GROWTH AND PRODUCTIVITY OF AEROBIC RICE (*Oryza sativa* L.) AS INFULENCED BY PINK PIGMENTED FACULTATIVE METHYLOTROPHS (PPFM)

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(2018-11-046)

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by

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THESIS

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KERALA, INDIA

2020

DECLARATION

I, hereby declare that this thesis entitled **"Growth and productivity of aerobic rice (*Oryza sativa* L.) as influenced by pink pigmented facultative methylotrophs (PPFM)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Growth and productivity of aerobic rice (*Oryza sativa* L.) as influenced by pink pigmented facultative methylotrophs (PPFM)"** is a record of research work done independently by Ms. Aswathy, J.C. under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS AND SYMBOLS

%	per cent
₹	Indian rupee
@	at the rate of
°C	degree Celsius
µg	microgram
B:C ratio	benefit cost ratio
CD	critical difference
cm	centimeter
DMP	dry matter production
dS m^{-1}	deci siemens per metre
EC	electrical conductivity
et al.	co-workers
Fig.	Figure
FYM	farmyard manure
g	gram
ha^{-1}	per hectare
i.e.	that is
K/ K_2O	potassium
PPFM	pink pigmented facultative methylotrophs

kg	kilogram
kg ha^{-1}	kilogram per hectare
ERI	emergence rate index
LAI	leaf area index
m	metre
m^{-2}	per square metre
mg	milligram
mm	millimetre
MOP	Muriate of potash
N	nitrogen
nos.	numbers
NS	not significant
P/ P_2O_5	phosphorus
RH	relative humidity
SE m	standard error of mean
t	tonnes
t ha^{-1}	tonnes per hectare
viz.	namely

APPENDIX

Introduction

1. INTRODUCTION

Rice is considered as the principal food crop of the world as it directly forms the food for more people than any other cultivated crop. Cultivation of rice dates back to 7000 to 5000 BC and the earliest archeological evidences comes from central and eastern China. As rice fulfills about 23 per cent of the caloric demands it is consumed by majority of the world population (Khush, 2005). All these have contributed to the honour conferred to this global grain in the form of two international years in its favour.

Rice is semi-aquatic in nature and is conventionally grown under flooded conditions. Food and water are considered as the basic requirements for survival of human beings. Water, nowadays is becoming a precious commodity due to its excessive use for household, industry and agriculture. About 75 per cent of the rice production is from the irrigated lowland systems, which accounts for 79 m ha. Irrigated rice consumes 3000 to 5000 L of water to produce 1kg of rice (Barker *et al*., 1998). There are predictions that many Asian countries will face serious water crisis by 2025. It is at this juncture that aerobic rice can help farmers who are unable to cultivate conventional lowland rice due to water shortage. Aerobic rice systems have been developed targeting the blending of drought tolerance and yield potential of upland rice and lowland rice respectively.

In the present scenario of climate change, crops are exposed frequently to abiotic stresses, *viz*., drought, elevated temperature, salinity, submergence and nutrient deficiencies. Among the different abiotic stress conditions water scarcity or drought is one of the most severe problems affecting crop production. Rice is one among those crops which has very little adaptation for water stress and shows remarkable sensitivity to drought (Kamoshita *et al*., 2008). On an average, 23 m ha area of rice is affected by insufficient water availability thus affecting the crop production significantly (Pandey *et al.*, 2007). It is of paramount importance that the drought tolerance behavior of crops need to be boosted so as to promote their satisfactory growth and productivity under water scarce situations.

Apart from the use of drought tolerant aerobic rice varieties for cultivation the use of certain microorganisms has also shown improved crop production ability under water scarce or drought conditions. Rhizosphere colonizing bacteria have a significant role in stress tolerance (Sandhya *et al.,* 2011). Plant growth promoting rhizhobacteria (PGPR) such as *Pseudomonas fluorescens* and *Bacillus subtilis* showed characteristics such as improved root colonizing capability, adaptability in catabolic processes and the ability to produce large number of enzymes and metabolites (Mayak *et al*., 2004; Saravanakumar and Samiyappan, 2007). Consequently, these organisms are widely used as inoculants under varied stress conditions to enable the plants to withstand the adverse conditions.

Phylloshpere colonizing *Methylobacterium* produces phytohormones such as cytokinin, auxins and the stress response enzyme aminocyclopropane-1-carboxylic acid (ACC) deaminase (Madhaiyan *et al*., 2005; Chinnadurai *et al.,* 2009). Pink-pigmented facultative methylotrophs (PPFM) is one among the various microorganisms that enable the crops to cope up with adverse environmental conditions, especially water stress. Pink-pigmented facultative methylotrophs, are a group of bacteria, capable of surviving on one-carbon compounds such as formate, formaldehyde, methanol, and methylamine in addition to a variety of C_2, C_3 and C_4 compounds and are classified as alpha-*Proteobacteria* (Lidstrom, 2001). Selective media containing methanol as the sole carbon source can be utilized for easily isolating them from plant tissues using (Corpe, 1985). They are identified by their pink color, which signifies them from other unrelated methylotrophic organisms normally seen on plant tissue.

The exogenous application of PPFM has been documented to counteract the adverse impact of drought through improved germination, growth, development, quality and yield of crops (Hayat *et al.*,2010) and in tomato, the field application of PPFM has been reported to enhance the photosynthetic rate, water status of the plant, compatible osmolytes like proline and anti-oxidant enzymes like catalase activity which protected the plant under drought stress condition (Sivakumar *et al*., 2017).

Considering the ability of PPFM to protect plants under drought stress condition, *in vitro* and *in vivo* screenings were conducted with several PPFM isolates at the Department of Agricultural Microbiology, College of Agriculture, Vellayani. Based on these studies, five isolates were identified for their ability in imparting moisture stress tolerance.

The present study entitled "Growth and productivity of rice (*Oryza sativa* L.) as influenced by pink pigmented facultative methylotrophs (PPFM)" was undertaken to study the field efficiency of the five superior Pink Pigmented Facultative Methylotroph (PPFM) isolates. The main objectives of the study were

- to study the effect of pink pigmented facultative methylotrophs (PPFM) on rice seedling emergence and vigour,
- to assess its effect on growth and yield of aerobic rice
- to work out the economics.

Review of Literature

2. REVIEW OF LITERATURE

Rice is the premier crop of the world which feeds more people than any other agricultural crop. As with the case of other commodities, the demand for rice is also increasing with increase in population. However, with shrinking resources like land and water, this is a daunting challenge. Traditionally, more than 50 per cent of irrigation water used in Asia is consumed by rice (Barker *et al*., 1998). Per capita water availability of 2500 m^3 $year^{-1}$ in 1990 has been expected to dwindle to 1500 m^3 $year^{-1}$ by 2025 suggesting considerable decline in availability of water.

The conventional wetland rice systems consume substantial quantities of water and are inherently labour intensive. In the current scenario of looming water crisis it has become imperative to frame strategies to adopt water saving rice production systems with higher water productivity. The technology of aerobic rice has been designed to make rice cultivation feasible with limited water. In aerobic system of cultivation, rice is raised in non-puddled, non-flooded and unsaturated soil conditions. However, when rice is raised aerobically during the summer season, moisture stress and high atmospheric temperature have found to affect the yield of the crop. Poor grain filling and consequently higher sterility percentage have identified as the prime cause for low yield of aerobic rice in summer. Thus much care is required for aerobic rice during summer season (Jana *et al*., 2013).

Exogenous application of plant growth regulators and plant growth promoting rhizobacteria has yielded promising results (Ashraf and Foolad, 2007). Pink pigmented facultative methylotrophs (PPFMs) are *Methylobaterium* species, whose exogenous application has been reported to help the crops by its action on moisture stress alleviation and improvement in germination, growth and yield (Hayat *et al*., 2010). During dry spell the osmoprotectant matrix produced by PPFM guard the plants from desiccation and high temperature (Irvine *et al*., 2012).

Research work done on the significance of aerobic rice and the effect of PPFM on the performance of crops are reviewed in this chapter. Since the studies conducted on the effect of PPFM on rice is limited, relevant literature on other economically important crops were also reviewed.

2.1.RICE CULTIVATION

Rice is the most extensively consumed staple food ofmore than half of the world's population, providing 21 per cent of global human per capita energy and 15 per cent of per capita protein (Khush, 2005).

In the world, rice (*Oryza sativa L.*) is one of the most important field crops that provide staple food to millions after wheat. It is considered as an indispensable source of calories for majority of the population within Asia. Asia which is the native of 60 per cent of Earth's population produces and consumes about 90 per cent of the rice in the world. Rice occupies an area of 167.13 m ha with a production of 782 million tonnes, covering all continents except Antarctica (FAOSTAT, 2018).

Plants are subjected to several harsh environmental stresses like drought, salinity, low and high temperatures, flood, pollutants, and radiation adversely affect growth, metabolism, and yield and ultimately the productivity of crops (Lawlor, 2002). Enhancing rice production assumes paramount importance despite the constraints and challenges faced by the rice farmers.

In India more than 70 per cent of the people consume rice and hence is the leading food crop and primary cereal as far as Indians are considered and therefore, national food security mainly depends on the production statistics of rice ecosystem. India ranks first in area and production among all the rice growing countries in the world next to China. In India rice alone is cultivated in 43.9 mha with production of around 110.15 million tonnes and a productivity of 2.50t ha^{-1}(GOI, 2016).

Though being the largest producer in rice, the productivity is the lowest among the developing countries which need to be improved. India alone would need about 122 million tonnes of rice for domestic consumption. Among cereal crops, rice consumes about 80 per cent of the total irrigated fresh water resources. The rice production in Asia has declined due to increasing water stress (Tao *et al*., 2004).

Owing to the semi aquatic phylogenetic origin of rice, the crop is more susceptible to drought when compared to other cereal crops. The response of plants to water stress depends on the duration and severity of the stress as well as the developmental stage of the plant (Bartels and Souer, 2004). In the case of rice, the stress during the flowering stage will result in severe yield losses (Liu *et al*., 2006). The water stress during the flowering stage will adversely affect the various physiological processes and it will ultimately lead to decreased spikelet fertility.

In Kerala also, the productivity of crops is adversely affected by drought. In 2009, a huge crop loss of Rs.14.40 crores from 4,000 hectares resulted from drought. Forty seven per cent of paddy cultivation was lost due to drought in 2016 (KSDMC, 2017).

2.2. AEROBIC RICE CULTIVATION

Of the global rice-growing area, about 31 per cent and 11 per cent are being occupied by lowland and upland rainfed rice respectively (Murty and Kondo, 2001; Kamoshita *et al*., 2008).

By 2025, physical water scarcity and economic water scarcity have been predicted in more than 17 m ha and 22 m ha respectively of Asia's irrigated rice. (Tuong and Bouman, 2003). In Asia, upland rice is raised with nominal inputs and is generally managed as a poor yielding subsistence crop in unfavorable upland condition. By 2025, many Asian countries are expected to face severe water scarcity problems. Aerobic rice system is an avenue for the farmers who do not have sufficient water for lowland rice.

The cultivation of aerobic rice offers a promising means to combat the looming water crisis. It can economise 50 per cent of irrigation water compared to the lowland rice (Huaqi *et al*., 2003). Farmers can use 30 to 40 per cent of less irrigation water by growing aerobic rice. However, yield decline to the tune of 10 to 25 per cent is a major concern in tropical aerobic rice (Ghosh *et al*., 2011).

Parthasarathi *et al.* (2012) reported that aerobic rice varieties are capable of producing yield of 4-6 t ha^{-1}even in soils where the moisture content of the soil is below the field capacity.

Aerobic rice is the one that grows well under dry conditions in the absence of flooding. Aerobic rice is a distinctively developed rice cultivation system which encompasses drought tolerance of upland rice and higher yield potential of lowland rice. Thus aerobic rice is designated as improved upland and low land rice in terms of yield potential and drought tolerance respectively (Lal *et al.*, 2013).

Jinsy *et al*. (2015) observed aerobic rice system to be a viable option in the lowlands of southern Kerala during the summer season. Though both upland rice as well as aerobic rice is adapted to aerobic soil conditions, aerobic rice varieties are more receptive to higher level of inputs resulting in enhanced yield than the traditional upland rice varieties. Apart from saving water without affecting the productivity, aerobic rice is found to curb the methane production also.

Thus aerobic rice cultivation is a sustainable rice production technology developed for tackling water scarcity on one hand and ensuring environmental safety arising due to global warming on the other.

2.3. MICROORGANISMS AND DROUGHT

In ecologically sustainable agricultural systems, the bacterial inoculants that provide cross protection against both biotic and abiotic stress would be extremely desirable (van Loon *et al.*, 1998).

Pseudomonas fluorescens and *Bacillus subtilis*, due to their exceptional ability to colonise roots, flexibility in their catabolic activity and their ability to produce a considerable range of metabolites and enzymes have obtained attention as inoculants to support plants under different biotic and abiotic stress conditions (Mayak *et al*., 2004; Saravanakumar and Samiyappan, 2007).

Hayat *et al*. (2010) opined that the exogenous application of PPFM counteracts the adverse effect of drought and improves germination, growth, development, quality and yield of crop plant. Shukla *et al*. (2012) reported that ability to tolerate drought stress and the water holding capacity of rice crop was enhanced by the application of *Trichoderma harzianum*.

Chandra *et al*. (2019) reported that PGPR containing ACC de- aminase serve as a bacterial inoculant for improving the plant growth, predominantly under unfavorable conditions such as floods, droughts, heavy metal toxicity, plant pathogens, drought and salinity.

2.4. PINK PIGMENTED FACULTATIVE METHYLOTROPHS (PPFMs)

Pink pigmented facultative methylotrophs can be easily identified by their pink color, which is not seen in other disparate methylotrophic organisms usually seen on plant tissue and is usually isolated from tissues utilizing specific media containing methanol as the sole carbon source (Corpe, 1985).

The genus *Methylobacterium* commonly known as Pink Pigmented Facultative Methylotrophs (PPFM) bacteria are of ubiquitous in nature. These bacteria are widely found on seeds, plant phyllospheres and in plant rhizosphere (Ivanova *et al*., 2001). The genus *Methylobacterium*comprises a group of pink pigmented facultatively methylotrophic bacteria which are capable of growing on one-carbon compounds like formate, formaldehyde, and methanol as sole carbon and energy sources, including a wide range of multi-carbon growth substrates (Lidstrom, 2001). These bacteria produce a variety of auxins (Ivanova *et al*., 2001) and cytokinins (Lidstrom and Chistoserdova, 2002) which can be utilized by the plants leading to an increased growth and yield.

Utilizing PPFM for crop growth promotion is gaining importance and exploiting the potential of the bacterium can lead to improved plant growth and yield in a sustainable way (Sundaram *et al*., 2002).

Madhaiyan *etal*. (2006) observed that some strains of *Methylobacterium* sp. are capable of synthesizing pectinase and cellulase enzymes which induces systemic resistance during plant colonization. *Methylobacterium* also help plant development by alleviating environmental stresses and breaking down toxic organic compounds, immobilizing heavy metals and restraining plant pathogens (Poorniammal *et al*., 2009) and hence plays a crucial role in balancing the microbes in plants as well as in plant development.

The key characteristic of PPFM is the capacity to oxidise methanol using methanol dehydrogenase enzyme (MDH) (Dourado *et al*., 2015).

Pink pigmented facultative methylobacteria (PPFM) are linked with the roots, leaves and seeds of nearly all terrestrial plants. They can utilize volatile C_1 compounds like methanol produced by plants during the cell division phase. They help to increase the stomatal carbon dioxide concentration resulting in enhanced photosynthetic rate and decreased rate of photorespiration in C_3 plants.

2.4.1. Effect of PPFM on Seed Germination and Seedling Growth

The effect of PPFM on germination of seeds was arbitrated by cytokinin or other plant growth promoters (Holland and Polacco, 1994). The cytokinin produced by PPFMs may be accountable for their impact on seed germination (Freyermuth *et al.,* 1996). Holland (1997) reported that PPFMs can enhance germination and vigour index when used as seed coatings.

Seed germination and early seedling growth are regarded as the most important phases of seed establishment, shaping successful crop production (Uniyal and Nautiyal, 1998).

Methylobacterium are capable of generating plant growth regulators such as auxin and cytokinin which induce cell division and cell elongation (Ivanova *et al.,* 2000).

Sundaram *et al*. (2002) observed that PPFMs affected seed germination and seedling growth in maize by the production of plant growth regulators like zeatin and related cytokinins.

It was reported that the three strains of *Methylobacterium* sp., *viz.*, PPFM-Os-07, *Mextorquens.*AM1 and *M extorquens mia A* mutant enhanced rice seed germination (Madhaiyan *et al*., 2004).Another important component that can influence the crop plant density and yield is the seed vigour index (Siddique and Wright, 2004).

Inoculation with PPFM resulted in increased seedling vigour, dry matter production and yield (Madhaiyan *et al.,* 2004).

PPFMs excrete plant growth hormones auxins and cytokinins that influence germination and root growth which in turn play critical role in a plant's response to stress condition (Doronina *et al*., 2002; Madhaiyan *et al*., 2005).

PPFM helped to improve seed germination through the generation of growth promoting substances like IAA, both in monocots and dicots (Anitha, 2010).

The germination percentage is greatly influenced by polyethylene glycol and mannitol content. A linear decrease in the percentage germination of canola, cauliflower and tomato was observed with an increase in polyethylene glycol and mannitol content. The lowest germination was recorded at highest concentration of polyethylene glycol or mannitol (Hadi *et al*., 2014).

Chandrasekaran *et al*. (2017) reported that under drought condition created by PEG 6000 in tomato, higher germination, greater root length and highest value of vigour index (690.45) were obtained with PPFM (2%) followed by gibberellic acid (617.28) and salicylic acid (551.67). They also observed enhanced shoot length (5.67 cm) when the seeds of tomato under drought condition (created by PEG 6000) were soaked in PPFM (2%). This was followed by gibberellic acid (5.40 cm) and salicylic acid (4.91 cm).

Paddy seeds (var. Jyothi) treated with PPFMs exhibited improved germination, biomass and seedling vigour index (Nysanth *et al*., 2018).Hundred per cent germination was recorded in PPFM 35 treated seeds.

Among the different isolates tested by Riyas (2019), PPFM 26 treated paddy seeds showed maximum germination percentage (87.50%), shoot length (9.47 cm) and seedling vigour index (2143.25).

2.4.2. Effect of PPFM on Growth and Growth Attributes of Rice

Pink Pigmented Facultative Methylotrophs manipulate plant growth by production of phytohormones such as IAA, cytokinins and vitamins (Basile *et al.,* 1985).

A significant enhancement in plant growth, biomass production and yield attributes of groundnut has been observed by the joint inoculation of PPFMs + *Rhizobium* on groundnut cultivar Co (Gn) 4. Maximum plant growth was observed in groundnut at the time of harvest by the inoculation of PPFMs-Ah (Reddy, 2002).

Madhaiyan (2003) observed a considerable increase in the plant height and dry matter production of cotton when inoculated with *Methylobacterium* and methanol than the untreated ones. The inoculation of *Methylotrophs extorquens*PPFMs-GO-71 along with methanol as spray on the phyllosphere appreciably increased the plant height when compared to the control. Plant height of tomato increased significantly by the foliar application of urea in conjunction with *Methylotrophs extorquees* PPFMs-Vm-11 when compared with the control under pot culture condition.

Madhaiyan *et al*. (2005) found that inoculation of PPFMs resulted in notable increase in plant growth, yield and sugar quality in sugarcane.

Lower crop growth rate recorded under stress induced at panicle initiation and flowering stages, resulted in lower crop recovery, leading to reduction in the grain yield (Thangamani, 2005).

Pink pigmented facultative methylotrophs can be regarded as a critical component for plant growth and yield due to their involvement and contribution to the metabolic profile of plants (Lee *et al*., 2006).

