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Pink pigmented facultative methylotrophic bacteria (PPFMs) as microbial farmers in small cardamom plantation

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Abstract
The non-infecting, plant associated bacteria have attracted increased attention for stimulating plant growth and as environmental friendly plant protecting agents in small cardamom. Pink-Pigmented Facultative Methylotrophic Bacteria (PPFM) (classified as methyl bacterium species) are persistent colonizers of plant leaf surface. As the leaves of most or all plants harbour PPFMs that utilize leaf methanol as their sole source of carbon and interacting partners in plant metabolism. Like little farmers, Methylotrophic bacteria play an important role in seedling establishment. They feed compounds (Cytokinin and Auxin) that stimulate plant growth. They protect their crop from pathogens and pests by induction of systemic resistance. PPFM is one such farmer and its relationship with plants illustrates many of these ideas. Krishi Vigyan Kendra has supported small cardamom farmers, by the following observations: (1) Foliar application of PPFMs to small cardamom during summer period which enhance the plant growth and increase yield, (2) Treated plants to decrease disease incidence of Fusarium disease caused by *Fusarium oxysporum* in small cardamom, (3) The PPFMs inoculation induced number of stomata, chlorophyll concentration and malic acid content and led to increased photosynthetic activity, (4) Screening of such kind of bacteria having immense plant growth promoting activities like nitrogen fixation, Phytohormone production, alleviating water stress to the plants can be successfully isolated and characterized and integration of such kind of organism in crop production will lead to 35% increased productivity in small cardamom plantation.

Keywords: Methylobacterium, nitrogen fixation, phytohormone production, water stress alleviation and fusarium wilt disease

Introduction
PPFMs are known to improve plant growth by adopting various mechanisms *viz*., nitrogen fixation and nodule formation, phosphate solubilization, plant growth regulators production (auxins, cytokinins, gibberellic acid), production of urease enzyme, vitamin B12 production, synthesis of siderophores. PPFM accountable for a diverse beneficial effect on plant includes fasten seed germination and seedling growth, accelerate vegetative growth by producing phytohormones, increase leaf area index and chlorophyll content, earliness in flowering, fruit set, and maturation, improves fruit quality, color, and seed weight, yield increase by 10% and Mitigate drought. Methylotrophic bacteria adapt to survive in stress conditions such as low nutrient, drought, and high temperature by producing biofilm, aggregate formation, and producing ultraviolet (UV)-protecting compounds. Additionally, PPFMs promote plant growth by producing an enzyme 1- aminocyclopropane-1-carboxylate (ACC) deaminase is responsible for drought management during the beneficial interaction with plants (Chinnadurai *et al.*, 2009) [10]. The benefit effect of gibberellins in maize (Tuna *et al.*, 2008), cytokinins in *Vigna radiata* [1], salicylic acid in tomato [2] and brassinosteroid in tomato [3] were documented. Exogenously applied brassinolide alleviated the detrimental effects of drought in maize and remarkably improved the chlorophyll contents, protein, relative leaf water contents (RLWC), proline, and enzymatic antioxidants [4]. Saruhan *et al.*, (2012) [5] reported that the exogenous salicylic acid reduced the adverse effects of drought stress in maize and might have a key role in providing tolerance to stress by decreasing water loss and inducing the antioxidant system in plants with leaf rolling, an alternative drought protection mechanism.

Application of cytokinin improves the physiological traits in terms of membrane stability index, RWC, and photosynthetic rate at the vegetative stage of wheat [6]. Based on this background knowledge available, the present investigation was carried out to assess the physiological basis of drought tolerance mediated by PPFM and other PGRs in tomato.

PPFM as Microbial Farmers on Small cardamom plantation like little farmers, methylotrophic bacteria play an important role in plant growth and 35 percentage yield increased. They feed compounds (cytokinin and auxin) that stimulate plant growth. They protect their crop from pathogens and pests by induction of systemic resistance. They reap a harvest of plant metabolites as methanol. Methylobacterium a pink-pigmented, facultative methylotrophs (or PPFM) is one such farmer and its relationship with plants illustrates many of these ideas [7].

Method of Application

The liquid formulation of PPFMs recommended for foliar spray (1% PPFM spray during morning or evening, recommended for all crops, spray at the critical stage of crop growth (or) 30-45 days interval).

