Original Research Article

Beneficial fungal root endophyte *Piriformospora indica* diminishes yield loss without compromising quality of banana fruits due to *Banana bract mosaic virus* infection through better soil nutrient mobilization

ABSTRACT

Aims: *Banana bract mosaic virus* (BBrMV) is a significant viral disease that adversely impacts the yield and quality of banana (*Musa acuminata*) fruits. To ensure plant health and productivity, improved nutrient management practices are essential. Conventional fertilizers in agriculture can impose substantial financial burdens due to their high costs. In addition to economic considerations, the negative impact of excessive fertilizer applications on the environment necessitates the introduction of sustainable alternatives. Endophytes have been recognized as a sustainable tool to enhance plant nutrient availability and uptake. *Piriformospora indica* is a beneficial fungal root endophyte that can improve nutrient utilization in banana plants. Efficient nutrient uptake and allocation within the plant play essential roles in enhancing fruit quality and yield. *P. indica*, is known for promoting plant growth and confer tolerance to diverse abiotic and biotic stresses. However, the effect of *P. indica* colonization on nutrient assimilation and fruit quality of BBrMV-infected banana plants still needs to be better understood, particularly in the humid tropical environments of southern laterites of Kerala. *P. indica* promotes both fruit size and quality, leading to an overall improvement of fruit attributes *viz*., fruit length, width, colour, TSS, acidity, moisture, and ash content etc., An experiment comprising four treatments with five replications revealed that *P. indica* colonization enhanced potassium concentration at the rhizosphere (603 Kg ha^{-1}), whereas decreased in the diseased plants (318 Kg ha^{-1}). The nutrient concentration in the rhizosphere recorded a substantial relation with disease severity. Compared to *P. indica* colonized plants, the uptake of N, P, K, Ca, Mg, S and B by BBrMV infected plants exhibited 36.5, 37.6, 57.5, 44.1, 23.3, 43.7 and 48.9 per cent reduction respectively. Hence, pre-colonization of *P. indica* improved the nutrient mobilization (in soil), uptake and accumulation (in fruit) in the BBrMV infected banana plants compared to the virus alone infected plant. Fruits from *P. indica* pre-colonized plants challenged with the virus exhibited an enhanced K (515.5 ± 6.25 mg 100g^{-1}) and Fe (0.42 ± 0.04 mg 100 g^{-1}) content along with high total soluble sugars (27.25 ^{o}Brix), titrable acidity (0.48 %) and ash content (1.14 %) compared to healthy and virus infected plants. Hence, this can instigate the appropriate sustainable virus disease management strategies in banana plants, emphasizing its improved fruit quality through nutrient assimilation.

Keywords: Banana, Banana bract mosaic virus, P. indica, Nutrient assimilation, Fruit quality

1. INTRODUCTION

In tropical and subtropical regions, bananas hold immense agricultural importance as a major fruit crop, with an annual production surpassing 120 million tonnes [1]. Bananas are a staple food in many regions and provide vital nutrients to millions of people worldwide.

Beyond their affordability, it is a reliable indicator plant for assessing soil nutrient deficiencies. The appearance and quality of both banana plants and their fruits can provide valuable insights into soil health and the effectiveness of fertilizer management practices. Notably, bananas exhibit a strong affinity for potassium, resulting in significant potassium accumulation in their fruits [2]. Understanding the dynamics of this essential nutrient is paramount for optimizing fruit quality and ensuring sustainable crop production. However, the dynamics of soil chemical qualities, influenced by factors including the quality and quantity of soil mineral matter, impact banana cultivation. Further, banana production is threatened by various biotic stress *viz*., bacterial, fungal and viral diseases, which reduce fruit yield and quality. Among virus diseases, *Banana bract mosaic virus* (BBrMV) characterizes by a single strand RNA genome responsible for a 5 to 40 per cent reduction in yield [3]. BBrMV displays characteristic symptoms like reddish spindle shaped lesions on pseudostem, chlorotic spindle streaks on leaves, traveller's palm appearance, poor bunch formation with long peduncle and in severe cases, the fingers become curled and deformed [4].