Foliar application of PPFM increased the leaf area index, tillering and consequently the crop growth rate of rice (Ajaykumar and Krishnasamy, 2018).This might be due to the result of increased leaf area index. CGR had positive association with leaf area index.

Nysanth (2018) observed that the PPFM inoculation in paddy had significant effect on growth parameters such as plant height, tiller production and leaf area as compared to the control.

In the study conducted by Riyas (2019), maximum plant height of 44.01 cm and 61.17 cm were recorded with PPFM 38 at 30 and 60 DAT whereas PPFM 37 recorded maximum plant height at 90 DAT (85.17 cm) under highest level of water stress condition. Maximum leaf area index of 4.01 and 5.02 were recorded with PPFM 37 at 30 and 60 DAT. The highest mean number of tillers per hill (5.56) was recorded with PPFM 37 at 60 DAT, among the different PPFM isolates tested.

2.4.2.1. Root Traits

The possession of extensive root system which permits access to water in the deeper layers of the soil profile was observed to be imperative in determining drought tolerance in upland rice and sizeable genetic variation was identified for this trait (O'Toole, 1982; Yoshida and Hasegawa, 1982; Ekanayake *et al.,* 1985; Chang *et al*., 1986; Fukai and Cooper, 1995).

Gowda *et al*. (2011) observed an increase in the concentrations of ABA in the roots during drought stress, which in turn helped in sustaining root growth and enhanced root hydraulic conductivity, whereby the effect of water stress could be reduced. Secondary traits such as deep, thick and highly ramified roots along with higher root to shoot ratio have been reported in rice as drought adaptation (Blum, 2011).

Sivakumar *et al*. (2018) observed highest root volume in absolute control (122.80 cm^3) whereas the lowest was recorded in control (97.90 cm^3) in tomato under drought condition. The root volume was increased by improving the lateral root growth through the foliar spray of PGRs and PPFM which in turn helped to alleviate drought.

Under water stress maximum root volume of 1.067 cm^3 was recorded in rice with PPFM 38 at 30 DAT, whereas highest mean root volume of 5.31 cm^3 was recorded with PPFM 37 at 60 DAT. Seeds treated with PPFM 15 gave the maximum root length of 18.38 cm among all the PPFM isolates tested (Riyas, 2019).

2.4.2.2. Root Shoot Ratio

Boyer (1985**)** reported that due to reduced shoot dry weight an increased root to shoot ratio was noted in plants subjected to soil moisture stress. Sharp *et al*. (1994) observed augmentation in the root to shoot dry weight ratio, hindrance in leaf area expansion and development of prolific and deeper roots, by the production of abscisic acid.

Cruz *et al*. (1986) presented that mild stress condition during vegetative stage in rice can decrease the root to shoot ratio by significantly reducing the root dry weight than shoot dry weight.

An increase in the root shoot ratio of rice seedlings has been reported by Nysanth (2018) when the seeds were treated with PPFM isolates. Seeds treated with PPFM 26 and PPFM 35 showed maximum root shoot ratio of 0.62.

Riyas (2019) observed the highest mean root shoot ratio of 0.506 with PPFM 38 and which was significantly higher when compared to all other treatments. The least mean root shoot ratio was observed with the treatment of PPFM 26.

2.4.2.3. Proline Content

Proline is one of the most important amino acids, which occur widely in higher plants that accumulates in large quantities normally in response to environmental stress (Ali *et al.*, 1999).

During oxidative stress proline act as scavenger of OH^- radical and plays a crucial role in osmotic adjustment (Anjum *et al.*,2000). The negative effect of ROS on the membrane lipid and protein, enzymes and DNA is reduced. Under water stress, proline plays an important role to sustain root growth. The concentration of proline has been shown to be generally higher in stress-tolerant than in stress-sensitive plants and the accumulation is mainly correlated with stress tolerance.

It has been suggested by Madhusudhan *et al*. (2002) that under conditions of water stress accumulation of proline contributes to maintain proper balance between extra and intra-cellular osmolarity.

Stress conditions triggered the energy flow of cells to get targeted towards protection mechanisms involving to synthesis of osmolytes like sugars, proline, etc. so that the cells are protected against fluctuations in osmotic conditions (Timmusk, 2003). These osmolytes accumulate at higher levels to alleviate the adverse effects of stress (Rasanen *et al*., 2004).

Proline serves as a compatible solute and a protective agent for cytoplasmic enzymes and structures. Uyprasert *et al.* (2004) stated that when rice is under water stress, the ability of genotypes to maintain water status and consequently delayed tissue death and leaf senescence could be directly linked to high proline accumulation.

In plant stress tolerance, proline accumulation is believed to play an important role (Ashraf and Foolad, 2007). Water stress induces a significant decrease in the metabolic factors such as decrease in the chlorophyll content and an increase in the proline accumulation (Din *et al.*, 2011).

Under drought condition, *Azospirillum* and arbuscular mycorrhizal inoculation was reported increased proline content in rice shoots when compared to control (Sanchez *et al*., 2011).

Sivakumar *et al.* (2017) suggested that the positive effect of PPFM might be due to the increment of osmolytes like proline that enhances the water uptake and maintain the water status of the plant. Foliar application of PPFM (2%) increased the proline content by 11.34 per cent followed by brassinolide and salicylic acid when compared to absolute control.

Phyllosphere isolates were observed to increase the content of proline, total sugars and total amino acids under drought conditions induced by poly ethylene glycol (PEG), as compared against stress-free condition (Kumar *et al*., 2017).

Riyas (2019) observed significantly higher proline content in PPFM 37 treated plants under different moisture levels. At FC, the maximum proline content of 90.18μg g^{-1} tissue was recorded with the PPFM 37 isolate.

2.4.3. Effect of PPFM on Yield Attributes and Yield

Rice is sensitive to moisture stress and the growth stages, *viz*., panicle initiation, anthesis and grain filling are observed to critical (Weisburg *et al*., 1991).

Yield losses are more severe when drought occurs during the reproductive phase as growth slows down during development of panicle, which in turn reduces number of grains and size of grain (Sarkarung, *et al.*,1995).

A higher number of nodules and maximum pod weight was observed in groundnut by the inoculation of PPFM isolate *Methylobacterium* sp. PPFM-Ah (Reddy, 2002).

Foliar application of PPFMs significantly increased the number of bolls, boll weight as well as the kapas yield in cotton (Madhaiyan, 2003). An increase in the seed weight of black gram (7.512 g per plant) was observed over the uninoculated control.

Water deficiency at vegetative and reproductive stages significantly reduced the grain yield of maize as compared to well-watered plant (Sah and Zamora, 2005). When compared to stress-free plants, a reduction of 19.5 per cent and 48.5 per cent were observed due to water deficit in vegetative and reproductive stages, respectively.

Madhaiyan *et al.* (2005) observed that the inoculation of PPFM resulted in appreciable increase in the sugar cane growth, cane yield and quality. In a study conducted by Raja and Sundaram, (2006) a higher boll number, boll weight per plant and seed cotton yield ha^{-1} was observed on the combined application of PPFM, *Azospirillum* and phosphorus solublising bacteria.

Highest maize cob yield was obtained by Radhika *et al*. (2008a) through the foliar application of PPFMs at the rate of 5L ha^{-1}. An enhanced productivity and quality parameters of baby corn was obtained when PPFM was applied at a rate of 5L ha^{-1} (Radhika *et al*., 2008b).

Jaleel *et al*. (2008) observed that drought is a serious environmental factor that adversely affect yield and quality of crops. But yield exhibited a positive response to the application of PGRs and PPFMs. They also observed that rice is sensitive to drought, specifically during flowering stage, resulting in severe yield losses. The yield loss observed with water stress was attributed to the loss in spikelet fertility due to impairment of physiological processes under stress.

Sridevi *et al*. (2017) observed that the foliar application of PPFM along with the recommended dose fertilizer and biofertilizers (Azophosmet as soil and seed treatment) in SRI system gave a higher grain as well as straw yield.

An increase in the fruit yield of tomato by about 35 per cent was observed by Sivakumar *et al*. (2018) on the application of PPFM (2%). Nysanth (2018) reported significant positive effect of PPFM isolates on the yield and yield attributes of paddy. Treatment with PPFM 11 recorded an increase in the yield to the tune of 37.59 per cent as compared against uninoculated control.

Under maximum water stress condition rice grain yield of 5.78 g per hill was recorded with PPFM 37, accounting for an increase of 67.05 per cent in grain yield over the water treated control and 16.53 per cent increase against the reference strain (PPFM 47). Maximum straw yield of 4.69 g per hill was recorded with PPFM 38 under maximum water stress condition (Riyas, 2019).

On the whole, PPFM was observed to possess growth enhancing effects by virtue of its ability to produce plant hormones like auxins and cytokinins and other growth promoting substances. Further, the microbial inoculants has also been reported to confer crops with drought tolerance capacity by inducing better root development and by regulating the osmotic balance within plants.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled "Growth and productivity of aerobic rice (*Oryza sativa* L.) as influenced by pink pigmented facultative methylotrophs (PPFM)" was undertaken, primarily to study the effect of PPFM on rice seedling emergence and vigour, to assess its effect on growth and yield of aerobic rice and to work out the economics. The present study was conducted as two experiments, *viz*., Experiment I: Effect of PPFM on seedling emergence and vigour of rice, and Experiment II: Effect of PPFM on growth and yield of aerobic rice. The materials used and the methods adopted in the conduct of the experiment are presented in this chapter.

3.1 MATERIALS

3.1.1 Experiment I: Effect of PPFM on Seedling Emergence and Vigour of Rice

The experiment was conducted to assess the effect of five superior isolates of PPFM on seedling emergence and vigour of rice.

3.1.1.1 PPFM Isolates

Five isolates of PPFM (PPFM 16, PPFM 26, PPFM 35, PPFM 37 and PPFM 38) from the Department of Agricultural Microbiology, College of Agriculture, Vellayani were tested for their effect on seedling emergence and seedling vigour of rice.

3.1.2 Experiment II: Effect of PPFM on Growth and Yield of Aerobic Rice.

3.1.2.1 Experimental Site

The experiment was conducted at the Integrated Farming System Research Station (IFSRS), Karamana, Thiruvananthapuram, Kerala, India. The experimental site was located at $8^{o}28^{'}25^{''}$N latitude and $76^{o}57^{'}32^{''}$E longitude at an altitude of 5 m above mean sea level.

3.1.2.2 Soil

A composite soil sample was collected from 0-15 cm depth before the experiment and analysed for its physico-chemical properties (Tables 1 and 2). The chemical properties of the soil were rated as per the package of practices recommendations of Kerala Agricultural University (KAU, 2016).

The soil of the experimental site was sandy clay loam in texture, moderately acidic in reaction, high in organic carbon and available phosphorus and low in available nitrogen and available potassium status.

Table 1. Mechanical composition of the soil of experimental site

Sl .No.	Fraction	Content in soil (%)	Method adopted
1	Coarse sand	65.4	Bouyoucos Hydrometer method (Bouyoucos, 1962)
2	Fine sand	6.6	
3	Silt	7.1	
4	Clay	20.0	

Textural class: Sandy clay loam

3.1.2.3 Climate and Season

The experimental site experiences warm humid tropical climate. The experiment was conducted during the summer season of 2019-‘20. The data on weather parameters like air temperature, relative humidity (RH) and rainfall during the cropping period were collected from the agrometeorological observatory maintained at IFSRS, Karamana. The standard week wise weather data during the cropping period are presented in Appendix I and graphically in Fig. 1.

The mean maximum temperature ranged between 31.9^{o}C and 34.1^{o}C, mean minimum temperature between 22.4^{o}C and 25.4^{o}C and mean RH I and RH II ranged between 87.9 per cent and 97.1 per cent and 61.8 per cent and 86.7 per cent, respectively. A total rainfall of 69.3 mm was recorded during the cropping period.

3.1.2.4 Cropping History of the Experimental Site

The area was previously under a bulk crop of rice.

Table 2. Chemical properties of the soil of experimental site

Sl. No	Parameter	Content	Rating	Method adopted
1	Soil reaction (pH)	6.05	Moderately acidic	1:2.5 soil solution ratio using pH meter (Jackson, 1973)
2	Electrical conductivity (dS m^{-1})	0.24	Normal	1:2.5 soil solution ratio using conductivity bridge (Jackson, 1973)
3	Organic carbon (%)	1.80	High	Walkley and Black rapid titration method (Jackson, 1973)
4	Available N (kg ha^{-1})	125.44	Low	Alkaline permanganate method (Subbiah and Asija, 1956)
5	Available P (kg ha^{-1})	32.26	High	Bray colorimetric method (Jackson,1973)
6	Available K (kg ha^{-1})	115.24	Low	Ammonium acetate method (Jackson, 1973)

3.1.2.5 Crop and Variety

The rice variety selected for the study was Uma (MO 16) released from the Rice Research Station, Moncompu, Alappuzha, Kerala. The salient varietal characters are given in Table 3.

Table 3. Important characters of rice variety Uma (MO 16)

Parameter	Character
Stature	Dwarf
Duration (days)	115-120
Tillering	Medium
Grain type	Medium, bold
Kernel colour	Red
Stress tolerance	Resistant to brown plant hopper and gall midge biotype-5
Special characteristics	High yielding variety with seed dormancy upto three weeks, non-lodging and photo insensitive.

(Nair *et al*., 2011; KAU, 2016)

3.1.2.5.1. Source of Seed Material

The seeds of rice variety, Uma (MO 16) was obtained from IFSRS, Karamana, Thiruvananthapuram, Kerala.

3.1.2.6 Manures and Fertilizers

Farmyard manure (FYM) containing 0.55 per cent N, 0.23 per cent P_2O_5 and 0.46 per cent K_2O was used as organic manure. Urea (46 per cent N), Rajphos (20 per cent P_2O_5) and Muriate of potash (60 per cent K_2O) were used as the inorganic sources to supply N, P and K respectively.

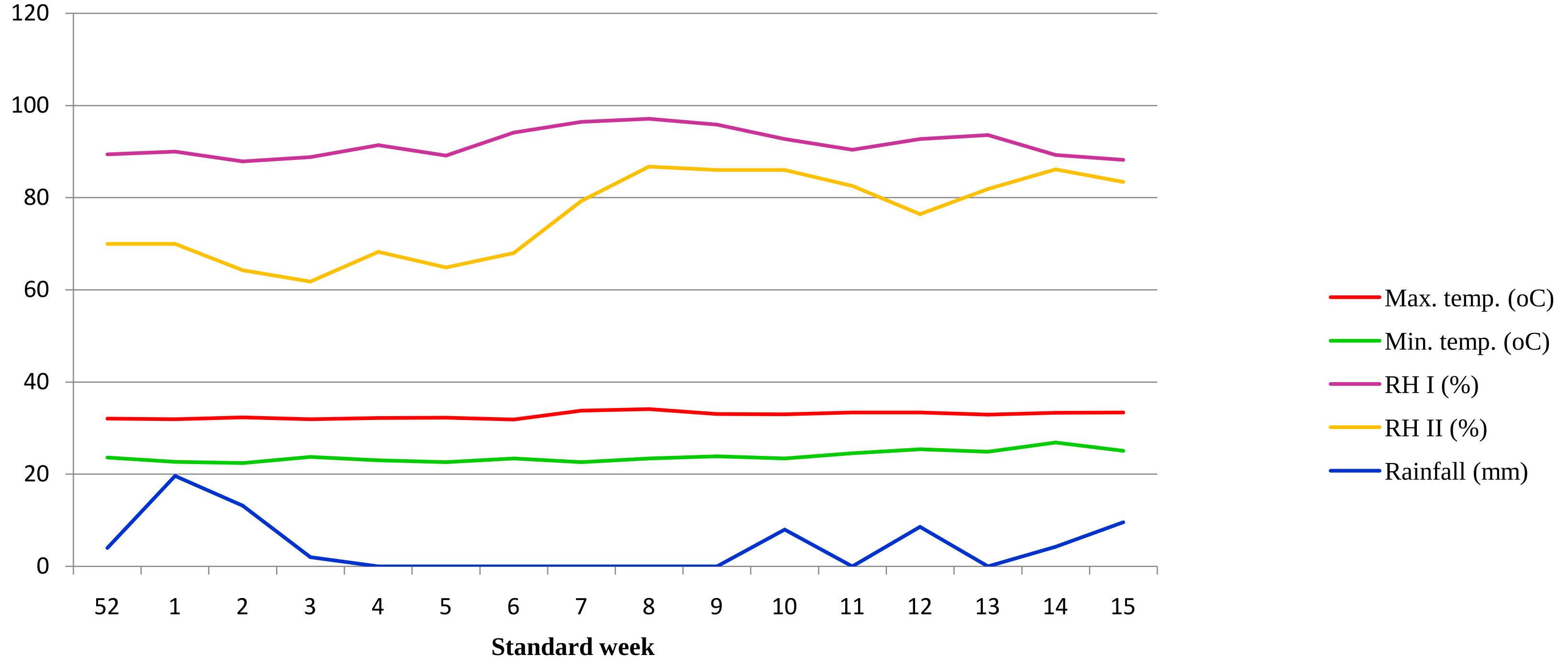

Fig. 1. Weather conditions during the cropping period.

3.2 METHODS

The efficiency of five isolates of PPFM was evaluated with the rice variety Uma (MO 16). The study was conducted as two simultaneous experiments.

3.2.1 Experiment – I : Effect of PPFM on Seedling Emergence and Vigour of Rice

A pot culture study was conducted to assess the effect of five isolates of PPFM on seedling emergence and seedling vigour of rice, as compared against a control.

3.2.1.1 Design and Layout

The experiment was laid out in completely randomised design (CRD) with six treatments replicated four times. The effect of five PPFM isolates was compared with distilled water as control.

The details of the layout are given below.

Design	:	Completely randomized design
Treatments	:	6
Replication	:	4
Variety	:	Uma (MO16)
Season	:	Summer (2019-’20)
Location	:	IFSRS, Karamana

3.2.1.2 Treatments

T_1 : PPFM 16

T_2 : PPFM 26

T_3 : PPFM 35

T_4 : PPFM 37

T_5 : PPFM 38

T_6 : Control

3.2.2 Experiment – II : Effect of PPFM on Growth and Yield of Aerobic Rice

The efficiency of the five PPFM isolates was compared against two controls (KAU POP and KAU POP + water spray).

3.2.2.1 Design and Layout

The details of the layout are given below.

Design	:	Factorial Randomised Block Design
Treatments	:	(5 x 2) + 2
Replication	:	3
Plot size	:	5 m x 4 m
Variety	:	Uma (MO 16)
Season	:	Summer (2019-’20)
Location	:	IFSRS, Karamana.

3.2.2.2 Treatments

Isolates of PPFM (P)

p_1- PPFM 16

p_2- PPFM 26

p_3- PPFM 35

p_4- PPFM 37

p_5- PPFM 38

Method of application (M)

m_1 - seed treatment (1%)

m_2- seed treatment (1%) + foliar application (2%) at 30 and 50 DAS

Control (C)

c_1 - KAU POP

c_2 - KAU POP + water spray at 30 and 50 DAS

The treatment combinations were as follows:

p_1m_1 : seed treatment with PPFM 16

p_1m_2 : seed treatment with PPFM 16 +foliar application at 30 and 50 DAS

p_2m_1 : seed treatment with PPFM 26

p_2m_2 : seed treatment with PPFM 26 + foliar application at 30 and 50 DAS

p_3m_1 : seed treatment with PPFM 35

p_3m_2 : seed treatment with PPFM 35 +foliar application at 30 and 50 DAS

p_4m_1 : seed treatment with PPFM 37

p_4m_2 : seed treatment with PPFM 37+foliar application at 30 and 50 DAS

p_5m_1 : seed treatment with PPFM 38

p_5m_2 : seed treatment with PPFM 38 +foliar application at 30 and 50 DAS

c_1 : KAU POP

c_2 : KAU POP + water spray at 30 and 50 DAS

3.2.3 Crop Management

3.2.3.1 Experiment I : Effect of PPFM on Seedling Emergence and Vigour of Rice

The study was conducted in plastic containers of 1L capacity, filled with the soil collected from the site of the field experiment. Paddy seeds were soaked overnight in the PPFM isolates at 1 per cent concentration and sown at the rate of 75 seeds per container. In control, the seeds were soaked overnight in distilled water and sown.