Validated Field Trials

Cardamom (*Elettaria cardamomum)* Maton) the Queen of Spices is indigenous to the southern stretch of evergreen forests of Western Ghats. In India, small cardamom is cultivated in the states of Kerala, Karnataka and Tami Nadu. It is also cultivated in parts of Guatemala, Tanzania, Sri Lanka, El Salvador, Vietnam, Laos, Cambodia and Papua New Guinea. Now Guatemala is offering stiff competition to Indian cardamom in the international market. Kerala is the largest producer of small cardamom and constitute lion share of Indian and world market. The methyl trophic bacterium offers new directions for phyllosphere management, as evidence d from the beneficial role of PPFMs on various crop plants [8, 9]. The small cardamom growth significantly increased as a result of PPFM inoculation spray give n at 30, 60 and 90 days every year, the treatment which provides more contact area for PPFMs to adhere for longer period facilitating their establishment on the surface. As hypothesized earlier, the production of cytokine in during symbiosis could be attributed to the indication of cytokine in synthesis in plants or synthesis of cytokine in compounds by PPFM [10], both the mechanisms requiring further investigation. The abaxial surface of leaves always registered higher population than adaxial surface, the population sizes varied in different field crops. This could be directly related to the methanol emission through stomata! Openings during leaf expansion by pectin demethylation [11], which may be utilized by PPFMs to survive in the surface of leaves. Conversely less population in the adaxial surface population could be attributed to several biotic and abiotic factors *viz*, rainfall, wind, irradiation, temperature, and humidity and nutrient competition between other epiphytic bacteria and fungi. The population density was higher in young leaves compared to matured leaves, which is directly attributed to higher methanol content and cytokine in levels in younger leave s than matured leaves. Rapid expansion of leaf area at the early growth of small cardamom plant as well as crop growth, influenced by methylotrophs inoculation in small cardamom.

Impact of PPFM on Drought Mitigation in Small Cardamom Plantation

PPFM is widely used in small cardamom plantation by recommended spraying of bacteria to mitigate drought and save crops. Foliar application of PPFMs to small cardamom during summer period which enhance the plant growth and increase yield, Treated plants to decrease the disease incidence of Fusarium caused by *Fusarium oxysporum* in small cardamom, The PPFMs inoculation induced number of stomata, chlorophyll concentration and malic acid content and led to increased photosynthetic activity and Screening of such kind of bacteria having immense plant growth promoting activities like nitrogen fixation, phytohormone production, alleviating water stress to the plants can be successfully isolated and characterized and integration of such kind of organism in crop production will lead to 35% increased productivity in small cardamom plantation. The evidence of that spraying of PPFM helps the small cardamom plantation remain green for 30 to 45 days.

Table 1: Horizontal Spread of the technology

S. No.	Villages	Area under PPFM technology (Before KVK intervention in ha.)	Increase in area PPFM technology after KVK intervention in ha.
1.	Senapathy	0	125
2.	Konnathady	0	260
3.	Santhanpara	0	310
4.	Bison valley	0	751
5.	Nedumkandam	0	490
6.	Kumily	0	225
7.	Rajakumari	0	192
8.	Rajakadu	0	79
10.	Kattappana	0	110

