of Agriculture, Ain Shams University, Cairo.

Pot experiment was designed in complete randomized blocks with 10 replicates, and contained 13 treatments, which were combinations between methanol concentrations and PPFM application as follows:

1- control
2- PPFM 3 spraying times
3- PPFM 4 spraying times
4- 10 % methanol 3 spraying times
5- 10 % methanol 4 spraying times
6- 20 % methanol 3 spraying times
7- 20 % methanol 4 spraying times
8- 10 % methanol +PPFM 3 spraying times
9- 10 % methanol +PPFM 4 spraying times
10- 20 % methanol +PPFM 3 spraying times
11- 20 % methanol +PPFM 4 spraying times
12- 20 % methanol +PPFM 1 spraying times
13- 30 % methanol 7 spraying times

Methanol and PPFM was sprayed with hand sprayer between 10.00 am and 14.00 pm. Water volume was estimated according to the growth stage of cotton plant to be 125, 150, 175 and 200 l/feddan for May, June, July and August spraying, respectively. Methanol sprayings schedule was started 45 days after planting and continued up to the end of bolls formation stage (at the 1st week of August). Data recorded on vegetative characteristics on mid of August and also two weeks from the last methanol and PPFM spraying.

A random sample was taken from each pot to estimate the following vegetative characteristics:

- Number of fruiting branches per plant
- The length of un-branched region (cm)
- Leaf area index LAI (cm^2/ plant)/ ground area (cm^2) according to **Beadle (1993)**.

Data recorded on yield and yield attributes were as follows:

- Average number of total bolls per plant
- Seed cotton yield per boll (g)
- Seed cotton yield per plant (g)

All the obtained data were exposed to the proper statistical analysis according to **Snedecor and Cochran (1980)**. The least significant differences at 0.05 level of significance were calculated.

RESULTS AND DISCUSSION

Isolation and purification of pink pigmented facultative methelotrophic bacteria (PPFM)

Seven isolates were obtained from the phylloplane of seven different plants (cotton- barley- wheat- tomato- corn- soybean- sunflower). The isolates were purified and then maintained on methanol ammonium mineral (AMS) agar medium **(Holland and Polacco, 1994)**.

Effect of methanol concentration on the growth of bacteria PPFM bacteria isolates

Data on application trials evaluating the effect of eight concentrations of methanol (0, 0.5, 0.75, 1, 5, 10, 20 and 30 %) on growth of seven PPFM isolates are recorded in **Table (1)**. It is clear that, the highest growth was obtained with PPFM isolates isolated from wheat plant treated with 1% methanol being 1.145. On the other hand, the lowest growth value was obtained with PPFM isolate isolated from cotton plants treated with 30% methanol being 0.086. Generally the growth rate of the isolates were decreased with increasing the methanol concentrations, PPFM isolates isolated from cotton plants show a best results with 10 and 20% methanol concentration, compared with other PPFM isolates at the same concentration of methanol .

Several investigations reported that PPFM bacteria were able to use methanol as a carbon source under obligate or restricted facultative or methelotrophic **(Tarta and Goodwin, 1985; Machiln *et al* 1988; Murrell and Dalton, 1992 and Lidstrom, 2002).**

Effect of foliar applications of methanol and PPFM on some growth parameters of cotton plants

Different concentrations of methanol with different time of foliar with or without PPFMs isolates were used to study their effect on un-branched region (cm) and LAI of cotton plant **Table (2)**.

Data recorded in **Table (2)** show that there are significant differences between un-branched region lengths (short length) of cotton plant treated with 30% methanol with 7 times compared other treatments. On the other the hand, 20% methanol plus PPFM 3and4 spraying times gave a relatively shorter length being 18.7 and 17.2 cm, respectively.. In this respect, **Kenda (2005)** suggested that foliar methanol application (under certain environmental conditions) may contrast auxin effect and disturb the hormonal balance in indeterminate plant. This suppress plant height and stimulate lateral branching on methanol treated plant. Similar trend was obtained by **Nonomura and Benson (1992); Cothern (1994) and Makhdum *et al* (2002).**

The same trend was also obtained in case of LAI. All treatments treated with methanol only or with PPFM were superior than the control. The highest value were obtained with cotton plant treated with 20% methanol, plus PPFM 3 and 4 spraying times and 30% methanol 7 times being 3.91, 3.31 and 3.86 respectively, compared with other treatments **(Table 2)**.