Plate 1. General view of experiment I

Plate 2.General view of experiment II

3.2.3.2 Experiment II : Effect of PPFM on Growth and Yield of Aerobic Rice

All cultural practices except water management were carried out as per the Package of Practices Recommendations of Kerala Agricultural University (KAU, 2016) for medium duration rice. Irrigation schedule standardized for aerobic rice by Jinsy (2014) was followed. Wherever foliar application of PPFM and water spray were done, irrigation was withheld for one week before and after the treatment.

3.2.3.2.1 Main Field Preparation

The land was thoroughly ploughed and levelled. Weeds and stubbles were removed. The experimental area was divided into three blocks of 12 plots each. The blocks and plots were separated with bunds of 30 cm width. Irrigation and drainage channels were provided for all the plots.

3.2.3.2.2 Seeds and Sowing

Seeds were soaked overnight in the PPFM isolates at 1 per cent concentration and dibbled at a spacing of 20 cm x 10 cm. With respect to the control treatments (c_1 and c_2) the seeds were soaked in water overnight and dibbled at the same spacing.

3.2.3.2.3 Application of Manures and Fertilizers

At the time of land preparation, well decomposed dry cow dung was applied to all the plots @ 5 t ha^{-1}. A nutrient recommendation of 90:45:45 kg NPK $ha^{-1}$was adopted uniformly for all the plots. N, P_2O_5 and K_2O were supplied by the application of Urea, Rajphos and Muriate of potash respectively. The entire dose of P, one-third N and half K were applied basally (10 days after sowing). The remaining N and K were applied in two splits, one-third N at tillering, one-third N and half K at seven days prior to panicle initiation (PI) stage (KAU, 2016). Gap filling and thinning were done two weeks after sowing, maintaining two seedlings per hill.

Plate 3. Seed treatment with PPFM (1%) and distilled water.

Plate 4. Foliar application of the treatments.

3.2.3.2.4 Irrigation

The irrigation method standardized for aerobic rice was followed. Accordingly, irrigation was given fixing the depth of irrigation as 2.24 cm (Jinsy, 2014). Irrigation was given once in three days up to panicle initiation stage. Thereafter daily irrigation was given. Irrigation was stopped one week prior to harvest, for promoting uniform maturity of grains. In the case of PFFM foliar treatments and KAU POP + water spray (c_2), irrigation was withheld one week before and after the spraying of PPFM / water.

3.2.3.2.5 Foliar Application of PPFM

The liquid cultures of the five isolates of PPFM were sprayed at 2 per cent concentration on high volume basis, as per the treatments proposed.

3.2.3.2.6 Weed Management

Proper weed management measures were adopted as and when required. The major method adopted was hand weeding.

3.2.3.2.7 Plant Protection

Incidence of caseworm and leaf roller was observed at 25 days after sowing. The pests were managed with two sprays of flubendiamide at the rate of 2mL per 10 L of water, at one week interval. A combined application of tetracycline (6g) and copperoxychloride (15g) per 10 L of water was given for controlling bacterial leaf blight.

3.2.3.2.8 Harvest

The crop was harvested when the grains attained maturity. Two border rows were left on all the sides and the net plot area was harvested, threshed, winnowed and dried separately. The weight of grains and straw from individual plots were recorded and expressed in kg ha^{-1}on dry weight basis.

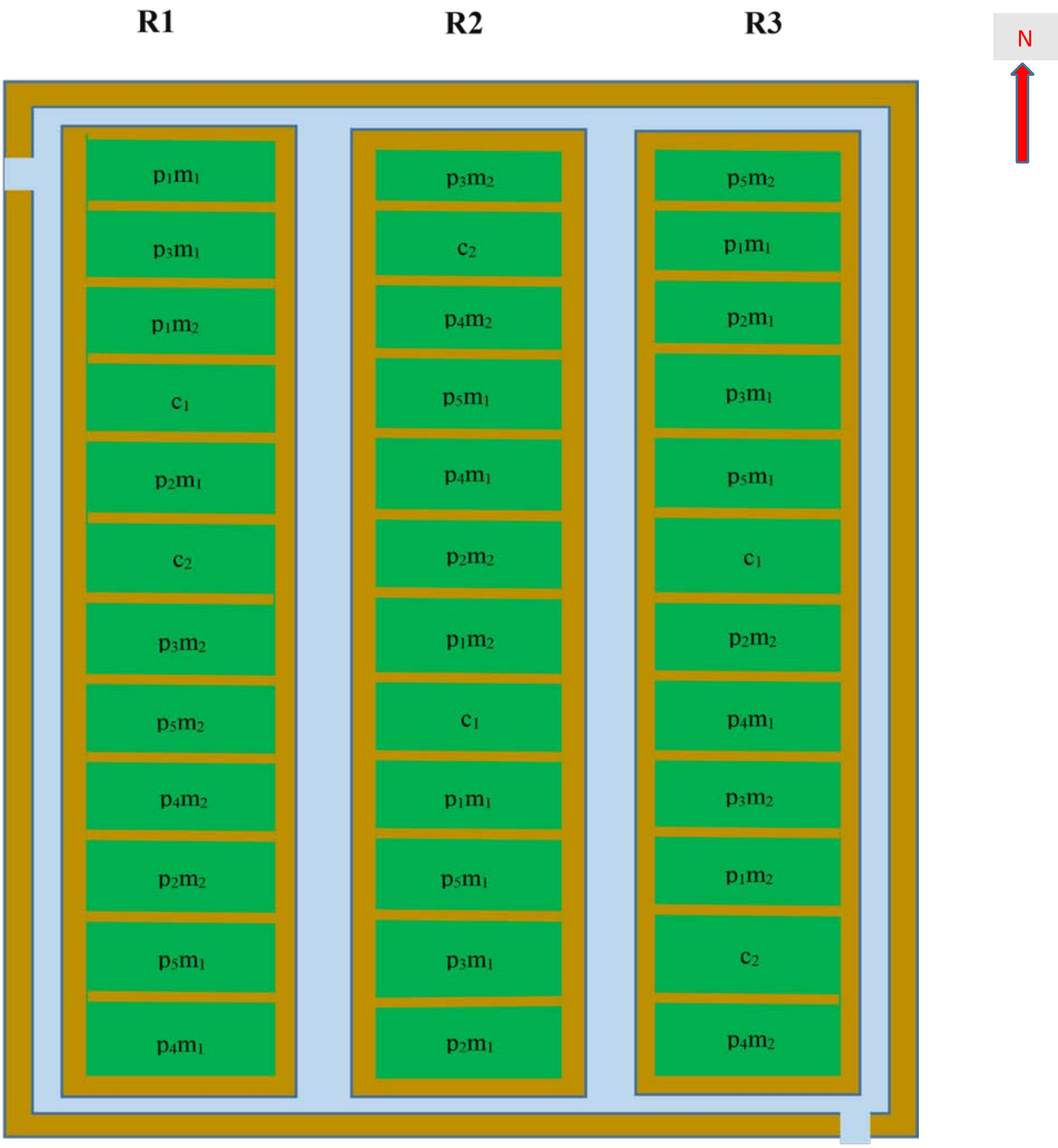

Fig. 2. Layout of the experimental field.

Channel

Bund

3.3 OBSERVATIONS

3.3.1 Experiment I : Effect of PPFM on Seedling Emergence and Vigour of Rice

3.3.1.1 Emergence Percentage

Emergence percentage was calculated after14 days.

$$\text{Emergence percentage (\%)} = \frac{\text{Number of seeds emerged x 100}}{\text{Total number of seeds sown}}$$

3.3.1.2 Rate of Emergence

Rate of emergence was expressed as emergence rate index (ERI) as proposed by Shmueli and Goldberg (1971)

$$\text{ERI}= \sum_{n=n0}^{n=c-1} Xn\ (c-n)$$

Xn - number of emerged seedling counted on day n.

c - number of days counted from sowing until day of emergence.

n - day of counting expressed as number of DAS

n_0 - day when emergence started expressed as number of DAS.

3.3.1.3 Shoot Length

Shoot length was measured from the collar region to the tip of the longest leaf at 14 days of growth. It was expressed in cm.

3.3.1.4 Root Length

Root length was measured from base of the stem to the tip of the root at 14 days of growth and was expressed in cm.

3.3.1.5 Dry Weight of Seedling

The dry weight of seedlings was taken after drying the whole plant samples at 60ºC in a hot air oven. It was expressed in mg.

3.3.1.6 Seedling Vigour Index

Seedling vigour index was calculated by using the equation proposed by Abdul-Baki and Anderson (1973).

Seedling vigour index I = (shoot length + root length) x dry matter production

Seedling vigour index II= (dry matter production) x germination percentage

3.3.2 Experiment – II : Effect of PPFM on Growth and Yield of Aerobic Rice

3.3.2.1 Growth and Growth Attributes

Two rows from all sides of each plot were left as border rows. Six hills were selected randomly from the net plot area of each plot and tagged as sample plants. The following observations were recorded from the sample plants and the mean values were worked out.

3.3.2.1.1 Plant Height

The height of the plant was measured from the base to the growing tip of the top most leaf at 20, 40, 60 DAS and from the base to the tip of the longest panicle at harvest. Plant height was recorded in centimeters (cm).

3.3.2.1.2 Tillers m^{-2}

Tiller count was recorded from the tagged hills at 20, 40, 60 DAS and harvest and the mean was worked out and expressed as tillers m^{-2}.

3.3.2.1.3 Root Volume

Root volume per plant was found out by displacement method and expressed in cm^{3} plant^{-1}.

3.3.2.1.4 Root Shoot Ratio

The shoot and root dry weight were recorded at harvest, after drying the sample plants at 60^{o}C in a hot air oven and the weights were expressed in g. Root shoot ratio was worked out from the dry weight of shoot and root at harvest, using the equation,

$$\text{Root shoot ratio} = \frac{\text{Dry weight of root (g)}}{\text{Dry weight of shoot (g)}}$$

3.3.2.1.5 Leaf Area Index

The leaf area index (LAI) was calculated at 20, 40, 60 DAS and at harvest stages using the method suggested by Yoshida *et al*. (1976). The maximum length 'l' and width 'w' of all the leaves of the middle tiller of the six sample hills were recorded from all the plots and leaf area index was calculated.

Leaf area index of a single leaf = l x w x k

k- Adjustment factor

(0.75 at seedling, maximum tillering, PI, and flowering stages and 0.67 at harvest).

LAI was calculated as follows

$$\text{LAI} = \frac{\text{Sum of leaf area/hill of 6 sample hill (cm}^2\text{)}}{\text{Area of land covered by the 6 sample hills (cm}^2\text{)}}$$

3.3.2.1.6 Dry Matter Production

At harvest, the sample hills were uprooted from each plot, washed and dried in shade. Grain and straw were oven dried at 65±5 C till constant weight was attained. Total dry matter production was computed as the sum of the dry weights of grain and straw for each treatment and expressed in kg ha^{-1}.

3.3.2.2 Yield Attributes and Yield

3.3.2.2.1 Productive Tillers m^{-2}

At harvest, the number of productive tillers was counted from the observation hills and expressed as number of productive tillers m^{-2}.

3.3.2.2.2 Grain Weight per Panicle

Grains from the 12 randomly selected panicles were removed, dried, weighed and the weight was recorded as grain weight per panicle and expressed in grams (g).

3.3.2.2.3 Filled Grains per Panicle

The filled grains were counted from the 12 randomly selected panicles from each plot and expressed as the mean number of filled grains per panicle.

3.3.2.2.4 Sterility Percentage

Sterility percentage was worked out using the following relationship

$$\text{Sterility percentage} = \frac{\text{Number of unfilled grains per panicle x 100}}{\text{Total number of grains per panicle}}$$

3.3.2.2.5 Thousand Grain Weight

One thousand bold grains were counted from the cleaned and dried produce from the net plot area of each plot and the weight of the grains was recorded in grams (g).

3.3.2.2.6 Grain Yield

The net plot area was harvested individually, threshed, winnowed, dried and weight was recorded and expressed in t ha^{-1} at 14 per cent moisture.

3.3.2.2.7 Straw Yield

The straw obtained from net plot area sun-dried to constant weight, and the weight was expressed in t ha^{-1}.

3.3.2.2.8 Harvest Index (HI)

Harvest index (HI) was calculated using the formula put forth by Donald and Hamblin (1976).

$$\text{HI} = \frac{\text{Economic yield}}{\text{Biological yield}}$$

3.3.2.3 Physiological Parameters

3.3.2.3.1 Chlorophyll Content

The chlorophyll content of fresh leaf sample was estimated using the DMSO (Di methyl sulphoxide) method suggested by Yoshida *et al*. (1976) at panicle initiation and flowering stages and expressed in mg g^{-1} of leaf tissue.

3.3.2.3.2 Proline Content

Proline content of the leaves was estimated by the method described by Bates *et al*. (1973) and expressed asµg mol^{-1} of fresh weight.

3.4 CHEMICAL ANALYSIS

3.4.1 Plant Analysis

The plant samples were collected after harvest, dried in a hot air oven at 65 ± 5 ^{o}C to constant weight, ground and sieved through a 0.5 mm sieve. The required quantity of samples were weighed, subjected to acid extraction and analysed. Grain and plant samples were analysed separately.

3.4.1.1 Uptake of Nitrogen

The nitrogen content was analysed by using the modified micro Kjeldahl method suggested by Jackson (1973). Nitrogen uptake was computed as the product of the nitrogen content and total dry matter production and expressed in kg ha^{-1}.

3.4.1.2 Uptake of Phosphorus

The phosphorus content was analysed by vanadomolybdo phosphoric yellow colour method (Jackson, 1973) and the uptake was computed in kg ha^{-1}.

3.4.1.3 Uptake of Potassium

The potassium content was analysed by using flame photometer method and the uptake was determined by multiplying it with total dry matter production and expressed in kg ha^{-1} (Jackson 1973)

3.4.1.4 Crude Protein Content

The nitrogen content of plant samples was estimated and then multiplied by a factor of 6.25 to obtain the crude protein content (Simpson *et al*., 1965) and expressed in percentage.

3.4.2 Soil Analysis

Before the start of the experiment, composite soil samples were collected and analysed to determine the soil reaction (pH), organic carbon, available nitrogen, available phosphorus and available potassium status of the soil. The mechanical composition of the soil was also determined. After the harvest of the crop, soil samples were taken separately from each plot and analysed for organic carbon available nitrogen, phosphorus and potassium status, adopting the procedures listed out in Table 2.

3.5 ECONOMIC ANALYSIS

The economics of cultivation was expressed in terms of gross returns, net returns and benefit cost ratio based on cost of cultivation and prevailing price of produce.

3.5.1 Gross Returns

The gross returns was worked out for all the treatments as the product of the quantity of produce (grain and straw) and the prevailing market price of the produce.

3.5.2 Net Returns

Cost of cultivation was deducted from gross returns to obtain the net income

$$\text{Net returns } (₹\ ha^{-1}) = \text{Gross returns } (₹\ ha^{-1}) - \text{Cost of cultivation } (₹\ ha^{-1})$$

3.5.3 B: C Ratio

The benefit-cost ratio (BCR) was worked out by using the following formula

$$\text{B:C Ratio} = \frac{\text{Gross Returns}}{\text{Cost of Cultivation}}$$

3.6 STATISTICAL ANALYSIS

EXPERIMENT – I

3.6.1 Effect of PPFM on Seedling Emergence and Vigour of Rice

Data generated were statistically analysed using analysis of variance (ANOVA) technique, as applied to completely randomized design. Critical difference was worked out at 5 per cent level of probability, wherever the treatment differences were found significant. The significance of treatment mean vs control mean was tested by calculating the f value. The f value was calculated by subtracting factorial sum of squares from treatment sum of squares and then dividing with error sum of squares.

EXPERIMENT – II

3.6.2 Effect of PPFM on Growth and Yield of Aerobic Rice

The data were analyzed using ANOVA as applicable to Randomized Block Design (Gomez and Gomez, 1984). Two way ANOVA for 2 factors (A- PPFM treatments, B-method of application) was carried out and critical difference (CD) was calculated based on their significance. The significance of treatment mean *vs* control mean was tested by calculating the f value. The f value was calculated by subtracting factorial sum of squares from treatment sum of squares and then dividing with error sum of squares.

Results

4. RESULTS

The experiment entitled "Growth and productivity of aerobic rice (*Oryza sativa* L.) as influenced by pink pigmented facultative methylotrophs (PPFM)" was undertaken, at IFSRS, Karamana, Thiruvananthapuram, Kerala, from December 2019 to April 2020. The objectives were to study the effect of PPFM on rice seedling emergence and vigour, to assess its effect on growth and yield of aerobic rice and to work out the economics. The study was conducted as two experiments, Experiment I: Effect of PPFM on seedling emergence and vigour of rice, and Experiment II: Effect of PPFM on growth and yield of aerobic rice. The results of the experiments are presented in this chapter.

4.1 EXPERIMENT – I

EFFECT OF PPFM ON SEEDLING EMERGENCE AND VIGOUR OF RICE

4.1.1Emergence Percentage

The results on the effect of PPFM on emergence percentage of rice are presented in Table 4.

Hundred per cent emergence was observed in all the PPFM treated seeds, irrespective of the isolate tested. The seeds treated with distilled water recorded 95 per cent emergence.

4.1.2 Emergence Rate Index

The results on the emergence rate index as influenced by PPFM are presented in Table 4.

The emergence rate index was recorded to be the highest (160) when the seeds were treated with PPFM 38, followed by PPFM 37 (156). The emergence rate index was the lowest for seeds treated with distilled water (control). The mean emergence rate index was observed to be 149.6 for PPFM treated seeds as against 121 recorded by the control.

4.1.3 Shoot Length

The data on the effect of PPFM on shoot length of rice seedlings are presented in Table 5.

Treating rice seeds with PPFM did not have significant effect on the shoot length of rice seedlings.

4.1.4 Root Length

The results on the variation in root length of rice seedlings as influenced by PPFM are presented in Table 5.

Root length of rice seedlings exhibited significant variation due to seed treatment with PPFM. Seed treatment with PPFM 38 recorded significantly longer roots during seedling stage (13.605 cm). It was on a par with the root length recorded with PPFM 16 and PPFM 26. On the whole the mean root length as influenced by PPFM was 12.246 cm. Among the different treatments, seed treatment with distilled water (control) produced seedlings with the lowest root length (10.475 cm).

4.1.5 Dry Weight of Seedling

The data pertaining to the effect of PPFM on dry weight of rice seedlings is presented in Table 6.

Pink pigmented facultative methylotrophs had significant effect on the dry weight of seedlings. The islolate, PPFM 38 resulted in significantly higher seedling dry weight (0.198 g per seedling), followed by PPFM 37. However it was comparable with all other isolates except PPFM 16. While seed treatment with PPFM recorded a mean seedling dry weight of 0.184 g per plant, the same was only 0.135 g per seedling with distilled water treated seeds.

4.1.6 Seedling Vigour Index

The results on the effect of PPFM on seedling vigour index, computed as vigour index I and II of rice are presented in Table 6.

Seedling vigour index I varied significantly with PPFM 38 recording higher value (7.64). However, it remained at par with the effect of all the other PPFM isolates tested. The seedling vigour index I of seeds treated with distilled water (control) was the lowest (4.62).

