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Impact of pink pigmented facultative methylotrophic bacteria and synthetic materials on small cardamom (*Elettaria cardamomum* Maton.) under drought

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Abstract
An experiment was conducted at the Cardamom Research Station, Kerala Agricultural University, Pampadumpara, Idukki, Kerala during 2017 summer (February-May) to evaluate the response of small cardamom crop to the foliar application of PPFM and synthetic materials under drought situation. Potassium di-hydrogen phosphate applied plants had higher chlorophyll stability index (90%) compared to others. The highest proline content was obtained with the exogenous application of naphthalene acetic acid, proline, potassium di-hydrogen phosphate and urea respectively. Ferrous sulphate, kaolin and potassium chloride enhanced the population of endophytic fungi. The results have confirmed that potassium di-hydrogen phosphate and potassium containing substances showed consistent superiority over other treatments in mitigating drought stress. The chlorophyll stability index of PPFM was significantly higher than the control and can be a good choice for the organic cardamom growers under drought situation.

Keywords: Small cardamom, drought, PPFM, synthetic materials, endophytic fungi, physico-chemical analyses

Introduction
Small cardamom (*Elettaria cardamomum* Maton), popularly called the queen of spices, is second only to pepper, the king of spices. It is the world's third most expensive spice surpassed in price per weight only by vanilla and saffron (Williams and Olivia, 2014) [13]. India is the second largest producer of small cardamom and plays an important role in the international trade of cardamom. The yield of cardamom is highly dependent on prevailing climatic conditions as the cardamom plant requires intermittent spells of rain and good sunshine during the growth stage. Cardamom production in the country during 2015-16 was estimated as 22 thousand tonnes compared to 18 thousand tonnes in 2014-15. Meanwhile in Kerala, cardamom production has increased by 21.8 per cent in 2015-16 despite the area under cultivation remaining stagnant (Anonymous, 2016) [2]. Cardamom cultivation is considered as a bed of roses with thorns, because both biotic and abiotic stresses play a vital role in lowering its productivity. Abiotic stresses such as rise in temperature, flood, drought and salinity have detrimental effect on yield of cardamom, among which, drought was not a serious problem in previous years. But, now it has became a great threat for cardamom cultivation owing to the failure of monsoon and summer showers. Drought is one of the greatest abiotic stresses to agriculture, inhibiting plant growth and thus reducing productivity (Zhang *et al.*, 2008) [15]. Endophytic microbes shown to have several beneficial effects on their host plant, including growth promoting activity, modulation of plant metabolism and phytohormone signalling that leads to adaptation to environmental abiotic or biotic stress. Probably for the first time in small cardamom, the evaluation of different materials on drought mitigation with special emphasis of their effect on foliar microflora and physico-chemical parameters has been advocated.

Materials and Methods
A field experiment was conducted to evaluate the effect of PPFM, nutrients, growth regulators, anti-transpirants and compatible solutes against the drought stress at Cardamom Research Station, Pampadumpara, Idukki District, Kerala, using cardamom variety Green Gold (*Njallani)* during 2016-2017 (Fig. 1). Two foliar applications were given at 30 days interval during April and May.

The index leaf (third leaf from top) was selected for analyses .The experiment was laid out in RBD with four replications. The experiment comprised of the following treatments.

S. No.	Treatment	Dose (lit^{-1})
1	Boric acid	3g
2	Diammonium Phosphate (DAP)	20g
3	Ferrous sulphate	5g
4	Kaoline	30g
5	Naphthalene Acetic Acid (NAA)	40 ppm
6	Potassium chloride (KCl)	10g
7	Potassium Dihydrogen Phosphate (KH_2PO_4)	10g
8	PPFM	1ml
9	Proline	100 ppm
10	Salicylic acid	500 ppm
11	Urea	10g
12	Zinc sulphate	5g
13	Control	-

Physiological and biochemical assessments

Chlorophyll Stability Index (CSI)

Leaf samples were selected randomly from the plants and homogenized in a mortar with 80 per cent acetone. The extract was centrifuged at 5000g for 5 minutes. Absorbance of the supernatant was recorded at 652 nm (total chlorophyll) spectrophotometrically. Chlorophyll content was determined by using the method of Sairam *et al*. (1997) [11] and calculated as follows.

$$CSI = \frac{\text{Total chlorophyll under stress}}{\text{Total chlorophyll under control}} \times 100$$

Proline content

The proline content was assayed by the method described by Bates *et al*. (1973) [4]. Freshly collected leaves (0.5g) were homogenized in 10 ml of 3% aqueous sulphosalicylic acid. The homogenate was filtered through Whatman No. 2 filter paper. Two ml of the filtrate was taken in a test tube and two ml of glacial acetic acid was added to it. Freshly prepared acid ninhydrin (2 ml) was added to this mixture. The final solution was heated for one hour in a boiling water bath. After one hour of boiling the reaction was terminated by placing the test tube in an ice bath. Toluene (4 ml) was added to the test tube and stirred for 20 - 30 seconds. Subsequently, the toluene layer was separated and the final mixture was again warmed to room temperature and the red colour (slightly red colour) was measured at 520 nm. The quantity of proline in the test sample with reference to standard curve can be expressed in terms of µ g/g fresh weight.

Isolation of endophytes

The isolation of endophytes was done as per the methodologies followed by (Dong *et al*., 1994) [5].

