INFLUENCE OF FOLIAR APPLICATION OF METHANOL AND PINK PIGMENTED FACULTATIVE METHYLOTROPHIC BACTERIA ON GROWTH AND PRODUCTIVITY OF COTTON

BY

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ABSTRACT

Kenda Hamade Al-Mohamad. Influence of Foliar Application of Methanol and Pink Pigmented Facultative Methylotrophic Bacteria on Growth and Productivity of Cotton. Unpublished Ph.D.thesis, Department of Agronomy, Faculty of Agriculture, Ain Shams University, 2009.

Laboratory, pots and field experiments were conducted in Faculty of Agriculture, Ain Shams University during 2006 – 2008 to isolate and evaluate the efficiency of PPFMs bacteria for methanol utilization and to investigate its effect alone or with methanol, both as foliar application on growth and productivity of Egyptian cotton Giza 85 cultivar.

Laboratory evaluation on the effect of methanol concentrations on growth of bacteria PPFM isolates from the phylloplane of seven different plants showed that the highest growth was obtained with PPFM isolates isolated from wheat plant treated with 1% methanol. On the other hand, the lowest growth value was obtained with PPFM isolate isolated from cotton plants treated with 30% methanol. However, PPFM isolates isolated from cotton plants show a best results with 10 and 20% methanol compared with other PPFM isolates at the same concentrations of methanol. Pots and field experiments results showed that foliar applications of methanol 20% alone or in combination with PPFM bacteria decreased height of cotton plant, and length of un-branched area on main stem but increased their number of leaves, number of vegetative and fruiting branches, LAI, number of bolls/plant, % of open bolls, seed cotton yield per boll and per plant and consequently seed cotton yield per feddan. The beneficial impact of methanol on growth, yield and yield attributes of cotton was mostly concomitant to increases in spraying times and methanol concentrations and to the presence of PPFM bacteria. Therefore, 20% methanol + PPFM sprayed 4 or 3 times treatments

exhibited the highest values of growth parameters, yield and yield components.

Key words: Cotton, Methanol, Pink Pigmented Facultative Methylotrophic Bacteria (PPFM); Foliar application, Growth, Yield, Yield attributes.

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INTRODUCTION

The process of photosynthesis by which green plants use sunlight energy to convert water and carbon dioxide into oxygen and organic compounds is the basis for virtually all life.

Production of biomass by plants depends to a great extent on environmental factors, such as water supply, air temperature, isolation and carbon dioxide concentration in the canopy. Under certain environmental conditions especially in warm, high solar radiation and quiet air conditions, CO_2 level in the air may limit photosynthesis and yield. Herein **Nonomura and Benson (1992)** explained that during the heat of the day, photosynthesis is not at its greatest because there was not enough carbon in the air to sustain C_3 plants growth and they were discarding much of the energy they had absorbed. He concluded that spraying them with methanol gave them a boost by supplying the carbon lacked, and thus inhibiting photorespiration in which plants reject some of the energy they have collected through photosynthesis.

Methanol, one of the simplest organic molecules, is also a natural product of plant metabolism, and it has now come to light that most plants also emit it from their leaves **(Madhaiyan *et al*., 2006).** Meanwhile foliage application of commercial methanol is used since **Nonomura and Benson (1992a and 1992b)** experiments as a precursor of CO_2 on plant to enhance growth and yield of C_3 plants. The benefits of methanol applications were most evident under high light intensity and high temperature (arid or semiarid conditions) and with C_3 crop species. This was supported by failure of C_4 plants to respond to foliar applied methanol **(Nonomura and Benson 1992a and 1992b).**

Recently many authors investigated the role of pink pigmented facultative methylotrophic bacteria (PPFM) in the mechanism of foliar applied methanol effects on crops **(Holland and Polacco, 1994; Faver and Gerik 1996, Munsanje *et al*. 1996 ; Elliot *et al*., 2002 and Larry**

and Gordan 2002). They 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 are ubiquitous in nature, living on plant leaves and use methanol as sole source of carbon, in turn secrete cytokinines, plant growth hormones and urease, which breaks down urea to NH_3 and CO_2 **(Holland and Polacco, 1994).** Leaves typically harbor PPFMs bacteria capable of metabolizing methanol **(Corpe and Rheem 1989 and Holland and Polacco 1994)** and these bacteria may be associated with leaf surface methanol emitted by the stomata of plant **(Nemecek-Marshall *et al*., 1995 and McGiffer and Manthey 1996).** Therefore, at this point numerous indications exist that these PPFMs interact with plants. Our investigation for M.Sc. degree **(Al-Mohamed, Kenda *et al*., 2005)** concluded that methanol has potential to improve productivity of cotton and other C_3 crops under our warm and high solar radiation Arabic climate.

So, this present study is aimed to (1) Isolate and evaluate the efficiency of PPFM strains for methanol utilization and cytokinin production, and (2) Recognize the influence of foliar applications of methanol rates, PPFM solution and their interaction on growth, and some physiochemical assessments, yield, yield components and fiber quality of Egyptian cotton (*Gossypium barbadense* L.)

REVIEW OF LITERATURE

The use of exogenous growth regulators to modify crop growth and enhance productivity has long interested researchers. Many have focused their attention on cotton and other C_3 crops owing to the inferior of net assimilation rate (NAR) of this group.