Seedling vigour index II was observed to be significantly higher (19.75) for seeds treated with PPFM 37, and the effect of all the PPFM isolates were comparable to one another. Seed treatment with distilled water recorded the lowest value (12.15) for seedling vigour index II.

4.2 EXPERIMENT – II

EFFECT OF PPFM ON GROWTH AND YIELD OF AEROBIC RICE

4.2.1 Growth and Growth Attributes

4.2.1.1 Plant Height

The results on the effect of PPFM and method of application on plant height of rice are presented in Table 7.

The PPFM isolates and method of application had no significant effect on plant height at 20 DAS, 40 DAS and 60 DAS. However, plant height recorded at harvest was observed to vary significantly in response to both these factors. Among the five isolates tested, PPFM 38 recorded significantly taller (98.64 cm) plants followed by PPFM 35. Between the two methods of application, seed treatment (1%) + foliar application (2%) at 30 and 50 DAS resulted in taller plants.

The interaction effect between PPFM and method of application was observed to be significant at 40 and 60 DAS and at harvest. The treatment combination p_5m_2 (PPFM 38 as seed treatment and foliar application) was significantly superior at all the three above growth stages. At 40 and 60 DAS p_5m_2 was on a par with p_5m_1, p_3m_2 and p_3m_1.

The PPFM treatments were significantly superior to the two controls, *viz*., KAU POP (c_1) and KAU POP + water spray (c_2).

Table 4. Effect of PPFM on emergence percentage and emergence rate index of rice

Treatment	Emergence percentage	Emergence rate index
T1 : PPFM 16	100	152
T2 : PPFM 26	100	133
T3 : PPFM 35	100	147
T4 : PPFM 37	100	156
T5 : PPFM 38	100	160
T6 :Control (distilled water)	95	121

Table 5. Effect of PPFM on shoot length and root length of rice seedlings, cm.

Treatment	Shoot length	Root length
T1 : PPFM 16	23.685	13.440
T2 : PPFM 26	22.840	12.435
T3 : PPFM 35	24.600	10.705
T4 : PPFM 37	26.425	11.045
T5 : PPFM 38	26.665	13.605
T6 :Control (distilled water)	23.710	10.475
SE m (±)	0.958	0.690
CD (0.05)	NS	2.0920

Table 6. Effect of PPFM on dry weight of seedling, seedling vigour index I and seedling vigour index II of rice

Treatment	Dry weight of seedling (g per seedling)	Seedling vigour index I	Seedling vigour index II
T1 : PPFM 16	0.165	6.20	16.75
T2 : PPFM 26	0.188	6.62	18.75
T3 : PPFM 35	0.178	6.24	17.75
T4 : PPFM 37	0.190	7.40	19.75
T5 : PPFM 38	0.198	7.64	19.00
T6 :Control (distilled water)	0.135	4.62	12.15
SE m (±)	0.011	0.43	1.05
CD (0.05)	0.0320	1.301	3.145

4.2.1.2 Tillers m^{-2}

The data on the number of tillers per square metre as influenced by PPFM and method of application are presented in Table 7.

Tiller production of rice recorded at 20, 40 and 60 DAS and at harvest were observed to vary significantly with PPFM and method of application. Number of tillers per square metre was significantly more when treated with PPFM 38. It was comparable with the effect of PPFM 26 at 20 and 60 DAS.

Method of application had significant effect on tiller production at 60 DAS and at harvest with m_2 (seed treatment – 1% + foliar application – 2% at 30 and 50 DAS) recording higher tiller count during these stages.

The combined effect of PPFM and method of application was significant at all the growth stages. The treatment combination p_5m_2 (PPFM 38 as seed treatment and foliar application) was superior at 20 and 40 DAS and at harvest. It was on par with p_5m_1, p_3m_1 and p_2m_2 at 20 DAS, p_5m_1, p_4m_2, p_3m_2 and p_1m_1 at 40 DAS and with p_5m_1 at harvest. At 60 DAS, seed treatment with PPFM 38 (p_5m_1) was significantly superior and was comparable with p_5m_2, p_4m_2 and p_2m_2.Number of tillers per square metre was significantly higher for PPFM treatment as compared to the two controls, *viz*., KAU POP (c_1) and KAU POP + water spray (c_2).

4.2.1.3 Leaf Area Index

The results on the response of leaf area index to PPFM and its method of application are presented in Table 8 and graphically in Fig. 3 and Fig. 4.

Leaf area index exhibited significant variation under the influence of the PPFM isolates tested with PPFM 38 proving to be superior at 20, 40 and 60 DAS and at harvest. At 60 DAS, the effect of PPFM 38 and PPFM 26 were comparable.

The significance of the effect of method of application was evident at 60 DAS. Application of PPFM as seed treatment (1%) + foliar application was found significant.

Table 7. Effect of PPFM and method of application on plant height and tillers m^{-2} of rice

Treatment	Plant height (cm)				Tillers m^{-2} (nos.)			
	20 DAS	40 DAS	60 DAS	At harvest	20 DAS	40 DAS	60 DAS	At harvest
PPFM (P)								
p_1 – PPFM 16	34.17	46.65	56.67	96.19	70.33	299.00	440.50	463.83
p_2 – PPFM 26	35.23	46.49	56.68	94.38	73.00	295.67	446.33	463.00
p_3 – PPFM 35	37.28	47.79	57.97	96.26	70.10	296.33	435.83	443.33
p_4 – PPFM 37	34.43	45.88	55.29	93.13	67.33	292.67	438.00	442.83
p_5 – PPFM 38	35.78	48.20	58.86	98.64	78.33	306.33	447.00	497.83
SE (m) ±	1.19	0.56	0.86	0.64	2.70	2.54	0.67	3.25
CD (0.05)	NS	NS	NS	1.347	5.678	5.339	1.406	6.842
Method of application (M)								
m_1 - seed treatment (1%)	34.94	46.69	56.97	95.32	72.20	296.53	442.46	466.07
m_2 - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	35.79	47.32	57.17	96.32	71.47	299.53	442.47	458.27
SE m (±)	1.19	0.56	0.86	0.64	2.70	2.70	0.67	3.25
CD (0.05)	NS	NS	NS	0.851	NS	NS	0.889	4.328
PPFM (P) x Method of application (M)								
p_1m_1 (PPFM 16 - seed treatment)	33.72	46.27	56.85	97.08	72.00	301.67	445.33	486.00
p_1m_2 (PPFM 16 - seed treatment + foliar application)	34.63	47.03	56.28	95.30	68.67	296.33	435.67	441.67
p_2m_1 (PPFM 26 - seed treatment)	35.53	46.44	57.30	96.72	71.67	296.00	444.33	461.00
p_2m_2 (PPFM 26 - seed treatment + foliar application)	34.92	46.54	56.05	92.03	74.33	295.33	448.33	465.00
p_3m_1 (PPFM 35 - seed treatment)	37.15	47.87	58.27	97.07	76.67	294.67	435.67	446.00
p_3m_2 (PPFM 35 - seed treatment + foliar application)	37.32	47.71	57.66	96.17	63.67	298.00	436.00	440.67
p_4m_1 (PPFM 37 - seed treatment)	33.06	45.05	54.37	88.29	63.33	282.00	429.67	440.67
p_4m_2 (PPFM 37 - seed treatment + foliar application)	35.80	46.71	56.22	97.96	71.33	303.33	446.33	445.00
p_5m_1 (PPFM 38 - seed treatment)	35.26	47.82	58.08	97.15	77.33	308.00	448.00	496.67
p_5m_2 (PPFM 38 - seed treatment + foliar application)	36.29	48.59	59.64	100.13	79.33	304.66	446.00	499.00
Control (C)								
c_1 - KAU POP	33.49	43.71	53.08	85.83	53.33	288.67	427.67	407.67
c_2 – KAU POP + water spray	33.83	44.40	54.00	86.21	65.33	285.33	425.67	407.33
SEm (±)	1.30	0.60	0.78	0.66	2.49	2.38	0.36	4.15
CD (0.05)	NS	1.766	2.277	1.931	7.310	6.976	1.997	12.198
Treatment *vs*. Control1	NS	S	S	S	S	S	S	S
Treatment *vs*. Control 2	NS	S	S	S	S	S	S	S

The interaction effect between PPFM and method of application was found to be significant at all the above growth stages. Among the combinations, the effect of p_5m_2 (PPFM 38 as seed treatment and foliar application) was superior at 20, 40 and 60 DAS and at harvest. It was on par with p_5m_1 at 20 and 40 DAS and at harvest and with p_4m_2 at 60 DAS.

The treatments were significantly superior to both the controls. Between the two controls, c1 (KAU POP) was marginally better than c_2 (KAU POP + water spray).

4.2.1.4 Dry Matter Production

The variation in dry matter production as influenced by PPFM and method of application is presented in Table 8 and graphically in Fig.5 and Fig. 6.

Among the PPFM isolates tested, treating rice with PPFM 38 resulted in significantly higher dry matter production at harvest (9287 kg ha^{-1}). The effect of all the other four isolates was at par.

Between the two methods of application, m_2 (seed treatment + foliar application) recorded higher dry matter production (8107 kg ha^{-1}) than seed treatment alone (m_1).

The interaction effect between PPFM and method of application was significant with respect to dry matter production of rice. Seed treatment followed by foliar application of PPFM 38 (p_5m_2) recorded significantly higher dry matter production (9607kg ha^{-1}) than the other treatment combinations, followed by p_5m_1 (8966kg ha^{-1}).

Between the two controls, KAU POP recorded higher dry matter production (7885 kg ha^{-1}) than KAU POP + water spray (6197 kg ha^{-1}).The dry matter production of the treatments and KAU POP (c_1) did not vary significantly. However, the treatments were significantly superior to KAU POP + water spray (c_2).

Table 8. Effect of PPFM and method of application on leaf area index and dry matter production of rice

Treatment	Leaf area index				Dry matter production (kg ha^{-1})
	20 DAS	40 DAS	60 DAS	At harvest	
PPFM (P)					
p_1 – PPFM 16	0.787	2.10	3.99	4.82	7768
p_2 – PPFM26	0.790	2.00	4.32	4.57	7660
p_3 – PPFM 35	0.775	2.02	4.19	4.51	7386
p_4 – PPFM 37	0.670	2.03	3.92	4.46	7217
p_5 – PPFM 38	0.860	2.32	4.57	5.18	9287
SE m (±)	0.008	0.03	0.11	0.13	353
CD (0.05)	0.0185	0.076	0.252	0.278	741.9
Method of application (M)					
m_1 - seed treatment (1%)	0.780	2.10	4.06	4.76	7620
m_2 - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	0.772	2.09	4.33	4.66	8107
SE m (±)	0.008	0.03	0.11	0.13	353
CD (0.05)	NS	NS	0.159	NS	469.2
PPFM (P) x Method of application (M)					
p_1m_1(PPFM 16 - seed treatment)	0.870	2.16	4.18	5.12	7456
p_1m_2(PPFM 16 - seed treatment + foliar application)	0.703	2.03	3.79	4.51	8080
p_2m_1(PPFM 26 - seed treatment)	0.853	2.04	4.36	4.69	7237
p_2m_2(PPFM 26 - seed treatment +foliar application)	0.727	1.97	4.28	4.46	7535
p_3m_1(PPFM 35 - seed treatment)	0.797	2.01	4.01	4.56	7456
p_3m_2(PPFM 35 - seed treatment +foliar application)	0.753	2.02	4.35	4.46	7863
p_4m_1(PPFM 37 - seed treatment)	0.527	1.97	3.34	4.32	6985
p_4m_2(PPFM 37 - seed treatment +foliar application)	0.813	2.10	4.50	4.60	7449
p_5m_1(PPFM 38 - seed treatment)	0.853	2.31	4.39	5.08	8966
p_5m_2(PPFM 38 - seed treatment +foliar application)	0.867	2.33	4.74	5.27	9607
Control (C)					
c_1 - KAU POP	0.623	1.72	3.42	3.59	7885
c_2 – KAU POP + water spray	0.613	1.68	3.41	3.57	6197
SE m (±)	0.008	0.05	0.11	0.12	384
CD (0.05)	0.0247	0.174	0.329	0.359	1126.3
Treatment *vs*. Control1	S	S	S	S	NS
Treatment *vs*. Control 2	S	S	S	S	S

4.2.1.5 Root Volume

The results on the effect of PPFM on the root volume of rice are presented in Table 9.

The isolate PPFM 16, showed significantly superior effect on root volume (33.33 cm^3 $hill^{-1}$) and it was comparable with that of PPFM 38, PPFM 26 and PPFM 37.

Root volume of rice did not vary significantly between the two methods of application tested for PPFM.

The results on the effect of PPFM over methods of application revealed significantly higher root volume for the treatment combination p_1m_1 (PPFM 16 as seed treatment). However, the root volume was comparable with that recorded by p_5m_1, p_5m_2, p_4m_2, p_1m_2 and p_2m_2.

The control c_2 (KAU POP + water spray) registered higher root volume (20 cm^3 $hill^{-1}$) than the KAU POP (16.66 cm^3 $hill^{-1}$).

Root volume of rice was significantly higher with PPFM treatment when compared to KAU POP (c_1) and KAU POP + water spray (c_2).

4.2.1.6 Root Shoot Ratio

The effect of PPFM and method of application on the root shoot ratio of rice is presented in Table 9.

Treating rice with PPFM 38 (p_5) was observed to record significantly higher root shoot ratio of 0.47. It was followed by PPFM 26 (0.38).

The treatment combination p_5m_2 (PPFM 38 as seed treatment + foliar application) recorded superior root shoot ratio (0.67).

Between the two controls root shoot ratio was higher for KAU POP (c_1) than KAU POP + water spray (c_2).

The treatment effect was significantly superior to both the controls.

4.2.2 Yield Attributes and Yield

4.2.2.1 Productive tillers m $^{-2}$

The variation in productive tillers per square metre as influenced by PPFM and its method of application is presented in Table 10.

Among the PPFM isolates tested, the treatment with PPFM 38 exhibited significantly higher number of productive tillers per square metre (423) followed by PPFM 16 (402.17). The effect of PPFM 16 was comparable with that of PPFM 35.

The method of application m_2 (seed treatment + foliar application) showed significantly higher number of productive tillers per square metre when compared to m_1 (seed treatment).

The treatment combination p_5m_2 (PPFM 38 as seed treatment + foliar application) resulted in significantly higher number of productive tillers per metre square (424.67) which was on par with p_5m_1.

Between the two controls, c_1 (KAU POP) exhibited significantly higher number of productive tillers per metre square thanc_2 (KAU POP + water spray). The treatment effect was significantly superior to both the controls.

4.2.2.2 Grain Weight per Panicle

The effect of PPFM and method of application on the grain weight per panicle of rice is presented in Table 10.

The isolate PPFM 38, showed a significantly superior grain weight per panicle (3.01 g) when compared to other isolates. It was followed by PPFM 37 (2.68 g) which was on par with PPFM 35.

Table 9. Effect of PPFM and method of application on root volume and root shoot ratio of rice

Treatment	Root volume (cm^3 $hill^{-1}$)	Root shoot ratio
PPFM (P)		
p_1 – PPFM 16	33.33	0.19
p_2 – PPFM 26	28.33	0.38
p_3 – PPFM 35	21.67	0.34
p_4 – PPFM 37	26.67	0.29
p_5 – PPFM 38	31.67	0.47
SE m (±)	3.57	0.02
CD (0.05)	7.492	0.049
Method of application (M)		
m_1 - seed treatment (1%)	28.00	0.32
m_2 - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	28.67	0.35
SE m (±)	3.57	0.02
CD (0.05)	NS	0.038
PPFM (P) x Method of application (M)		
p_1m_1 (PPFM 16 - seed treatment)	36.67	0.17
p_1m_2 (PPFM 16 - seed treatment + foliar application)	30.00	0.21
p_2m_1 (PPFM 26 - seed treatment)	26.67	0.39
p_2m_2 (PPFM 26 - seed treatment + foliar application)	30.00	0.37
p_3m_1 (PPFM 35 - seed treatment)	20.00	0.49
p_3m_2 (PPFM 35 - seed treatment + foliar application)	23.33	0.20
p_4m_1 (PPFM 37 - seed treatment)	23.33	0.27
p_4m_2 (PPFM 37 - seed treatment + foliar application)	30.00	0.31
p_5m_1 (PPFM 38 - seed treatment)	33.33	0.46
p_5m_2 (PPFM 38 - seed treatment + foliar application)	30.00	0.67
Control (C)		
c_1 - KAU POP	16.66	0.24
c_2 - KAU POP + water spray	20.00	0.21
SE m (±)	3.45	0.02
CD (0.05)	10.104	0.068
Treatment *vs*. Control1	S	S
Treatment *vs*. Control 2	S	S

Seed treatment followed by foliar application of PPFM isolates (m_2) recorded significantly higher grain weight per panicle (2.69 g) when compared to m_1 (seed treatment).

The combined effect of PPFM and method of application was significantly higher for p_5m_2 (PPFM 38 as seed treatment + foliar application) with respect to the grain weight per panicle. It was at par with p_5m_1 (PPFM 38 as seed treatment).

KAU POP (c_1)registered significantly higher grain weight per panicle when compared to KAU POP + water spray (c_2). The treatment effect was significantly superior to c_2 (KAU POP + water spray), whereas there was no significant difference between the treatment andc_1 (KAU POP).

4.2.2.3 Filled Grains per Panicle

The response of number of filled grains per panicle to PPFM and its method of application is presented in Table 11.

Treating rice with PPFM 38 resulted in higher number of filled grains per panicle (111.33) which was comparable to that with PPFM 16 (110.33).

Number of filled grains per panicle did not vary significantly with the method of application of PPFM.

The interaction effect of PPFM and method of application was significant. Filled grains per panicle were higher in p_5m_1 and p_3m_2. Both these treatment combinations were on par with p_1m_1, p_2m_2, p_2m_1, p_3m_1, p_5m_2 and p_4m_2.

Between the controls, KAU POP (c_1) recorded significantly higher number of filled grains per panicle when compared toc_2 (KAU POP + water spray). The effect of treatment was significantly superior to both the controls.

4.2.2.4 Sterility Percentage

The results on the effect of PPFM and method of application on the sterility percentage of rice are presented in Table 11.

Treating rice with PPFM 38 resulted in lower sterility percentage (14.68 %) when compared to others. It was at par with PPFM 35 and PPFM 16.

The method of application did not show any significant variation in sterility percentage of rice.

The treatment combination p_1m_1 recorded lower sterility percentage (13.94%) and was comparable with p_5m_1, p_3m_2, p_5m_2, p_3m_1, p_4m_2, p_2m_1.

The sterility percentage was significantly lower for c_2 (KAU POP + water spray).

The treatments when compared against the controls proved to be superior with lower sterility percentage.

4.2.2.5 Thousand Grain Weight

The results on the effect of PPFM and its method of application on thousand grain weight of rice are presented in Table 11.

The effect of PPFM 38 was significantly superior with respect to the thousand grain weight of rice (24.12 g). It was followed by PPFM 35 (23.32 g).

There was no significant effect for the method of application of PPFM on thousand grain weight of rice.

The treatment combination, p_5m_1 (PPFM 38 as seed treatment) recorded higher thousand grain weight, comparable with p_5m_2.

KAU POP (c_1) recorded significantly higher thousand grain weight when compared to c_2(KAU POP + water spray). The treatment effect was superior to both the controls.