Effect of foliar applications of methanol and PPFM on numbers of fruiting branches and total bolls per plant and seed cotton yield per boll and per plant (g)

Data calculated in **Table (3)** show in general that all treatments treated with different concentration of methanol alone or with PPFM gave a higher yield parameters under investigation compared with control, except cotton plant treated with 20% methanol +PPFM 1 spraying time. The highest value of cotton yield were obtained from plant treated with 20% methanol plus PPFM bacteria 3 or 4 times. Such two potent treatments increased number of fruiting branches / plant by 53.1 and 58.0 %; number of total bolls / plant by 38.1 and 43.0 %; seed cotton weight per boll 37.1 and 48.2% and seed cotton yield per plant by 46.1 and 50.8%, respectively than the control. Increases in seed cotton yield per plant mainly attributed to significant increase in number of fruiting branches and bolls per plant and seed cotton weight per boll **(Table 3)**.

Growth and yield improvement occurred in methanol and PPFM bacteria treated cotton plant was explained by the role of methanol as a carbon source to increase carboxylation reaction and enhanced photosynthetic rate of treated plant **(Faver and Gerik, 1996; Cothern, 1994; Gerik and Faver, 1994; Kumar *et al* 1999 and Zbiec *et al* 1999).** Moreover, CO2 resulting from rapid oxidation of methanol can successfully compete with

Table 1. Effect of methanol concentration on growth of pink pigmented facultative methylotrophic bacteria PPFM

Treatments	Cotton	Wheat	Barley	Sunflower	Soybean	Corn	Tomato
0%	0.309	0.277	0.188	0.262	0.333	0.625	0.347
0.5%	0.580	0.285	0.456	0.472	0.356	0.392	0.539
0.75%	0.580	0.661	0.687	1.081	0.545	0.599	0.520
1%	0.408	1.145	0.373	0.253	0.407	0.541	0.406
5%	0.420	0.407	0.382	0.150	0.291	0.204	0.238
10%	0.310	0.255	0.337	0.403	0.258	0.265	0.271
20%	0.174	0.113	0.144	0.084	0.095	0.269	0.133
30%	0.086	0.121	0.166	0.144	0.208	0.089	0.186
LSD 0.05	0.068	0.051	0.089	0.062	0.087	0.065	0.054

Table 2. Effect of foliar application methanol and PPFM on length of un-branched region (cm) and LAI of cotton plant

Treatments	Length of un-branched region (cm)	LAI*
10% Methanol, 3 times	22.5	2.55
10% Methanol, 4times	22.7	2.61
20% Methanol, 3 times	21.0	2.75
20% Methanol, 4 times	20.1	2.79
10% Methanol + PPFM 3 times	19.8	2.91
10% Methanol + PPFM 4 times	19.2	2.82
20% Methanol + PPFM 3 times	18.7	3.80
20% Methanol, + PPFM 4 times	17.2	3.91
20% Methanol + PPFM 1 times	22.7	2.60
PPFM 3 times	20.6	3.00
PPFM 4 times	19.1	3.01
30% Methanol, 7 times	15.6	3.31
Control	22.5	2.51
LSD 0.05	0.564	0.165

* LAI: Leaf area index, Cm^2/plant

Table 3. Effects of foliar application of methanol and PPFM on numbers of fruiting branches and total bolls/plant and seed cotton yield per boll and per plant (g)

Treatments	No. of fruiting branches/plant	No. of bolls/plant	Seed cotton yield per boll (g)	Seed cotton yield per plant (g)
10% Methanol, 3 times	8.4	22.4	1.42	27.9
10% Methanol, 4 times	8.6	22.5	1.43	28.2
20% Methanol, 3 times	9.9	25.3	1.67	30.4
20% Methanol, 4 times	9.1	25.9	1.69	32.2
10% Methanol, + PPFM 3 times	10.1	29.1	1.76	33.3
10% Methanol, + PPFM 4 times	10.5	28.5	1.89	34.1
20% Methanol, + PPFM 3 times	12.4	30.8	1.96	37.7
20% Methanol, + PPFM 4 times	12.8	31.9	2.12	38.9
20% Methanol, + PPFM 1 times	8.0	22.0	1.41	26.1
PPFM 3 times	8.9	23.1	1.58	27.7
PPFM 4 times	9.4	23.5	1.59	27.9
30% Methanol, 7 times	11.7	30.3	1.92	35.7
Control	8.1	22.3	1.43	25.8
LSD 0.05	0.621	0.410	0.087	2.012

CO_2 for ribulose 1,5 diphosphate and consequently depress photorespiration rate in C3 plant **(Nonomura and Benson, 1992 and Zbiec *et al* 1999).** In addition, PPFM bacteria use methanol as sole source of carbon and secrete cytokinins plant growth hormones which stimulate translocation of minerals and organic compounds in leaves **(Elliot *et al* 2000 and Larry and Gordan 2002)** and consequently improve growth and productivity of cotton plant.

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