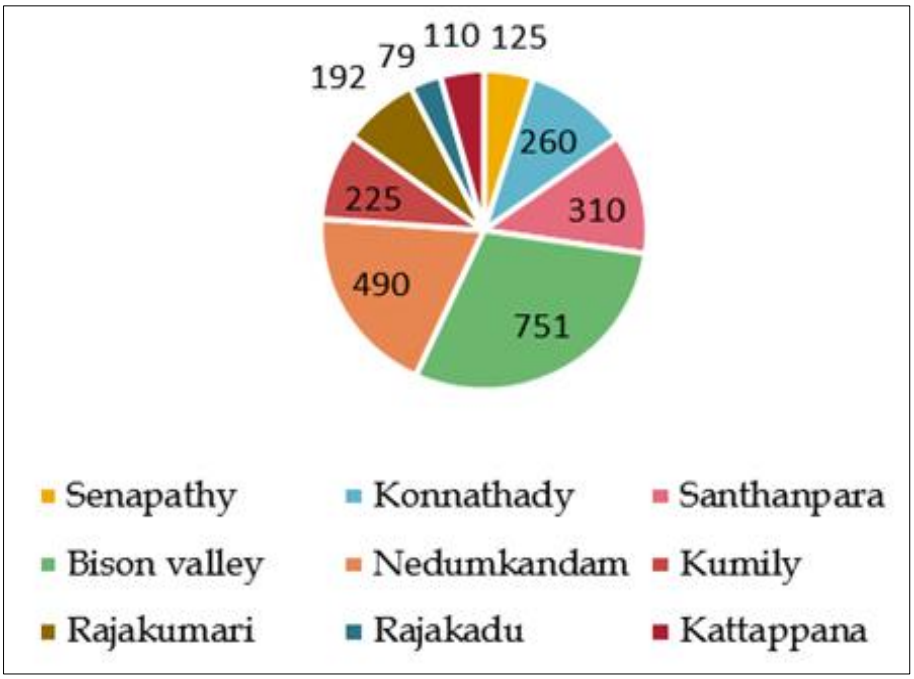

Fig 1: Increase in area PPFM technology after KVK intervention in ha

Table 2: Vertical Spread of the technology

S.no	Year	Production in kg (ha)	Productivity (qtl./ha)
1	2015-16	1050	4.38
2	2016-17	1205	4.71
3	2017-18	1312	5.42
4	2018-19	921**	3.10
5	2019-20	1310	4.90
6	2020-21	1406	5.80

Table 3: Comparison of demonstration and farmers practice

Parameters	Demonstration	Farmers practice
% reduction in Fusarium disease	45	32
Days of Irrigation	45 days once during summer	7 days once during summer
Number tiller/plant	57-62 per plant	48-51
Number panicle/plant	228	192
Number capsule/panicle	576	475
Gross cost (Rupees/ha)	322000	354000
Gross Return (Rupees/ha)	736000	477000
BCR	2.28	1.34

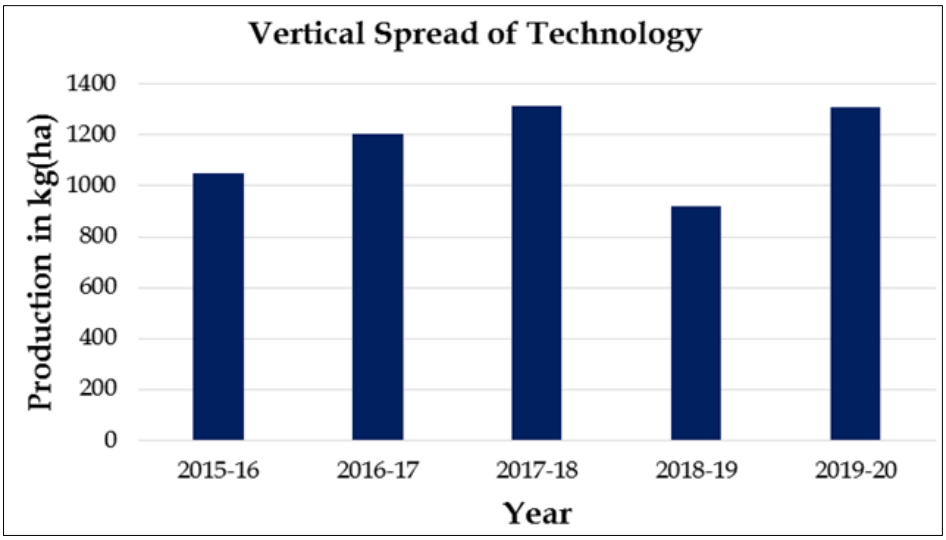

Fig 2: Vertical spread of Technology (Production (Kg/ha.)

Conclusion

The results of the present investigation indicated in general, inoculation of efficient strains of PPFM along with PSB showed increased new shoot as well as total dry capsule production. PPFM association with plant growth can be exploited for eco-friendly and cost-effective practices to promote sustainable agriculture. They employ multiple mechanisms to promote plant growth like phytohormones production, nodulation, nitrogen fixation, and nutrient acquisition. Similarly, methylotrophic bacteria also offer drought-tolerant mechanisms by nitrogen fixation, phytohormones production, ACC deaminase production, and phosphate solubilization. PPFMs could be potential bio-inoculants to increase plant growth and drought stress tolerance in sustainable small cardamom production.

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