Table 10. Effect of PPFM and method of application on productive tillers m^{-2} and grain weight per panicle

Treatment	Productive tillers m^{-2} (nos.)	Grain weight per panicle (g)
PPFM (P)		
p_1 – PPFM 16	402.17	2.40
p_2 – PPFM 26	391.83	2.47
p_3 – PPFM 35	397.83	2.56
p_4 – PPFM 37	382.83	2.68
p_5 – PPFM 38	423.00	3.01
SE m (±)	4.35	0.09
CD (0.05)	9.141	0.208
Method of application (M)		
m_1 - seed treatment (1%)	396.60	2.55
m_2 - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	402.47	2.69
SE m (±)	4.35	0.09
CD (0.05)	5.781	0.132
PPFM (P) x Method of application (M)		
p_1m_1 (PPFM 16 - seed treatment)	410.33	2.49
p_1m_2 (PPFM 16 - seed treatment + foliar application)	394.00	2.30
p_2m_1 (PPFM 26 - seed treatment)	383.33	2.37
p_2m_2 (PPFM 26 - seed treatment + foliar application)	400.33	2.56
p_3m_1 (PPFM 35 - seed treatment)	400.67	2.54
p_3m_2 (PPFM 35 - seed treatment + foliar application)	395.00	2.58
p_4m_1 (PPFM 37 - seed treatment)	367.33	2.37
p_4m_2 (PPFM 37 - seed treatment + foliar application)	398.33	2.98
p_5m_1 (PPFM 38 - seed treatment)	421.33	2.99
p_5m_2 (PPFM 38 - seed treatment + foliar application)	424.67	3.03
Control (C)		
c_1 - KAU POP	366.33	2.44
c_2 – KAU POP + water spray	363.33	2.30
SE m (±)	4.06	0.11
CD (0.05)	11.900	0.313
Treatment *vs*. Control1	S	NS
Treatment *vs*. Control 2	S	S

4.2.2.6 Grain Yield

The data on grain yield as influenced by PPFM and method of application are presented in Table 12 and graphically in Fig. 7 and Fig. 8.

Among the five isolates tested, PPFM 38 recorded significantly superior grain yield (4.43 t ha^{-1}) and it was followed by PPFM 35 (3.43 t ha^{-1}).Method of application of PPFM had no significant effect on the grain yield of rice.

The combined effect on the grain yield was found to be higher for p_5m_2 (PPFM 38 as seed treatment + foliar application) which was at par with p_5m_1 (PPFM 38 as seed treatment).

The control c_1 (KAU POP) recorded significantly higher grain yield when compared to c_2 (KAU POP + water spray).

The effect of the treatments and KAU POP was comparable. However, the control c_2 (KAU POP + water spray) recorded significantly lower grain yield than the treatments.

4.2.2.7 Straw Yield

The response of straw yield of rice to PPFM and its method of application is presented in Table 12 and graphically in Fig. 7 and Fig. 8.

The results revealed that the isolate PPFM 38 was significantly superior (5.31 t ha^{-1}) to the other isolates with respect to the straw yield. Between the methods of application, seed treatment + foliar application (m_2) recorded significantly higher straw yield when compared to seed treatment alone (m_1).

The interaction effect between PPFM and method of application was significant. Seed treatment followed by foliar application of PPFM 38 (p_5m_2) recorded higher straw yield which was comparable to p_5m_1, p_1m_2 and p_3m_2.

KAU POP (c_1) resulted in significantly higher straw yield when compared to KAU POP + water spray (c_2). No significant difference was observed between the treatments and control with respect to straw yield.

Table 11. Effect of PPFM and method of application on filled grains per panicle, sterility percentage and thousand grain weight

Treatment	Filled grains per panicle (nos.)	Sterility percentage (%)	Thousand grain weight (g)
PPFM (P)			
p_1 – PPFM 16	110.33	15.30	23.17
p_2 – PPFM 26	109.50	16.84	23.15
p_3 – PPFM 35	111.17	14.82	23.32
p_4 – PPFM 37	109.00	16.64	23.22
p_5 – PPFM 38	111.33	14.68	24.12
SE m (±)	0.58	0.65	0.26
CD (0.05)	1.215	1.369	0.542
Method of application (M)			
m_1 - seed treatment (1%)	110.27	15.46	23.43
m_2 - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	110.13	15.85	23.36
SE m (±)	0.58	0.65	0.26
CD (0.05)	NS	NS	NS
PPFM (P) x Method of application (M)			
p_1m_1 (PPFM 16 - seed treatment)	110.67	13.94	23.17
p_1m_2 (PPFM 16 - seed treatment + foliar application)	110.00	16.65	23.17
p_2m_1 (PPFM 26 - seed treatment)	110.67	15.77	23.23
p_2m_2 (PPFM 26 - seed treatment + foliar application)	108.33	17.90	23.06
p_3m_1 (PPFM 35 - seed treatment)	110.67	15.03	23.20
p_3m_2 (PPFM 35 - seed treatment + foliar application)	111.67	14.61	23.43
p_4m_1 (PPFM 37 - seed treatment)	107.67	18.00	23.30
p_4m_2 (PPFM 37 - seed treatment + foliar application)	110.33	15.27	23.13
p_5m_1 (PPFM 38 - seed treatment)	111.67	14.55	24.23
p_5m_2 (PPFM 38 - seed treatment + foliar application)	111.00	14.81	24.00
Control (C)			
c_1 - KAU POP	107.00	18.65	22.37
c_2 – KAU POP + water spray	106.00	17.39	22.03
SE m (±)	0.63	0.66	0.27
CD (0.05)	1.857	1.942	0.788
Treatment *vs*. Control1	S	S	S
Treatment *vs*. Control 2	S	S	S

4.2.2.8 Harvest Index

The results of the effect of PPFM and its method of application on harvest index of rice are presented in Table 12.

Treating rice with PPFM 38 (p_5) resulted in significantly higher harvest index (0.46) than the other isolates. Harvest index did not vary significantly between the two methods of application of PPFM.

The treatment combination p_5m_1 (PPFM 38 as seed treatment) recorded higher harvest index which was on par with p_5m_2 (PPFM 38 as seed treatment + foliar application).

Between the controls, c_1 (KAU POP) resulted in significantly higher harvest index than c_2 (KAU POP + water spray. The treatment effect was significantly superior to both the controls.

4.2.3 Physiological Observations

4.2.3.1 Chlorophyll content

The variation in the chlorophyll content with PPFM and method of application is presented in Table 13.

The isolate PPFM 38 resulted in higher chlorophyll content at both panicle initiation (1.109 mg g^{-1} of leaf tissue) and flowering (1.151 mg g^{-1} of leaf tissue) stages. However, the effect of PPFM 38 was comparable with PPFM 37 at both the growth stages. Method of application did not show any significant difference in the chlorophyll content of rice.

The interaction effect of PPFM and method of application was higher for PPFM 38 as seed treatment followed by foliar application at both panicle initiation (1.27 mg g^{-1} of leaf tissue) and flowering (1.29 mg g^{-1} of leaf tissue) stages. It was at par with p_5m_1 and p_4m_2 during both the above growth stages.

Table 12. Effect of PPFM and method of application on grain yield, straw yield and harvest index

Treatment	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Harvest index
PPFM (P)			
p_1 – PPFM 16	3.39	4.77	0.42
p_2 – PPFM 26	3.25	4.51	0.42
p_3 – PPFM 35	3.43	4.62	0.43
p_4 – PPFM 37	3.19	4.53	0.40
p_5 – PPFM 38	4.43	5.31	0.46
SE m (±)	0.14	0.25	0.01
CD (0.05)	0.287	0.515	0.012
Method of application (M)			
m_1 - seed treatment (1%)	3.49	4.58	0.43
m_2 - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	3.60	4.91	0.42
SE m (±)	0.14	0.25	0.01
CD (0.05)	NS	0.326	NS
PPFM (P) x Method of application (M)			
p_1m_1 (PPFM 16 - seed treatment)	3.39	4.44	0.43
p_1m_2 (PPFM 16 - seed treatment + foliar application)	3.39	5.10	0.40
p_2m_1 (PPFM 26 - seed treatment)	3.11	4.49	0.41
p_2m_2 (PPFM 26 - seed treatment + foliar application)	3.39	4.52	0.43
p_3m_1 (PPFM 35 - seed treatment)	3.39	4.43	0.43
p_3m_2 (PPFM 35 - seed treatment + foliar application)	3.46	4.79	0.42
p_4m_1 (PPFM 37 - seed treatment)	3.26	4.41	0.40
p_4m_2 (PPFM 37 - seed treatment + foliar application)	3.18	4.64	0.41
p_5m_1 (PPFM 38 - seed treatment)	4.29	5.11	0.46
p_5m_2 (PPFM 38 - seed treatment + foliar application)	4.57	5.50	0.45
Control (C)			
c_1 - KAU POP	3.36	4.92	0.41
c_2 - KAU POP + water spray	2.62	3.88	0.40
SE m (±)	0.14	0.27	0.01
CD (0.05)	0.422	0.788	0.018
Treatment *vs*. Control1	NS	NS	S
Treatment *vs*. Control 2	S	NS	S

KAU POP (c_1) was found to show significantly higher chlorophyll content when compared to KAU POP + water spray (c_2) at panicle initiation and flowering stages.

The treatments when compared against the controls revealed significance at panicle initiation stage, with KAU POP (c_1) recording higher chlorophyll content. However there was no significant difference between the treatment effect and controls at flowering stage.

4.2.3.2 Proline Accumulation

The effect of PPFM and its method of application on proline accumulation is presented in Table13.

Treating rice with PPFM 38 (p_5) resulted in significantly higher proline accumulation of 0.87 µg mol^{-1} each at panicle initiation and flowering stages.

Seed treatment with PPFM (m_1) recorded a significantly higher proline content when compared to seed treatment followed by foliar application (m_2).

The combined effect of PPFM and method of application was observed to be superior for proline content with significantly higher content at p_5m_2 both at panicle initiation and flowering stages. It was followed by p_4m_1.

Between the controls, c_2 (KAU POP + water spray) showed significantly higher proline content at both the stages compared to c_1 (KAU POP). Proline content was observed to be significantly higher in the treatments than the controls, at panicle initiation and flowering stages.

4.2.4 Plant Analysis

4.2.4.1 Uptake of Nitrogen

The response of nitrogen uptake to PPFM and its method of application is presented in Table 14.

The nitrogen uptake was significantly superior with PPFM 38 (73.37 kg ha^{-1}) and it was followed by PPFM 16 (59.71 kg ha^{-1}). Seed treatment followed by foliar application (m_2) showed significantly higher uptake of nitrogen than the seed treatment alone (m_1).

The interaction effect of PPFM and its method of application was significantly superior with p_5m_2 (PPFM 38 as seed treatment and foliar application) recording higher value (76.08 kg ha^{-1}) which was on par with p_5m_1.

The control, KAU POP (c_1) showed significantly higher nitrogen uptake when compared to KAU POP + water spray (c_2).The treatment effect was comparable with c_1 (KAU POP)but varied significantly with c_2 (KAU POP + water spray).

4.2.4.2 Uptake of Phosphorus

The results on the effect of PPFM and its method of application on the uptake of phosphorus are presented in Table 14.

The treatment with PPFM 38 resulted in significantly higher phosphorus uptake (27.59 kg ha^{-1}) in rice. Seed treatment followed by foliar application (m_2) recorded significantly higher phosphorus uptake (24.83 kg ha^{-1}).

Seed treatment with PPFM 38 followed by its foliar application (p_5m_2) resulted in higher uptake of phosphorus (28.94 kg ha^{-1}). It was at par with seed treatment with PPFM 38 (p_5m_1).

Between the controls, c_1 (KAU POP) resulted in significantly higher phosphorus uptake than c_2(KAU POP + water spray). The treatments exhibited significant variation with c_2 whereas no significant variation was observed with c_1.

Table 13. Effect of PPFM and method of application on chlorophyll content and proline accumulation

Treatment	Chlorophyll content (mg g^{-1} of leaf tissue)		Proline accumulation (μg mol^{-1} of fresh weight)	
	Panicle initiation	Flowering	Panicle initiation	Flowering
PPFM (P)				
p_1 – PPFM 16	0.620	0.687	0.551	0.529
p_2 – PPFM26	0.517	0.552	0.403	0.404
p_3 – PPFM 35	0.494	0.506	0.705	0.702
p_4 – PPFM 37	0.790	0.862	0.716	0.697
p_5 – PPFM 38	1.109	1.151	0.870	0.870
SE m (±)	0.164	0.163	0.006	0.012
CD (0.05)	0.3437	0.3421	0.0137	0.0267
Method of application (M)				
m_1 - seed treatment (1%)	0.655	0.698	0.673	0.656
m_2 - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	0.757	0.805	0.624	0.625
SE m (±)	0.164	0.163	0.006	0.012
CD (0.05)	NS	NS	0.0087	0.0169
PPFM (P) x Method of application (M)				
p_1m_1(PPFM 16 - seed treatment)	0.692	0.727	0.678	0.656
p_1m_2(PPFM 16 - seed treatment + foliar application)	0.547	0.647	0.423	0.402
p_2m_1(PPFM 26 - seed treatment)	0.400	0.430	0.394	0.390
p_2m_2(PPFM 26 - seed treatment +foliar application)	0.634	0.674	0.413	0.420
p_3m_1(PPFM 35 - seed treatment)	0.510	0.524	0.689	0.682
p_3m_2(PPFM 35 - seed treatment +foliar application)	0.478	0.487	0.720	0.722
p_4m_1(PPFM 37 - seed treatment)	0.720	0.789	0.915	0.880
p_4m_2(PPFM 37 - seed treatment +foliar application)	0.859	0.926	0.516	0.513
p_5m_1(PPFM 38 - seed treatment)	0.952	1.011	0.689	0.672
p_5m_2(PPFM 38 - seed treatment +foliar application)	1.266	1.290	1.051	1.067
Control (C)				
c_1 - KAU POP	1.048	1.080	0.205	0.246
c_2 – KAU POP + water spray	0.876	0.915	0.367	0.320
SE m (±)	0.150	0.150	0.020	0.010
CD (0.05)	0.4497	0.4580	0.0446	0.0341
Treatment *vs*. Control1	S	NS	S	S
Treatment *vs*. Control 2	NS	NS	S	S

4.2.4.3 Uptake of Potassium

The data of potassium uptake as influenced by PPFM and its method of application are presented in Table 14.

Among the PPFM isolates, rice treated with PPFM 38 exhibited significantly higher uptake of potassium (141.96 kg ha^{-1}). Seed treatment followed by foliar application (m_2) showed significantly higher potassium uptake when compared to seed treatment alone.

The treatment combination, p_5m_2 (PPFM 38 as seed treatment + foliar spray) recorded significantly higher potassium uptake when compared with others.

KAU POP (c_1) was found to show significantly higher potassium uptake when compared to KAU POP + water spray (c_2). The treatment effect was comparable with KAU POP (c_1) but varied significantly with KAU POP + water spray (c_2).

4.2.4.4 Crude Protein Content

The crude protein content of rice as affected by PPFM and its method of application is presented in Table 14.

The PPFM isolates, method application and their interaction had no significant effect on the crude protein content of rice. The controls and treatments also failed to exhibit significant variation in crude protein content.

4.2.5 Soil Analysis

4.2.5.1 Organic Carbon

The effect of PPFM and its method of application on soil organic carbon content is presented in Table 15.

The soil organic carbon content did not show any significant variation among the treatments and between the treatments and controls.

4.2.5.2 Available Nitrogen

The results on the available nitrogen status of the soil after the experiment as influenced by PPFM and its method of application are presented in Table 15.

The treatments did not have any significant effect on the available nitrogen status of the soil. The comparison made between the treatments and controls also proved to be non-significant.

4.2.5.3 Available Phosphorus

The data on the effect of PPFM and its method of application on available phosphorus status of the soil after the experiment are presented in Table 15.

Available phosphorus status of soil after the experiment was observed to remain unaffected either by PPFM or its method of application. Significant variation was also not observed between the two controls and between the treatments and controls.

4.2.5.3 Available Potassium

The results on the effect of PPFM and its method of application on the available potassium of soil after the experiment are presented in Table 15.

Treating rice with PPFM 35 (p_3) resulted in higher potassium content in soil after the experiment. It was on par with PPFM 16 and PPFM 38.

There was no significant difference between the methods of application of PPFM isolates. The interaction effect between PPFM and method of application was also not significant. Further, the treatments and controls failed to exhibit significant difference in the available potassium status of soil after the experiment.

Table 14. Effect of PPFM and method of application on nutrient uptake and crude protein content of rice

Treatment	Nutrient uptake (kg ha^{-1})			Crude protein (%)
	Nitrogen	Phosphorus	Potassium	
PPFM (P)				
p_1 – PPFM 16	59.71	24.69	116.79	5.77
p_2 – PPFM26	55.07	22.38	123.31	5.25
p_3 – PPFM 35	57.96	22.63	119.27	5.15
p_4 – PPFM 37	58.88	22.34	111.30	5.53
p_5 – PPFM 38	73.37	27.59	141.96	5.60
SE m (±)	2.75	0.96	2.52	0.24
CD (0.05)	5.774	2.007	5.297	NS
Method of application (M)				
m_1 - seed treatment (1%)	55.97	23.02	119.15	5.56
m_2 - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	66.03	24.83	125.90	5.36
SE m (±)	2.75	0.96	2.52	0.24
CD (0.05)	3.652	1.269	3.350	NS
PPFM (P) x Method of application (M)				
p_1m_1(PPFM 16 - seed treatment)	55.36	24.17	108.22	5.77
p_1m_2(PPFM 16 - seed treatment + foliar application)	64.06	25.20	125.35	5.78
p_2m_1(PPFM 26 - seed treatment)	50.51	20.91	125.13	5.21
p_2m_2(PPFM 26 - seed treatment +foliar application)	59.63	23.86	121.50	5.29
p_3m_1(PPFM 35 - seed treatment)	51.01	22.20	115.97	5.24
p_3m_2(PPFM 35 - seed treatment +foliar application)	64.90	23.07	112.56	5.07
p_4m_1(PPFM 37 - seed treatment)	52.30	21.60	105.28	6.04
p_4m_2(PPFM 37 - seed treatment +foliar application)	65.46	23.09	117.31	5.02
p_5m_1(PPFM 38 - seed treatment)	70.66	26.24	141.16	5.56
p_5m_2(PPFM 38 - seed treatment +foliar application)	76.08	28.94	142.75	5.63
Control (C)				
c_1 - KAU POP	62.25	122.05	23.37	5.31
c_2 – KAU POP + water spray	44.04	97.82	19.76	4.97
SE m (±)	3.04	2.69	1.07	0.23
CD (0.05)	8.917	7.879	3.137	NS
Treatment *vs*. Control1	NS	NS	NS	NS
Treatment *vs*. Control 2	S	S	S	NS

4.3 ECONOMIC ANALYSIS

4.3.1 Gross Returns

The results on gross returns due to PPFM and its method of application are presented in Table 16.

Among the PPFM isolates, the treatment with PPFM 38 resulted in significantly higher gross returns when compared to others. The method of application of PPFM did not show any significant variation in the gross returns of rice.

The interaction effect of PPFM and its method of application was significantly superior for p_5m_2(PPFM 38 as seed treatment + foliar application) when compared to others.

Between the two controls, KAU POP (c_1) recorded significantly higher gross returns than KAU POP + water spray (c_2). The treatment effect varied significantly with KAU POP + water spray (c_2) but did not show any significant variation with KAU POP (c_1).

4.3.2 Net Returns

The effect of PPFM and method of application on net returns of rice is presented in Table 16.

Treating rice with PPFM 38 resulted in significantly higher net returns for rice (₹ 75,639 ha^{-1}). Net returns did not vary significantly between the two methods of application.

Seed treatment followed by foliar application of PPFM 38 (p_5m_2) recorded significantly higher net returns, followed by seed treatment of PPFM 38 (p_5m_1).

KAU POP (c_1) recorded significantly higher net returns than KAU POP + water spray (c_2). Further the net returns of the treatments were significantly higher than the controls.