Mineral nutrients are essential to plant health and play an important role in plant viral disease management. It is widely recognized that, upto a certain extent, a balanced supply of macro- and micronutrients is necessary for plants to defend against viral infections. Selvarajan and Balasubramanian corroborated the statement and resulted in 125 to 150 per cent more recommended doses of fertilizer that could tackle the yield loss developed by BBrMV infection in banana plants [5].

Soil is a non-renewable resource, requires considerable time for its natural replenishment of nutrients [6]. Crop intensification and monocropping deteriorated the soil quality, reducing plant nutrient availability. Poor nutrient uptake consequently lowers photosynthetic ability, negatively impact plant fitness and increases susceptibility to disease [7]. Fertilizer application studies demonstrated that a twofold increase in fertilizer application could mitigate the yield losses resulting from BBrMV infection to a certain degree [8]. As BBrMV is a vector-borne viral disease, implementing insecticide-based measures to control aphids can also effectively reduce the disease spread up to a certain limit. In contrast, it is expensive and harmful to the environment. Under such condition, it is imperative to find alternatives suitable to provide benefits of diseases suppression and enhanced quality parameters. Effective management of viral diseases is essential for the sustainable banana production. Numerous management strategies, such as cultural practices, chemical and biological control have been developed to manage plant virus disease. Cultural practices such as crop roguing and field sanitation can help to reduce virus spread which is a time consuming process. Similarly, chemical control can also be used, but it is often costly and can adversely affect the environment. Biological control techniques *viz*., introducing beneficial endophytes and natural predators, are one of the most significant and eco-friendly methods for effective control of virus diseases in plants [9]. Research has shown that endophytes also play a crucial role in mineral nutrition and biological regulation. This is significant because, endophytes have been found to facilitate nutrient uptake by the plants, thereby promoting overall plant development [10]. The potential of endophytes as valuable assets in sustainable agriculture and ecosystem management, offering promising solutions to enhance crop productivity and reduce reliance on fertilizers.

The endophytic fungus *Piriformospora indica*, a member of the Sebacinales (Basidiomycota), was discovered from the roots of xerophytic woody plants such as *Zizyphus nummularia* and *Prosopis juliflora* in the Thar Desert of India [11] living asymptomatically within plants. They are known to attribute resistance against biotic and abiotic stresses and can extract, mobilize and transport primary nutrients *viz*., Nitrogen (N), phosphorus (P), potassium (K); secondary nutrients *viz*., calcium (Ca), sulphur (S),

magnesium (Mg); and micronutrients like iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn). In plant growth and development, considerable amounts of phosphorus are required to fulfil various regulatory, structural and energy transfer functions [12]. Considering the potential application in agriculture, one of the critical processes being investigated is enhancing plant nutrient uptake, particularly in striving towards a sustainable agriculture paradigm that mitigates the effects of climate change and incorporates soil regeneration cycles. In addition, with expanding knowledge regarding the applications of the endophyte population in plant nutrient acquisition [13], the role of *P. indica* colonization on banana plants and enhanced fruit yield in virus infected plant through improved nutrient assimilation have yet to be identified.

2. MATERIAL AND METHODS

2.1 Experimental Site

An experiment was laid out during 2019–2021 at Coconut Research Station, Balaramapuram (geographically situated at 8.40° N latitude and 77.01° E longitude). The area belongs to Agro-Ecological Unit 8 (AEU 8). The region experiences a tropical monsoon climate, with a mean rainfall of 110.40 mm from April to December [14], a relative humidity ranging between 61- 85% and an average temperature varying from 28- 32°C.

2.2 Maintenance and mass multiplication of *P. indica* culture

Potato dextrose agar (PDA) medium (pH 6.5 to 6.8) was used for the *P. indica* culture. Fungal discs of 5 mm size from an actively developing region of three weeks-old *P. indica* culture were inoculated to PDA and potato dextrose broth (PDB) in Petri plates and conical flasks respectively; and incubated in dark at room temperature (28±2°C).