Statistical analysis

The data gathered and mean values were taken from measurements of four replicates and standard error of the means was calculated. The values were subjected to statistical scrutiny and transformed in to angular or square-root values, wherever necessary (Gomez and Gomez, 1984) [8].

Results and Discussion

Effect on Chlorophyll Stability Index

Potassium di-hydrogen phosphate sprayed plants showed higher (90.0%) CSI followed by proline (79.0%) (Table 1). Ferrous sulphate (67.7%), boric acid (66.8%), kaolin (66.4%) and $ZnSO_4$ (63.3%) treated leaves were found to be the next best treatments. PPFM had significantly higher CSI (60.3%) compared to control (15.90%) and was on par with salicylic acid (59.2%) and was better than di-ammonium phosphate, naphthalene acetic acid and potassium chloride. Hussein *et al*. (2012) [9] reported that the foliar application of 100 ppm potassium mono-phosphate resulted in significant increase of chlorophyll 'a' content of pepper. Al-shaheen and Soh (2016) [1] revealed that the exogenous application of proline @ 100 and 200 ppm elevated the chlorophyll content in wheat leaves. Srivastava *et al*. (2014) [12] depicted that the foliar application of iron and zinc increases the chlorophyll content in mung bean. El-Feky *et al*. (2012) [7] proved that, 0.5 and 1.5 mg l^{-1} boron significantly increased Chlorophyll a and Chlorophyll b contents by 3% and 7% at vegetative and flowering stages, respectively on barley. Kaolin application has positive effect on chlorophyll and water content of the leaves (Azizi *et al*., 2013) [3]. All these findings support our present investigation results.

Effect on proline content

Naphthalene acetic acid treated leaves had higher proline content (18.704 µ moles /g sample) followed by exogenous application of proline (10.736 µ moles /g sample), potassium di-hydrogen phosphate (10.736 µ moles /g sample), urea (10.736 µ moles /g sample), ferrous sulphate (10.390 µ moles /g sample) and zinc sulphate (10.044 µ moles /g sample). Even though the proline content of PPFM treated plants was significantly less compared to other chemical treatments; it was higher than that of control. The untreated plants possessed least proline content (1.732 µ moles /g sample). A study by Durrani *et al*. (2010) [6] conveyed that, the application of NAA increases the seed protein content in spinach. Similarly, iron and zinc sprays resulted in the increase of protein content on wheat (Monjezi *et al*., 2013) [10]. Parallel finding by Zafar *et al*. (2014) [14] showed that the zinc application enhanced the proline content in sunflower. Kaolin application, alone, did not have any significant effect on proline content, but by increasing the irrigation period, accompanied by increasing kaolin application resulted in show up trend for proline (Azizi *et al*., 2013) [3]. These are in agreement with the present results obtained.

Effect on endophytes

Ferrous sulphate, kaolin and potassium chloride enhanced the population of endophytic fungi in treated plants whereas; all other treatments did not affect its population and is on par with that of control.

The results have confirmed that potassium di-hydrogen phosphate and potassium containing materials showed consistent superiority over other treatments in mitigating drought stress (Fig. 2) because, plants treated with these chemicals showed high level of drought mitigating factors like higher chlorophyll stability index and proline content with no inhibition of endophytic fungi. The effect of PPFM on drought mitigation was through the improved CSI in the treated plants under stress (drought) condition. Hence, the research work came out with some valuable findings (*ie.* foliar application of potassium di-hydrogen phosphate /

potassium chloride (10g/l) or naphthalene acetic acid (40 ppm) or boric acid (20g/l) or kaolin (30g/l) at fortnightly intervals) could help cardamom farmers for the effective mitigation of drought and those farmers following organic management practices can go with the application of PPFM.

Table 1: Effect of PPFM and synthetic materials on the physico-chemical parameters on leaves and the endophytic microorganisms.

S. No.	Treatment	Chlorophyll Stability Index (%) **	Proline content (µ moles /g sample)	Endophytic Fungi
1	Boric acid	66.78^{c}	6.93^{bcd}	0.00^{c}
2	Diammonium Phosphate (DAP)	53.70^{g}	6.93^{bcd}	0.00^{c}
3	Ferrous sulphate	67.72^{c}	10.39^{ab}	1.00^{a}
4	Kaoline	66.39^{cd}	7.97^{bc}	1.00^{a}
5	Naphthalene Acetic Acid (NAA)	51.29^{h}	18.70^{a}	0.00^{c}
6	Potassium chloride (KCl)	35.00^{i}	9.70^{abc}	0.50^{b}
7	Potassium Dihydrogen Phosphate (KH_2PO_4)	89.72^{a}	10.736^{ab}	0.00^{c}
8	PPFM	60.31^{f}	2.43^{de}	0.00^{c}
9	Proline	78.98^{b}	10.74^{ab}	0.00^{c}
10	Salicylic acid	59.24^{f}	3.46^{cde}	0.00^{c}
11	Urea	64.48^{de}	10.74^{ab}	0.00^{c}
12	Zinc sulphate	63.29^{e}	10.04^{ab}	0.00^{c}
13	Control	23.50^{j}	1.73^{e}	0.00^{c}
	CD (P=0.5)	1.93	1.21	0.222
	CV	1.48	20.18	12.589
	SEm±	0.78	0.31	0.013

*The figures are square root transformed values ** The figures are arc sine transformed values.

Fig 1: Experimental field

Potassium di-hydogen phosphate

Potassium chloride

Control

Naphthalene Acetic Acid

Boric acid

Fig 2: Best treatments *vs* Control

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