Table 15. Effect of PPFM and method of application on soil nutrient status after the experiment

Treatment	Organic carbon (%)	Available nitrogen (kg ha^{-1})	Available phosphorus (kg ha^{-1})	Available potassium (kg ha^{-1})
PPFM (P)				
p_1 – PPFM 16	1.82	152.62	30.56	100.38
p_2 – PPFM26	1.82	154.65	30.52	69.53
p_3 – PPFM 35	1.70	150.52	30.80	137.28
p_4 – PPFM 37	1.72	183.98	30.04	76.00
p_5 – PPFM 38	1.68	163.07	30.39	101.61
SE m (±)	0.07	26.36	0.33	19.02
CD (0.05)	NS	NS	NS	39.962
Method of application (M)				
m_1 - seed treatment (1%)	1.78	158.03	30.38	100.00
m_2 - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	1.71	163.91	30.55	93.92
SE m (±)	0.07	26.36	0.33	19.02
CD (0.05)	NS	NS	NS	NS
PPFM (P) x Method of application (M)				
p_1m_1(PPFM 16 - seed treatment)	1.77	154.71	30.46	107.81
p_1m_2(PPFM 16 - seed treatment + foliar application)	1.87	150.52	30.65	92.96
p_2m_1(PPFM 26 - seed treatment)	1.83	137.86	30.35	74.18
p_2m_2(PPFM 26 - seed treatment +foliar application)	1.80	171.43	30.69	64.88
p_3m_1(PPFM 35 - seed treatment)	1.73	158.89	30.62	155.53
p_3m_2(PPFM 35 - seed treatment +foliar application)	1.67	142.16	30.99	119.02
p_4m_1(PPFM 37 - seed treatment)	1.77	188.16	30.13	69.37
p_4m_2(PPFM 37 - seed treatment +foliar application)	1.67	179.80	29.94	82.63
p_5m_1(PPFM 38 - seed treatment)	1.80	150.53	30.32	93.11
p_5m_2(PPFM 38 - seed treatment +foliar application)	1.57	175.61	30.46	110.10
Control (C)				
c_1 - KAU POP	1.80	154.70	30.17	86.13
c_2 – KAU POP + water spray	1.87	165.79	31.12	123.09
SE m (±)	0.07	27.43	0.97	18.22
CD (0.05)	NS	NS	NS	NS
Treatment *vs*. Control1	NS	NS	NS	NS
Treatment *vs*. Control 2	NS	NS	NS	NS

4.3.3 B:C Ratio

The variation in B:C ratio due to PPFM and its method of application is presented in Table 16 and Fig. 9.

The B:C ratio of rice was observed to be significantly higher when rice was treated with PPFM 38 (1.59). The effect of method of application was not significant.

The treatment combination p_5m_2 (PPFM 38 as seed treatment + foliar application) resulted in a higher B: C ratio and was on par with p_5m_1.

A significantly higher B: C ratio was recorded with KAU POP (c_1) than with KAU POP + water spray (c_2). However, the treatments were significantly superior to both the controls.

Table 16. Effect of PPFM and method of application on gross returns, net returns and B:C ratio

Treatment	Gross returns (₹ ha^{-1})	Net returns (₹ ha^{-1})	B:C ratio
PPFM (P)			
p_1 – PPFM 16	159390	31276	1.25
p_2 – PPFM 26	152510	24396	1.19
p_3 – PPFM 35	160024	31910	1.25
p_4 – PPFM 37	151504	23390	1.19
p_5 – PPFM 38	203753	75639	1.59
SE m (±)	6547	6547	0.05
CD (0.05)	13755.0	13755.0	0.107
Method of application (M)			
m_1 - seed treatment (1%)	162428	36888	1.30
m_2 - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	168445	37756	1.29
SE m (±)	6547	6547	0.05
CD (0.05)	NS	NS	NS
PPFM (P) x Method of application (M)			
p_1m_1 (PPFM 16 - seed treatment)	157736	32197	1.26
p_1m_2 (PPFM 16 - seed treatment + foliar application)	161044	30355	1.23
p_2m_1 (PPFM 26 - seed treatment)	146880	21341	1.17
p_2m_2 (PPFM 26 - seed treatment + foliar application)	158140	27451	1.21
p_3m_1 (PPFM 35 - seed treatment)	157736	32197	1.27
p_3m_2 (PPFM 35 - seed treatment + foliar application)	162312	31623	1.24
p_4m_1 (PPFM 37 - seed treatment)	152582	27043	1.22
p_4m_2 (PPFM 37 - seed treatment + foliar application)	150426	19737	1.15
p_5m_1 (PPFM 38 - seed treatment)	197204	71665	1.57
p_5m_2 (PPFM 38 - seed treatment + foliar application)	210301	79612	1.61
Control (C)			
c_1 - KAU POP	159028	23539	1.17
c_2 – KAU POP + water spray	124405	-1084	0.99
SE m (±)	6979	6979	0.05
CD (0.05)	2046.0	2046.0	0.159
Treatment *vs*. Control1	NS	S	S
Treatment *vs*. Control 2	S	S	S

Discussion

5. DISCUSSION

The experiment entitled "Growth and productivity of aerobic rice (*Oryza sativa* L.) as influenced by pink pigmented facultative methylotrophs (PPFM)" was undertaken to study the effect of PPFM on rice seedling emergence and vigour, to assess its effect on growth and yield of aerobic rice and to work out the economics. The study was conducted as two simultaneous experiments, Experiment I: Effect of PPFM on seedling emergence and vigour of rice, and Experiment II: Effect of PPFM on growth and yield of aerobic rice. The results of the study are discussed briefly in this chapter.

5.1 EXPERIMENT – I

EFFECT OF PPFM ON SEEDLING EMERGENCE AND VIGOUR OF RICE

5.1.1 Emergence Percentage and Emergence Rate Index

The results on the effect of PPFM on emergence percentage of rice revealed 100 per cent emergence in response to seed treatment with PPFM, irrespective of the isolate tested, as compared to 95 per cent emergence recorded with distilled water (control). The emergence rate index (ERI), which is a function of number of days taken for seedling emergence was also greater with PPFM treatment. The isolate PPFM 38 recorded the highest ERI (160) followed by PPFM 37. The mean ERI of PPFM treated rice seeds was 149.6 compared against an ERI of 121 for seeds treated with distilled water.

The effect of seed treatment with PPFM in enhancing the germination and emergence has been reported by Holland (1997). The stimulatory effect of PPFM is presumably due to their ability to produce plant growth regulators and Vitamin B_{12} (Basile *et al*., 1985; Freyermuth *et al*., 1996). Chandrasekaran *et al*. (2017) also observed similar results in tomato where seed treatment with 2 per cent PPFM enhanced the germination and emergence in tomato.

5.1.2 Shoot Length, Root length and Dry Weight of Seedling

Treating rice seeds with PPFM did not have significant effect on the shoot length of rice seedlings. However, seedling root length was observed to vary significantly with PPFM treatment. Seedling root length (13.605 cm) and dry weight of seedling (0.198 g per seedling) were greater with PPFM 38. While it remained at par with PPFM 16 and PPFM 26 with respect to root length, it was comparable with the effect of PPFM 37 for seedling dry weight. Seed treatment with PPFM resulted in greater mean seedling root length (12.246 cm) than the control (10.475 cm).

Longer roots produced under the effect of PPFM could be attributed to the ability of methylobacterium to grow on carbon compounds and produce plant growth regulators like auxin and cytokinin (Ivanova *et al*., 2000). Auxins and cytokinins promote cell division and cell elongation, resulting in longer roots. Methylobacterium was studied by Subhaswaraj *et al*. (2017) and established the production of indole acetic acid and its effect on plant growth promotion. Nysanth *et al.* (2018) reported that PPFM inoculation had significant effect on the seedling biomass. Accumulation of plant hormones like trans-zeatin riboside, isopentenyladenosine, and indole -3 acetic acid has been reported in seedlings treated with PPFMs (Lee *et al*., 2006). The increase in the root length and dry weight of rice seedlings might be the result of the effect of these growth promoting hormones.

5.1.3 Seedling Vigour Index

Both, seedling vigour index I and II varied significantly with PPFM treatment. While PPFM 38 recording the highest value (7.64) for seedling vigour index I, the same was recorded by PPFM 37 for seedling vigour index II. However, the effect of all the PPFM isolates tested was comparable. The seedling vigour index I and II of seeds treated with distilled water (control) were the lowest and it was 4.62 and 12.15 respectively. Microbes like PPFM have been reported to play an important role in seed germination and seedling establishment of plants (Holland *et al*., 2002). Further methylotrophic bacteria are capable of secreting certain diffusible substances like other than plant growth substances into the growing medium (Koenig *et al*., 2002). The results corroborate with those reported by Madhaiyan*et al*. (2004) who observed enhanced seedling vigour in rice seeds treated with PPFM.

The results of the present study illustrated the effectiveness of PPFM in increasing the emergence percentage by 5.26 per cent, emergence rate index by 9.9 to 32.2 per cent, seedling root length by 22.2 to 29.9 per cent, seedling dry weight by 24.4 to 46.7 per cent, seedling vigour index I by 34.2 to 65.4 and seedling vigour index II by 37.9 to 62.6 per cent over the control (distilled water). Similar results have been reported by Radha *et al*. (2009) in soybean and Meena *et al*. (2012) in wheat.

5.2 EXPERIMENT – II

EFFECT OF PPFM ON GROWTH AND YIELD OF AEROBIC RICE

5.2.1 Growth and Growth Attributes

The PPFM isolates and method of application had no significant effect on plant height at 20, 40 and 60 DAS. Among the five isolates tested, PPFM 38 recorded significantly taller (98.64 cm) plants at harvest, followed by PPFM 35. Tiller production of rice was observed to respond significantly to PPFM and method of application. Number of tillers per square metre was significantly more when treated with PPFM 38.

Between the two methods of application, seed treatment (1%) + foliar application (2%) at 30 and 50 DAS resulted in taller plants and more number of tillers per square metre. The treatment combination p_5m_2 (PPFM 38 as seed treatment and foliar application) was significantly superior at all the three above growth stages with respect to plant height and tiller count.

In general, plant height is a genetically controlled growth attribute which plays an important role in productivity. Methylotrophs might have favoured an increase in plant height by the production of growth promoters like zeatin, auxins and cytokinins. High production of cytokinins in the apical tissues and rhizosphere soil has been reported by Poonguzhali *et al*. (2017) as the reason for the increase in plant height of green gram in response to PPFM application. The coordination between auxins and cytokinins has been identified as crucial for promoting balanced growth of shoot and root system. The action of auxins help in the production of extensive root system and consequently cytokinins signals the shoot system to produce more tillers (Raghavendra and Santhosh, 2019). These results are in conformity with those of Holland (1997) and Raja and Sundaram (2006). The effectiveness of supplementing seed treatment with foliar application of PPFM could be attributed to the efficacy of foliar sprays in easy and quick absorption as suggested by Umamageswari *et al*. (2019). The superiority of PPFM treatments was also evident from significant variation in plant height and tiller production as compared to the two controls *viz*., KAU POP (c_1) and KAU POP + water spray (c_2).

Leaf area index and dry matter production were found to be significantly superior on treating rice with PPFM 38 and with seed treatment (1%) followed by foliar application (2%). The interaction effect of the combination, p_5m_2 (PPFM 38 as seed treatment and foliar application) was significantly superior with respect to leaf area index and dry matter production. Leaf area index of the treatments were significantly superior to both the controls. However, the mean dry matter production of the treatments and KAU POP (c_1) did not vary significantly. But the treatments were significantly superior to KAU POP + water spray (c_2).

The growth promoting substances produced by PPFM might have increased the number of leaves and the rate of leaf elongation as reported by Li *et al*. (2009). Similar increase in leaf area index due to foliar application of PPFM has been reported by Ajaykumar and Krishnasamy (2019). The higher leaf area index could be attributed to the higher tiller count in response to PPFM which led to more number of leaves and consequently higher leaf area index. Dry matter production is a function of leaf area index and radiation use efficiency. The higher dry matter production recorded in the treatments might be due to increase in plant height, more number of tillers per square metre and better root development. Further, the growth promoting activity mediated by PPFM might have also aided in the accumulation of photo assimilates in various sinks leading to higher dry matter production. Similar results have been reported by

Thakur *et al*. (1995), Singh *et al*. (2004) and Li *et al*. (2009). The lack of significant variation in dry matter production between treatments and KAU POP might be because of the fact that the crop maintained under KAU POP received copious irrigation without stress at the critical growth stages.

The isolate PPFM 16, showed significantly superior effect on root volume and it was comparable with that of PPFM 38. However, significantly superior root shoot ratio was recorded with PPFM 38. Seed treatment (1%) followed by foliar application (2%) at 30 and 50 DAS (m_2) resulted in significantly higher root shoot ratio. The interaction effect of PPFM and methods of application revealed significantly higher root volume for the treatment combination p_1m_1 (PPFM 16 as seed treatment) which was on par with $p_5m_{2.}$ The treatment combination p_5m_2 (PPFM 38 as seed treatment + foliar application) showed significantly superior root shoot ratio. Between the two controls root shoot ratio was higher for KAU POP (c_1) than KAU POP + water spray (c_2) whereas the root volume was significantly higher for KAU POP + water spray.

Root volume and root shoot ratio was significantly higher for the treatments when compared against both the controls.

Indole-3-acetic acid (IAA) is the main auxin that controls physiological processes during root formation and further development (Aloni *et al*., 2006). The ability of *Methylobacterium* to produce IAA has been reported by Ivanova *et al*. (2001). This suggest that PPFM treatment can enhance IAA concentrations in plant and induce root development. Ethylene is another compound that regulates root development (Madhaiyan *et al*., 2007) and this is in turn related to the biosynthesis pathway of auxin (Hardoim *et al*., 2008). Ethylene concentration increases under stress and inhibits root elongation. Methylobacterium – plant interaction modulates the synthesis of ethylene. Sivakumar *et al*. (2018) have also reported higher root volume in tomato with foliar spray of PPFM due to improved lateral root growth.

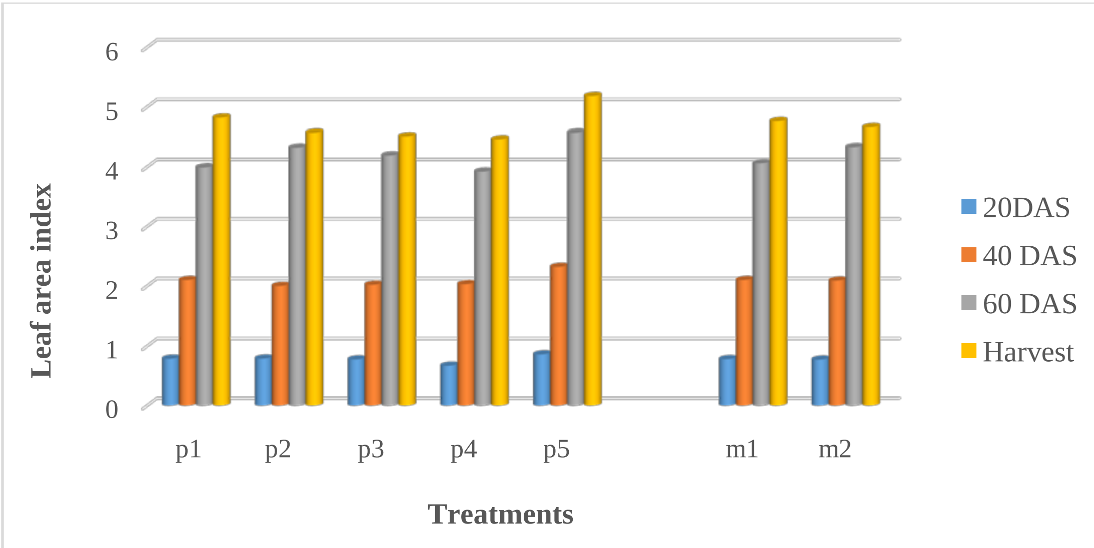

Fig. 3. Effect of PPFM and method of application on leaf area index.

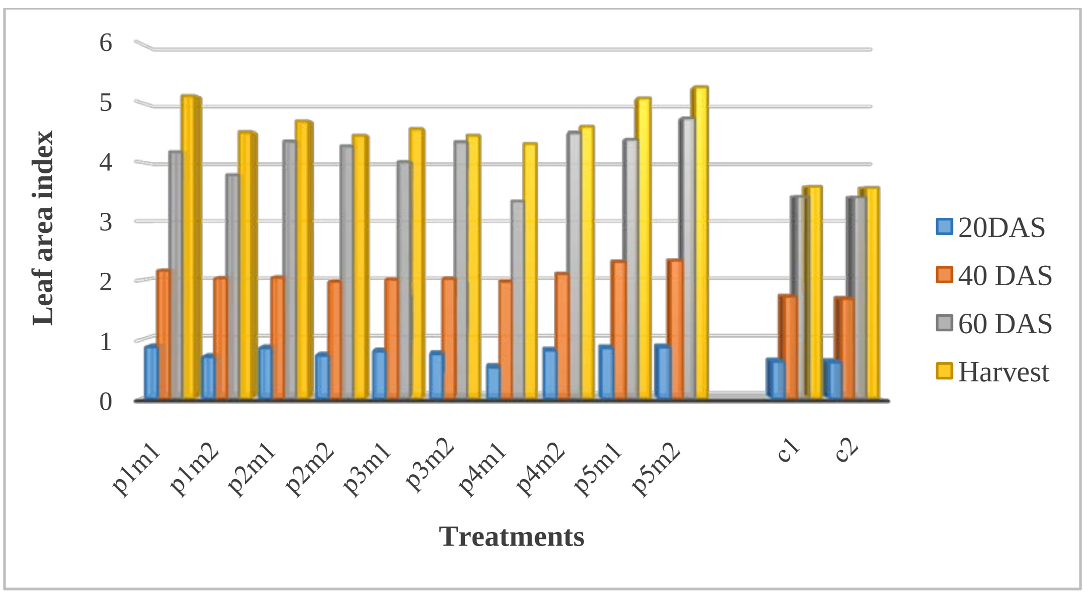

Fig. 4. Effect of PPFM x method of application on leaf area index.

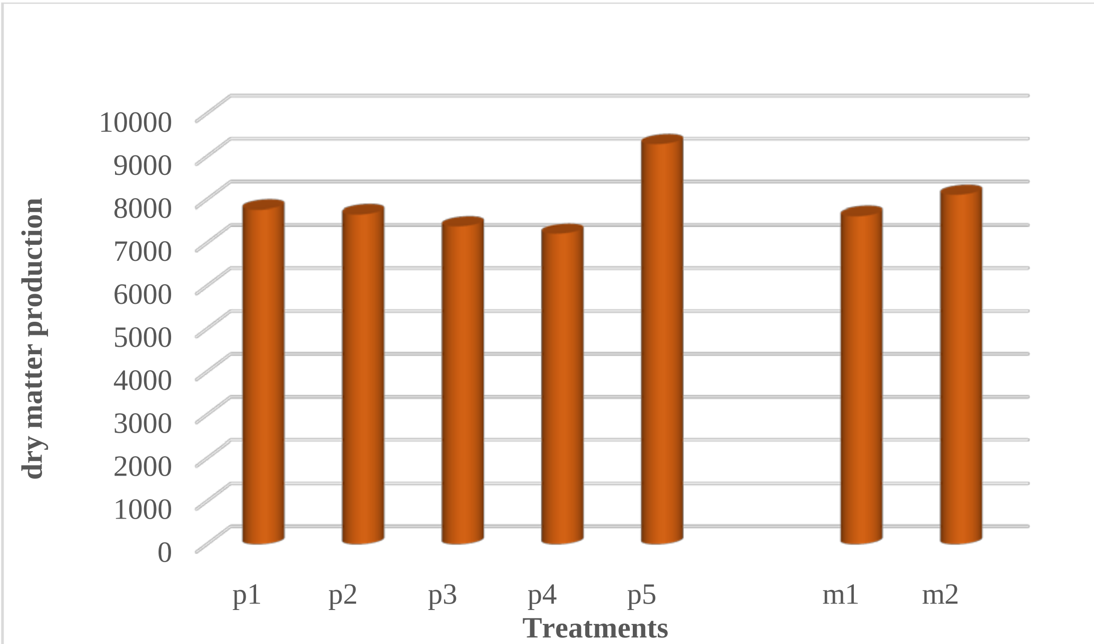

Fig. 5. Effect of PPFM and method of application on dry matter production, kg ha^{-1}.