Collected literature related to the influence of exogenous application of methanol and pink pigmented facultative methylotropic (PPFM) bacteria and their interaction on growth and productivity of cotton and other C_3 crop plants will be reviewed under the following main headings:

1. Influence of pink pigmented facultative methylotrophic (PPFMs) bacteria.
   1.1. Identification studies of PPFMs bacteria.
   1.2. Relation between PPFMs and plants.
2. Influence of methanol foliar application.
   2.1. On growth of plants.
   2.2. On yield, and yield attributes.
   2.3. On yield quality.
3. Interaction between PPFMs bacteria and methanol foliar application.

1. Influence of pink pigmented facultative methylotropic (PPFMs) bacteia.

1.1. Identification studies of PPFMs bacteria :

Historical data pointed out that the first methylotrophic bacterium was described by **Leow (1892)** who reported that this pink pigmented bacterium named *Bacillus methylicus*. Members of the genus *Methylobacterium* have been isolated from soil, dust, fresh water, lake sediments, leaf surfaces and nodules, rice grains, air and hospital environments **(Lynch *et al*., 1980).** The most abundant group of methylotrophs isolated from surfaces of green plants was pink-pigmented facultative methylotrophic bacteria (PPFMs) **(Corpe and Basile 1982).**

PPFMs bacterium can be easily isolated from plant tissues using selective media containing methanol as the sole carbon source **(Corpe, 1985)** and identified by their pink color, which distinguishes them from the other unrelated methylotrophic organisms normally encountered on plant tissue. They are abundant and non-pathogenic, distributed ubiquitously in the plant phyllosphere, and have been isolated from more 100 species of plants, ranging from liverworts and mosses to angiosperms and gymnosperms **(Corpe and Basile,1982; Dunleavy, 1990 and 1998).**

Lidstrom (1992) reported that bacteria of the genus Methylobacterium are well-studied examples of facultative methylotrophs. These bacteria are classified as α-proteobacteria and are capable of growth on methanol and methylamine as well as on variety of C_2, C_3 and C_4 compounds. Pink-pigmented bacterium named *Bacillus methylicus* can grew on methanol, methylamine, formic acid and several multicarbon compounds was identical in many characteristics to *Methylobacterium* species and other PPFMs isolated many years later in several laboratories **(Quayle, 1987).** PPFMs are common inhabitants of variety of terrestrial and aquatic environments **(Green *et al*., 1988).**

Corpe and Rheem (1989) confirmed that PPFMs are soil plant associated bacteria that are distributed on plant surfaces. However, **Corp and Jensen (1991)** demonstrated the distribution and colonization of leaf surface by PPFMs of the genus *Methylobacterium*. **Hirano and Upper (1992)** found them to represent greater than 90% of the phylloplane microflora of *Phaseolus vulgaris* throughout the growing season.

Bacteria of the genus *Methylobacterium* (PPFMs, pink-pigmented facultative methylotrophic bacteria) are strict aerobic, Gram-negative rods, able to grow on C1 compounds **(Green, 1992 and Trotsenko *et al*., 2001).**

Chanprame *et al*., (1996) revealed that PPFMs bacteria were found on the leaf surface of all 40 plants (38 species) tested. Likewise, **Romanovskaya *et al*. (1996)** studied the ecology of PPFMs of genus

Methylobacterium presented in the phyllosphere of many plants using leaves of 200 various plants, since most of the species and strains of PPFMs studied were isolated from plant tissues **(Holland 1997).** Similarly, **Koeing *et al*. (2002)** found that PPFMs are persistent colonizers of plant surfaces. In addition to their ability to colonize the phyllosphere, members of *Methylobacterium* were also able to colonize the rhizosphere of tested plant species.

Morphological description studies showed that *Methylobacterium* was short rods, 0.8 – 1.0 x 1.0 – 8.0 µm occurring singly or occasionally in rosettes, branched and pleomorphic, motile by single polar, sub polar or lateral flagella, although some strains are non motile, Gram negative, although many strains are grown variable. Colonieson methanol- salt agar are more uniformed pale pink. The pigment is insoluble and probably carotenoids. In static liquid media, cells grow as a pink pellicle **(Green and Bousfield 1983 andCorpe 1985).**

Hoon and Kim (1989) revealed that after several times of selection with methanol, a PPFM isolate was stabilized morphologically by color of the colonies and by the cell shape. The colonies were bright pink, slimy, glistening, raised convex with entire margin. The cell was Gram-negative, slightly curved rod, with dimention of 1.0 – 1.2 x 3.0 – 4.0 µm. It was motile and had a single polar inserted flagellum. **Romanovskaya *et al*. (1996)** stated that the PPFM bacteria were Gram-negative, non-spore forming, and rod shape. It formed circular, convex and opaque colonies on nutrient agar.

Pnnk-pigmented facultative methylotrophic bacteria (PPFMs) are widely distributed in nature. Over 150 strains of PPFMs have been studied taxonomically **(Green and Bousfield, 1982, 1983; Hood *et al*., 1987 and Orf-Heba *et al*., 2005).**

1.2. Relation between PPFMs bacterium and plants :

The stem and plant leaf surface, or above-ground phytosphere, is a suitable habitat for a variety of microorganisms. Under natural conditions,