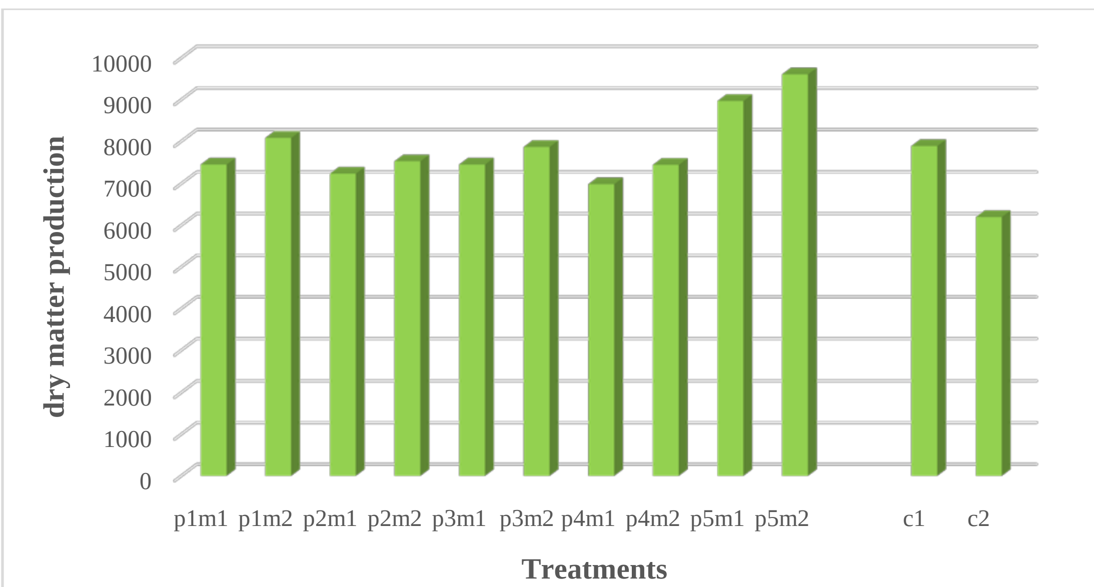

Fig. 6. Effect of PPFM x method of application on dry matter production, kg ha^{-1}.

The higher root volume recorded in KAU POP + water spray (c_2) might be due to the tendency of roots to produce more lateral roots with increased diameter and surface area under stress conditions (Fenta *et al.*, 2014) so that water and nutrients can be absorbed from the deeper layers of the soil for maintaining photosynthesis (Comas *et al.*, 2013). The higher root shoot ratio recorded in KAU POP as compared to KAU POP + water spray might be due the higher root weight supported by moisture stress free condition. Similar observations were made by Nejad (2011).

5.2.2 Yield Attributes

Among the PPFM isolates tested, the treatment with PPFM 38 exhibited significantly higher number of productive tillers per square metre, grain weight per panicle, number of filled grains per panicle, thousand grain weight and lower sterility percentage. Seed treatment followed by foliar application of PPFM isolates (m_2) recorded significantly higher number of productive tillers per square metre and grain weight per panicle. The combined effect of PPFM and method of application was significantly higher for p_5m_2 (PPFM 38 as seed treatment + foliar application) with respect to number of productive tillers per square metre and grain weight per panicle. The number of filled grains per panicle was significantly higher for p_5m_1 and $p_3m_2$which were at par with p_5m_2. However, the treatment combination p_1m_1 recorded significantly lower sterility percentage. It was at par with p_5m_2. The thousand grain weight was significantly higher for p_5m_1 which was also at par with p_5m_2. Between the two controls, c_1 (KAU POP) exhibited significantly higher number of productive tillers per metre square, higher grain weight per panicle, number of filled grains per panicle, thousand grain weight and lower sterility percentage. The treatments were superior to both the controls with respect to productive tillers per square metre, filled grains per panicle, thousand grain weight and sterility percentage of rice. However, in the case of grain weight per panicle, the treatment effect was significantly superior to c_2 (KAU POP + water spray), whereas there was no significant difference between treatments and c_1 (KAU POP).

The higher leaf area index and dry matter production supported by PPFM treatment might have resulted in superiority in the various yield attributes. Lidstrom and Chistoserdova (2002) had observed increase in yield attributes of maize with exogenous application of *Methylobacterium* species and attributed this to the effect of cytokinins and auxins. Cytokinins promotes cell division, delays senescence, counteracts apical dominance and helps in the better translocation of assimilates and consequently help to improve the yield potential of plants (Madhaiyan *et al*., 2005). Foliar spray of PPFM was reported to improve the yield attributes of cotton (Madhaiyan, 2003).The superiority of KAU POP with respect to yield attributes was due to the fact that the crop was maintained without any moisture stress during the entire growth phase, especially the panicle initiation and flowering stages, during which daily irrigation was given.

5.2.3 Yield

Treating rice with PPFM 38, resulted in significantly superior grain yield, straw yield and harvest index. Between the methods of application, seed treatment + foliar application (m_2) recorded significantly higher straw yield. The combined effect of PPFM and method of application on the grain yield and straw yield was found to be significantly higher for p_5m_2 (PPFM 38 as seed treatment + foliar application) whereas p_5m_1 recorded the highest harvest index, on par with p_5m_2 (PPFM 38 as seed treatment + foliar application). Between the controls, c_1 (KAU POP) recorded significantly higher grain yield, straw yield and harvest index. The treatment effects were comparable with KAU POP with respect to grain yield whereas straw yield was comparable with both the controls. The treatment effect was superior to both the controls with respect to the harvest index.

Grain yield is a function of yield attributes like number of productive tillers, filled grains per panicle and grain weight. The effect of PPFM was observed to be significant for majority of the yield attributes. This might have been reflected in the increased grain yield. Crop yield hinges on the ability of plants to carry out photosynthesis, which in turn depends on the carbondioxide concentration. In C_3 crops like rice, foliar application of

PPFM will help to increase carboxylation reaction and enhance photosynthetic rate (Kumar *et al*., 1999). Further, the PPFM bacteria use single carbon compounds leached out from the leaves and release carbon dioxide. This carbon dioxide can compete with atmospheric carbon dioxide for ribulose1,5 diphosphate and consequently reduce photorespiration rate in C_3 plants (Nonomura and Benson, 1992; Zbiec *et al*., 1999). Further, the cytokinins secreted by PPFM also stimulate better translocation of assimilates from the leaves resulting in enhanced yield of the crop (Elliot *et al*., 2000). The results of the present study are in line with that of Kenda *et al*. (2009). The present study revealed that seed treatment of PPFM supplemented with foliar application at 30 and 50 DAS (m_2) resulted in higher grain yield. This might be because of the positive effect of foliar application at the active tillering and panicle initiation stages. While active tillering is regarded as a phase of rapid growth and development in rice, panicle initiation is the most critical stage for rice. Thus foliar application might have aided the crop not only with the advantages of PPFM but also served as an additional source of water helping the crop in better absorption and translocation of nutrients.

As discussed earlier the KAU POP for aerobic rice warranted irrigation once in three days up to panicle initiation stage and daily irrigation thereafter until one week prior to harvest. But in KAU POP + water spray irrigation was withheld one week prior to and after the water spray treatment at 30 DAS and 50 DAS. Thus the stress imposed upon the crop, especially before panicle initiation might have affected the yield of the crop negatively.

5.2.4 Physiological Observations

Chlorophyll content and proline accumulation estimated at panicle initiation and flowering stages exhibited remarkable variation with PPFM treatment. Seed treatment with PPFM (m_1) recorded significantly higher proline content when compared to seed treatment followed by foliar application (m_2). The interaction effect of PPFM and method of application was observed to be superior at both panicle initiation and flowering stages with respect to chlorophyll content and proline accumulation. KAU POP (c_1) was found to show significantly higher chlorophyll content at panicle initiation and flowering stages. The proline content was significantly higher with c_2 (KAU POP + water spray) at both the stages. Higher chlorophyll content was observed with the treatments when compared against the controls at panicle initiation stage whereas proline content significantly higher in the treatments than the controls, at panicle initiation and flowering stages.

The effect of PPFM in increasing the chlorophyll content has been reported by several workers. Methylobacterium inoculation has been reported to increase the photosynthetic activity by increasing the stomatal count, chlorophyll content and malic acid content in crops (Madhaiyan *et al*., 2004). Satyan *et al*. (2018) have also observed an increase the chlorophyll stability index of small cardamom due to PPFM inoculation. In the present study also, a similar trend was observed as evidenced by the higher chlorophyll content in the treatments compared to the controls. Between the two controls, KAU POP + water spray recorded a lower chlorophyll content. This is possibly due to the effect of periodical moisture stress imposed. Moisture stress induces reduction in chlorophyll level, stomatal conductance and photosynthesis (Sanchez *et al*., 1983). The decrease in chlorophyll in response to stress has been attributed to enhanced catabolism of the chlorophyll complex or due to severe retardation in chlorophyll synthesis. Greening under rewatering was observed to be inhibited by at least 50 per cent even under mild moisture stress (Alberte *et al*., 1975).

Proline is a non-protein amino acid that has been observed to accumulate in plants when subjected to moisture stress (Yoshiba *et al*., 1997). Increase in proline content in response to moisture stress might be the result of the transcriptional activation of P5CS (delta-1 pyrroline-5 carboxylate synthase) as reported by Babiychuk *et al*. (1996) and the associated increase in protease activity, which causes the degradation of proteins under water stress conditions (Jain *et al*., 1996). Proline is switter ionic and highly hydrophilic in nature and hence acts as a compatible solute, stabilizes sub cellular structures (Chain and Dandekar, 1995) and help in the solubility of proteins and other bio polymers. This in turn aids in maintenance of turgour pressure of both root and shoot even when plant is under moisture stress (Ludlow and Muchaw, 1990).

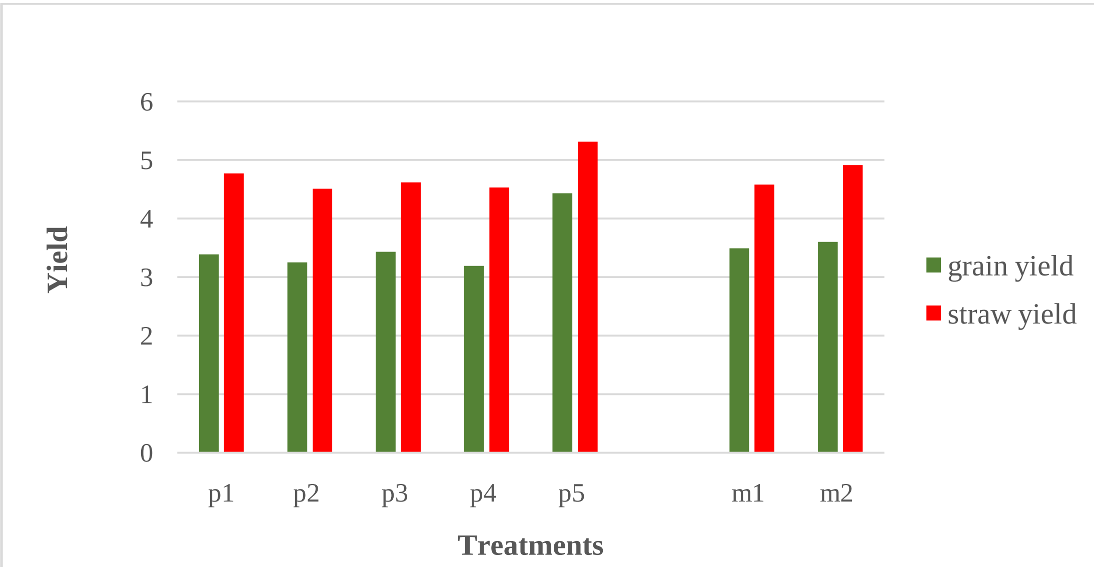

Fig. 7. Effect of PPFM and method of application on grain yield and straw yield, t ha^{-1}.

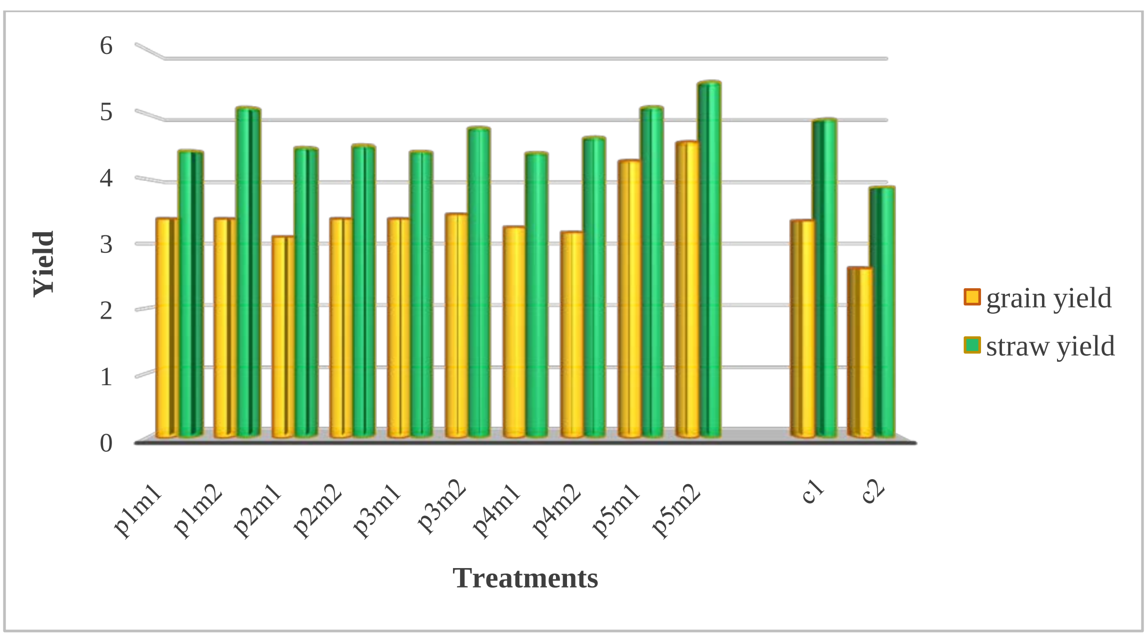

Fig. 8. Effect of PPFM x method of application on grain yield and straw yield, t ha^{-1}.

Similar findings have been reported by Gowri (2005) and Jinsy (2014). The higher proline accumulation observed with KAU POP + water spray might be due to the moisture stress imposed before and after the spraying water.

5.2.5 Nutrient Uptake

Significantly higher uptake of nitrogen, phosphorus and potassium was observed consequent to treatment with PPFM 38. Between the two methods of application, seed treatment followed by foliar application(m_2) showed significantly higher uptake of all the three major nutrients than seed treatment alone (m_1). The interaction effect of PPFM and its method of application was significantly superior with p_5m_2 (PPFM 38 as seed treatment and foliar application) recording higher uptake of N, P and K. Uptake of nitrogen and phosphorus was on par with seed treatment with PPFM 38 (p_5m_1). The control, KAU POP (c_1) showed significantly higher nutrient uptake when compared to KAU POP + water spray (c_2). The mean nutrient uptake recorded by the treatments was comparable with c_1 (KAU POP).

The significant increase in NPK uptake of rice could be attributed to the efficient and well developed root system as indicated by higher root shoot ratio. Physiological responses due to PPFM included better root shoot development, increased leaf area and chlorophyll content which might have retarded senescence and enabled the crop to absorb nutrients better. It was observed that nutrient uptake followed the same pattern as dry matter production. Nutrient uptake is partly a function of dry matter production and concentration of nutrients in the plant. Thus, the higher total dry matter production observed with PPFM treatment might have contributed towards better nutrient uptake. This is in accordance with the findings of Fageria and Baligar (2005) who have stated that nutrient accumulation pattern in plants followed dry matter accumulation.

5.2.6 Crude Protein Content

Crude protein content of grain did not show any significant variation due to PPFM treatment, methods of application and its interaction. This might be because of the fact that crude protein content is partly a genetically controlled aspect of crops.

5.2.7 Soil Analysis

Analysis of the soil after the experiment revealed that the PPFM isolates tested, methods of application and their interaction had no significant effect on the organic carbon content, available nitrogen and available phosphorus status of the soil. However, available potassium status was significantly higher with PPFM 35. But the effect of method of application and interaction was not significant. None of the soil parameters tested had significant difference between the controls and among the treatments and controls.

The data presented in Table 15 revealed an erratic trend in the available potassium status in response to PPFM treatments. Though previous reports could be found for the effect of PPFM on nitrogen fixation and phosphorus solubilisation, no reference could be found towards its effect on potassium. Hence the trend observed in the present study could be attributed to the dynamic behavior of potassium in the soil.

5.2.8 Economic Analysis

The treatment with PPFM 38 resulted in significantly higher gross returns, net returns and B:C ratio, whereas no significant variation could be observed between the methods of application of PPFM. The combined effect of PPFM and its method of application was significantly superior for p_5m_2 (PPFM 38 as seed treatment + foliar application) with respect to gross returns, net returns and B:C ratio. KAU POP (c_1) recorded significantly higher values than KAU POP + water spray (c_2). The mean gross returns of the treatments was significantly greater than KAU POP + water spray (c_2). However, it was comparable with KAU POP (c_1). The mean net returns and B:C ratio of the treatments were greater than both the controls, KAU POP (c_1) and KAU POP + water spray (c_2).

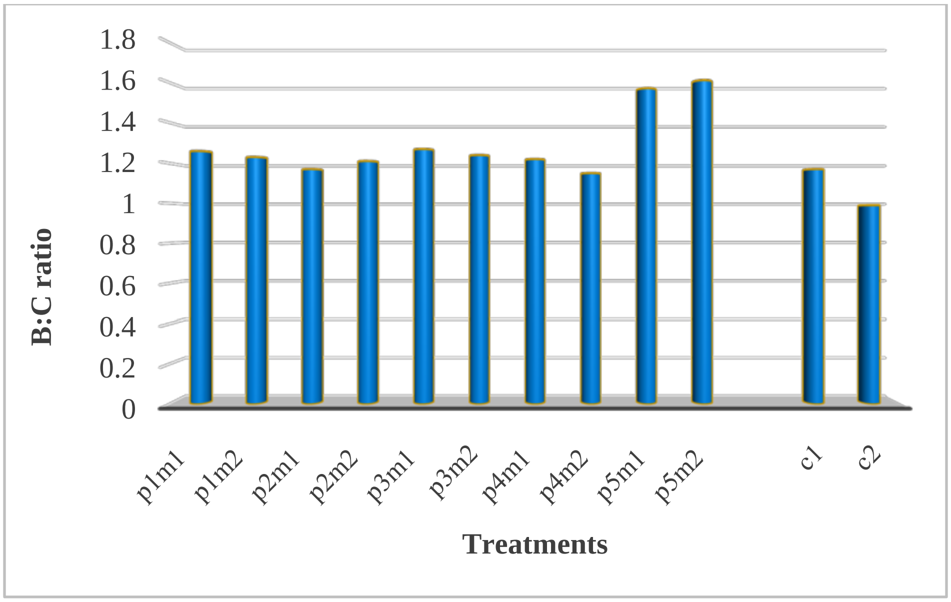

Fig. 9. Effect of PPFM x method of application on B:C ratio of rice.

Gross returns followed the same pattern as grain yield and straw yield as it is computed based on market price of the produce. The mean gross returns realized from the treatments remained comparable with that of KAU POP since the grain yield variation between the two was not significant. The superiority of KAU POP (c_1) over KAU POP + water spray (c_2) could be attributed to the lower yield recorded by c_2 compared to c_1. The higher mean net returns and B:C ratio of treatments compared to KAU POP is due to the higher cost of cultivation for KAU POP where daily irrigation is given from panicle initiation stage onwards.

The present study revealed that seed treatment with pink pigmented facultative methylotrophs, PPFM 38 at 1 per cent (p_5) resulted in vigorous seedlings as evidenced by the greater root length, dry weight and seedling vigour index. Seed treatment (1%) followed by foliar application (2%) of PPFM 38, twice, at 30 and 50 days after sowing (p_5m_2) enhanced the growth, yield and economics of aerobic rice.

Summary

6. SUMMARY

The experiment entitled “Growth and productivity of aerobic rice (*Oryza sativa* L.) as influenced by pink pigmented facultative methylotrophs (PPFM)” was undertaken at IFSRS, Karamana, Thiruvananthapuram, Kerala, from December 2019 to April 2020, with the objectives to study the effect of PPFM on rice seedling emergence and vigour, to assess its effect on growth and yield of aerobic rice and to work out the economics.

The study was conducted as two simultaneous experiments. Experiment I was undertaken to study the effect of PPFM on seedling emergence and vigour of rice. The efficacy of seed treatment (1%) with five superior isolates of PPFM *viz*., PPFM 16, PPFM 26, PPFM 35, PPFM 37 and PPFM 38 was tested against distilled water as control. The study was laid out in completely randomised design with six treatments and four replications.

Experiment II was undertaken to study the effect of PPFM on growth and yield of aerobic rice and to work out the economics. The experiment was carried out in factorial randomised block design with [(5 x 2) + 2] treatments replicated thrice. The treatments comprised combinations of five PPFM isolates (p_1 – PPFM 16; p_2 – PPFM 26; p_3 – PPFM 35; p_4 – PPFM 37; p_5 – PPFM 38) and two methods of application [m_1 – seed treatment (1%); m_2 – seed treatment (1%) + foliar application (2%) at 30 and 50 DAS] compared against two controls (c_1 – KAU POP ; c_2 – KAU POP + water spray). The variety selected for the study was MO 16 (Uma).

The results of experiment I revealed that seed treatment with all the five isolates of PPFM resulted in 100 per cent emergence as against the control where the emergence was only 95 per cent. The highest emergence rate index (160) was recorded with PPFM 38 whereas as the lowest was observed in control (distilled water). Pink pigmented facultative methylotrophs (PPFM) had significant effect on seedling characters such as root length, dry weight of seedling and seedling vigour index. The seed treatment of rice with PPFM 38 exhibited relatively longer roots (13.605 cm), higher seedling dry weight

(0.198 g per seedling) and greater seedling vigour index I (7.4). Seedling vigour index II was found to be higher for seeds treated with PPFM 37 (19.75). However, PPFM had no significant effect on the shoot length of rice seedlings.

The results of experiment II proved that the PPFM isolates and its method of application had significant effect on the growth and growth attributes of rice. Among the PPFM isolates tested, PPFM 38 (p_5) resulted in taller plants (98.64 cm) at harvest and more number of tillers per square metre at 20, 40 and 60 DAS and at harvest. Further, dry matter production (9287 kg ha^{-1}) at harvest, leaf area index at 20, 40 and 60 DAS and at harvest and root shoot ratio were significantly superior for PPFM 38. However, root volume of rice was observed to be higher (33.33 cm^3 $hill^{-1}$) with PPFM 16. Treating rice seeds with PPFM at 1 per cent followed by foliar application (2%) at 30 and 50 DAS (m_2) had significantly superior effect on the above mentioned growth attributes except root volume. No significant variation in the root volume was observed with respect to the method of application.

The interaction effect of PPFM and its method of application was observed to be significantly superior for all the above mentioned growth attributes. The treatment combination p_5m_2(PPFM 38 as seed treatment and foliar application) recorded significantly taller plants, more number of tillers per square metre, higher dry matter production and greater root shoot ratio. The root volume was higher in p_1m_1 (PPFM 16 as seed treatment), which was comparable to p_5m_2.

Between the controls, KAU POP (c_1) was significantly superior to KAU POP + water spray (c_2) with respect to all the growth attributes except root volume. Root volume was higher (20 cm^3 $hill^{-1}$) in KAU POP + water spray. Growth and growth attributes of rice was significantly superior with the PPFM treatments than both the controls.

Yield attributes of rice exhibited significant variation in response to PPFM treatments. Among the five PPFM isolates tested, treatment with PPFM 38 recorded significantly higher productive tiller count (423.00), grain weight per panicle (3.01 g), filled grains per panicle (111.33) and thousand grain weight (24.12 g) and significantly lower sterility percentage (14.68%). Grain yield (4.43 t ha^{-1}), straw yield (5.31 t ha^{-1}) and harvest index were also superior with PPFM 38. Between the two methods of application, m_2 (seed treatment + foliar application) showed significantly higher number of productive tillers per square metre, grain weight per panicle, grain yield and straw yield. However, the method of application of PPFM could not elicit significant variation in the number of filled grains per panicle, sterility percentage, thousand grain weight and harvest index.

Seed treatment (1%) followed by foliar application (2%) of PPFM 38 at 30 and 50 DAS (p_5m_2) resulted in higher number of productive tillers per square metre (424.67), grain weight per panicle, grain yield and straw yield of rice. However, number of filled grains per panicle, thousand grain weight and harvest index were higher with p_5m_1 (PPFM 38 as seed treatment). Sterility percentage was significantly lower (13.94%) with seed treatment of PPFM 16 (p_1m_1).

Between the two controls, yield attributes and yield were significantly superior in KAU POP (c_1) than KAU POP + water spray (c_2).

The treatment effect of PPFM on productive tillers per metre square, sterility percentage, number of filled grains and harvest index was superior to both the controls. While the grain weight per panicle and grain yield registered by the treatments were comparable with c_1 (KAU POP), they were significantly superior to c_2 (KAU POP + water spray). Treatment *vs*. controls was not significant with respect to straw yield.

Chlorophyll content and proline accumulation recorded at panicle initiation and flowering stages were significantly higher with PPFM 38. Between the two methods of application of PPFM, seed treatment (1%) with PPFM (m_1) recorded superiority in proline content. But no significant variation was observed in chlorophyll content.

Among the treatment combinations, PPFM 38 as seed treatment (1%) + foliar application (2%) at 30 and 50 DAS (p_5m_2) was observed to engender significantly higher contents of chlorophyll and proline, both at panicle initiation and flowering stages. Between the controls, KAU POP recorded higher chlorophyll content whereas proline accumulation was greater with KAU POP + water spray. At panicle initiation stage, chlorophyll content was significantly higher in KAU POP (c_1) than the treatments. On the other hand, proline content was significantly higher in treatments than the controls, during both the growth stages.

The uptake of nitrogen (73.37 kg ha^{-1}), phosphorus (27.59 kg ha^{-1}) and potassium (141.96 kg ha^{-1}) was significantly higher when treated with PPFM 38. Seed treatment followed by foliar application(m_2) showed significantly higher uptake of nitrogen, phosphorus and potassium than seed treatment alone (m_1).

The interaction effect of PPFM and method of application was significantly superior, with p_5m_2 (PPFM 38 as seed treatment + foliar application) recording higher uptake of nitrogen (76.08 kg ha^{-1}), phosphorus (28.94 kg ha^{-1}) and potassium (142.75 kg ha^{-1}). Between the controls, c_1 (KAU POP) resulted in significantly higher nutrient uptake than c_2(KAU POP + water spray). The treatments exhibited significant variation with c_2 whereas no significant variation was observed with c_1. Crude protein content of rice was not affected by the treatments.

The soil analysis after the experiment revealed that the treatments failed to generate significant variation in the organic carbon and available nutrient status of soil. However, treating rice with PPFM 35 resulted in significantly higher potassium content in soil after the experiment. The comparisons made among the treatment combinations, between the two controls and between the treatments and controls also proved to be non-significant.

Among the five PPFM isolates tested, gross returns, net returns and B:C ratio were significantly higher for treatment with PPFM 38. The effect of method of application was not significant. The treatment combination p_5m_2(PPFM 38 as seed treatment + foliar application) resulted in significantly higher gross returns, net returns and B: C ratio. Between the controls, KAU POP (c_1) registered better economics than

KAU POP + water spray (c_2). Gross returns of treatments and KAU POP was comparable. However, net returns and B:C ratio were significantly higher for the treatments.

Pink pigmented facultative methylotrophs, PPFM 38 (p_5) as seed treatment (1%) resulted in vigorous seedlings as evidenced by the greater root length, seedling dry weight and seedling vigor index. Seed treatment (1%) followed by foliar application (2%) of PPFM 38, twice, at 30 and 50 days after sowing (p_5m_2) enhanced the growth, yield and economics of aerobic rice.

FUTURE LINE OF WORK

- The effect of the superior isolate can be tested against isolates from other parts of country.
- The effect of PPFM can be tested in other economically important crops.
- The efficacy of PPFM in soil nutrient mobilization could be explored.
- The extent of water saving possibility may be worked out.

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Appendices

APPENDIX I

Weather data during the cropping period

(December 2019 to April 2020)

Standard week	Temperature (^{o}C)		Relative humidity (%)		Rainfall (mm)
	Maximum	Minimum	RH I	RH II	
52	32.1	23.6	89.4	70.0	4.0
01	31.9	22.7	90.0	70.0	19.6
02	32.4	22.4	87.9	64.3	13.2
03	31.9	23.8	88.8	61.8	2.0
04	32.2	23.0	91.4	68.3	0.0
05	32.3	22.6	89.1	64.9	0.0
06	31.9	23.4	94.1	68.0	0.0
07	33.8	22.6	96.4	79.3	0.0
08	34.1	23.4	97.1	86.7	0.0
09	33.1	23.9	95.9	86.0	0.0
10	33.0	23.4	92.7	86.0	8.0
11	33.4	24.6	90.4	82.6	0.0
12	33.4	25.4	92.7	76.4	8.6
13	32.9	24.9	93.6	81.9	0.0
14	33.4	26.9	89.3	86.1	4.3
15	33.4	25.1	88.2	83.4	9.6

APPENDIX II

Average input cost and market price of produce

Sl. No	Items	Cost (Rs)
	INPUT	
A	Seed	45 per kg
B	Labour	
1	Man	700 per day
2	Woman	500 per day
C	Cost of manures, fertilizers and PPFM	
1	FYM	5 per kg
2	Lime	15 per kg
3	Urea	8 per kg
4	Rock phosphate	10 per kg
5	Muriate of potash	17 per kg
6	PPFM	50 per L
9	OUTPUT	
A	Market price of grain	40 per kg
B	Market price of straw	5 per kg

GROWTH AND PRODUCTIVITY OF AEROBIC RICE

(*Oryza sativa* L.) AS INFLUENCED BY

PINK PIGMENTED FACULTATIVE METHYLOTROPHS (PPFM)

by

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ABSTRACT

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ABSTRACT

The study entitled "Growth and productivity of aerobic rice (*Oryza sativa* L.) as influenced by pink pigmented facultative methylotrophs (PPFM)" was undertaken at College of Agriculture, Vellayani, during 2018 – 2020. The main objectives were to study the effect of PPFM on rice seedling emergence and vigour, to assess its effect on growth and yield of aerobic rice and to work out the economics.

The study was carried out as two experiments at the Integrated Farming System Research Station, Karamana, during December, 2019 to April, 2020. The test variety of rice was Uma (MO 16). The first experiment was a pot culture study in completely randomised design with six treatments and four replications. The treatments comprised seed treatment of rice with five superior isolates of PPFM from the Department of Agricultural Microbiology (T_1 - PPFM 16, T_2 - PPFM 26, T_3 - PPFM 35, T_4 - PPFM 37, T_5 - PPFM 38) and T_6 - control (distilled water).

The second experiment was conducted to assess the effect of the five PPFM isolates on growth and yield of aerobic rice. The experiment was laid out in randomised block design with [(5 x 2) + 2] treatment combinations, replicated thrice. The treatments were the five PPFM isolates (p_1 to p_5) and two methods of application, *viz*., m_1 – seed treatment (1%), m_2 – seed treatment (1%) + foliar application (2%) at 30 and 50 days after sowing (DAS), compared against two controls (c_1 – KAU POP, c_2 – KAU POP + water spray at 30 and 50 DAS).

The results of experiment I revealed uniform effect of PPFM isolates on emergence percentage. However, emergence rate index (ERI) was the highest for PPFM 38 (160), followed by PPFM 37 (156). Seed treatment of rice with PPFM 38 recorded significantly longer seedling roots, higher seedling dry weight and greater seedling vigour index I. Seedling vigour index II was higher for seeds treated with PPFM 37.

The results of experiment II showed that among the PPFM isolates tested, PPFM 38 (p_5) resulted in taller plants, more number of tillers per square metre, leaf area index, dry matter production, root shoot ratio and yield attributes. However, root volume of rice

was observed to be higher with PPFM 16 and remained at par with PPFM 38. Grain yield (4.43 t ha^{-1}), straw yield (5.31 t ha^{-1}) and harvest index were also superior with PPFM 38.

Between the two methods of application, treating rice seeds with PPFM (1%) + foliar application (2%) at 30 and 50 DAS (m_2) elicited superior effect on growth attributes including dry matter production and yield attributes like productive tiller count, grain weight per panicle and straw yield . However, root volume was not affected by the method of application of PPFM. Growth and growth attributes and yield attributes and yield were superior with the treatment combination p_5m_2 (PPFM 38 as seed treatment + foliar application). Sterility percentage was significantly lower (13.94%) with seed treatment of PPFM 16 (p_1m_1), and it was on par with p_5m_2.

Between the controls, KAU POP (c_1) was significantly superior to KAU POP + water spray (c_2) with respect to growth and yield. PPFM treatment was superior to both the controls. However, the mean grain weight per panicle and mean grain yield recorded by the treatments were comparable with that of KAU POP.

Chlorophyll content and proline accumulation recorded at panicle initiation and flowering stages were significantly higher with PPFM 38. Seed treatment (m_1) recorded superiority in proline content. The treatment combination, p_5m_2 recorded significantly higher chlorophyll and proline at both the growth stages. While KAU POP (c_1) recorded higher chlorophyll content, proline accumulation was greater with KAU POP + water spray (c_2). Nutrient uptake followed the same trend as dry matter production.

Gross returns, net returns and B:C ratio were significantly higher for treatment with PPFM 38. The effect of method of application was not significant. The treatment combination p_5m_2 resulted in better economics. Mean gross returns of treatments and KAU POP was comparable. However, net returns and B:C ratio were significantly higher for the treatments. The present study revealed that treating rice seeds with PPFM 38 (p_5) at 1 per cent resulted in vigorous seedlings as evidenced by superior seedling characters. Seed treatment (1%) followed by foliar application (2%) of PPFM 38 (p_5m_2), twice, at 30 and 50 days after sowing enhanced the growth, yield and economics of aerobic rice.

സംഗ്രഹം

നെൽക്കൃഷിയിൽ പിങ്ക് പിഗ്മെന്റഡ് ഫാക്കൽറ്റേറ്റീവ് മീതൈലോട്രോഫ്സ് (പിപിഎഫ്എം)-ന്റെ പ്രഭാവം

പിപിഎഫ്എം എന്ന ചുരുക്ക പേരിൽ അറിയപ്പെടുന്ന പിങ്ക് പിഗ്മെന്റഡ് ഫാക്കൽറ്റേറ്റീവ് മീതൈലോട്രോഫ്സ്, ബാക്ടീരിയ ഗണത്തിൽ പെടുന്ന സൂക്ഷ്മ ജീവികളാണ്. നെല്ലിന്റെ വളർച്ചയിലും വിളവിലും ഇവയുടെ പ്രഭാവം അിറയുന്നതിലേക്കായി ഒരു പഠനം നടത്തുകയുണ്ടായി. ഞാറിന്റെ വളർച്ചയിലും കരുത്തിലും, തുടർന്നുള്ള നെല്ലിന്റെ വിളവിലും, ആദായത്തിലും പിപിഎഫ്എം-ന്റെ പ്രഭാവം പഠിക്കുക എന്നതായിരുന്നു പ്രധാന ലക്ഷ്യങ്ങൾ.

തിരുവനന്തപുരം, കരമനയിൽ സ്ഥിതിചെയ്യുന്ന സംയോജിത കൃഷിസമ്പ്രദായ ഗവേഷണകേന്ദ്രത്തിൽ, 2019 ഡിസംബർ മുതൽ 2020 -ഏപ്രിൽ വരെ ആയിരുന്നു പഠനം. നെൽകർഷകർക്കിടയിൽ ഏറെ പ്രചാരത്തിലുള്ള ഉമ എന്ന നെല്ലിനത്തിൽ രണ്ട് ഭാഗങ്ങളായാണ് പരീക്ഷണ നിരീക്ഷണങ്ങൾ നടത്തിയത്. ഇതിനായി വെള്ളായണി കാർഷിക കോളേജിലെ അഗ്രിക്കൾച്ചറൽ മൈക്രോബയോളജി വിഭാഗത്തിൽ നിന്നും ലഭ്യമാക്കിയ പിപിഎഫ്എം-16, 26, 35, 37, 38 എന്നീ അഞ്ച് ഐസൊലേറ്റുകളുടെ കാര്യക്ഷമത, വെള്ളവുമായി താരതമ്യം ചെയ്യുകയുണ്ടായി.

പിപിഎഫ്എം-38 ഉപയോഗിച്ചുള്ള വിത്തുപചാരം ഞാറിന്റെ വേരുകളുടെ നീളവും, കരുത്തും വർദ്ധിപ്പിക്കുന്നതായി കണ്ടു. പ്രസ്തുത ഐസൊലേറ്റിന്റെ ഉപയോഗം നെല്ലിന്റെ ഉയരം, ചിനപ്പുകളുടെ എണ്ണം, ഇലകളുടെ വിസ്തൃതി, വിളവ്, അറ്റാദായം എന്നിവ വർദ്ധിപ്പിക്കാൻ സഹായകമാണ് എന്ന് കണ്ടെത്തി. രണ്ടു പ്രയോഗരീതികൾ പരീക്ഷി

ച്ചതിൽ, 1 ശതമാനം പിപിഎഫ്എം വിത്തുപചാരം വഴിയും തുടർന്ന് 2 ശതമാനം പത്രപോഷണമായി വിത്ത് വിതച്ച് 30 ദിവസം കഴിഞ്ഞും, 50 ദിവസം കഴിഞ്ഞും നൽകുന്നതാണ് ഉത്തമം എന്ന് തെളിഞ്ഞു.

പൊടിവിത അവലംബിക്കുന്ന നെൽകൃഷി സമ്പ്രദായത്തിൽ പിപിഎഫ്എം-38, 1 ശതമാനം ഉപയോഗിച്ച് വിത്തുപചാരം നടത്തുകയും തുടർന്ന് 2 ശതമാനം പത്രപോഷണം മുഖേന വിത്ത് വിതച്ച് 30 ദിവസം കഴിഞ്ഞും, 50 ദിവസം കഴിഞ്ഞും നൽകുന്നത് നെല്ലിന്റെ വളർച്ചയും, വിളവും, അറ്റാദായവും വർദ്ധിപ്പിക്കുവാൻ ഉപകാരപ്രദമാണെന്ന് കണ്ടെത്തി.