Twenty one day old mycelial mats grown in PDB were used for the mass multiplication of *P. indica* in cocopeat: dried farm yard manure medium (1:1) amended with 0.02 per cent gram flour [15]. Cocopeat of electrical conductivity less than 0.4 mS. cm^{-1} were soaked overnight in tap water and washed three times in running tap water to remove excess lignin and salt. Decanted cocopeat was spread on floor under shade to drain the excess moisture. Dried and pounded farm yard manure was mixed with cocopeat at a 1:1 ratio (w/w) and gram flour was added @ 2 per cent (w/w). The mixture was filled in polypropylene bags and sterilized at 121°C with 15 psi for 2 h. The sterilization was repeated for three consequent days to completely sterilize the medium and destroy the resting spores or seeds, if any. The sterilized medium was spread aseptically on surface sterilized plastic trays and mixed with a 21-day-old mycelial mat of *P. indica* grown in PDB @ 0.2 per cent (w/w) under a laminar airflow chamber. To completely grow the fungus in the medium, the inoculated (covered with cling film) trays were placed at room temperature (28±2°C) for 15 days with a light and dark cycle of 12 hours each and relative humidity of 75 to 80 per cent.

2.3 Co-cultivation of *P. indica* with sword suckers and planting of banana in the field

The virus free sword suckers var. Nendran were used for the co-cultivation study. Sword suckers were randomly confirmed for their virus free nature serologically with polyclonal antibody of BBrMV coat protein (National Research Centre, Tiruchirapalli, Tamil Nadu) and molecularly with BBrMV coat protein specific primers (BBrMV F - ATGTCAGCTCCATCTTCATC and BBrMV R -TATCACGCTTCACATCTTCA). The rhizomes of sword suckers were surface-sterilized for 30 seconds with 0.1 per cent mercuric chloride,

then washed twice with distilled water; and then with 1 per cent sodium hypochlorite for 30 seconds followed by two washing in distilled water. Rhizomes of sword suckers were smeared with 30 per cent (w/v) *P. indica*-mass multiplied medium in distilled water (slurry coating) containing 2 x 10^6 cfu ml^{-1} and allowed to shade dry for two days. Whereas, control plants were treated with 30 per cent slurry made with cocopeat: dried farm yard manure medium amended with 0.02 per cent gram flour without *P. indica*. The treated suckers were planted in a randomized block design, with a spacing of 2 m × 2 m between each plant. The plant was raised with standard fertilizer recommendations [16]. An experiment laid out with four treatments *viz*., Healthy, BBrMV infected, *P. indica* colonized healthy plant and *P. indica* colonized plant challenged with BBrMV with five replications. The biometric observations of plants were carried out six months after planting. Similarly, the morphological observations were recorded at ripening stage 1 (fully green) and qualitative studies were carried out at ripening stage 6 (fully yellow) of banana fruit [17] as the fruits attain high market value at stage six [18].

2.4 Examination of *P. indica* colonization

To examine the colonization of *P. indica*, one month-old *P. indica*-colonized roots of banana suckers were randomly collected at different intervals. The roots were meticulously cleaned before being divided into 1 cm-long segments. Standardized protocol for the endophyte staining was followed [19] to ensure *P. indica*-colonization in the roots of bananas. The roots and newly emerged roots were washed thoroughly to remove the debris; subsequently, the roots were expertly sectioned into uniform 1 cm bits and soaked overnight with 10 per cent potassium hydroxide (KOH) solution at room temperature. Following the overnight treatment, the root sections are meticulously cleansed with distilled water and the rinsing process is repeated five times to ensure the complete removal of residual KOH then acidified with 1 per cent Hydrochloric acid (HCl). After five washings in distilled water, the root bits were stained with 0.02 per cent trypan blue. Molecular confirmation of *P. indica* colonization was carried out using Pitef primer (Pitef F- TCGTCGCTGTCAACAAGATGand Pitef R-GAGGGCTCGAGCATGTTGT) [20].

2.5 BBrMV infection and Diseases severity

Five months old banana plants were artificially inoculated with aphids. Aphids subjected to were transferred individually onto BBrMV severely infected banana plants. Aphids were given a 5-minutes acquisition access period, later removed from BBrMV infected plants and directly transferred a group of 20 aphids onto the protected test plants. Aphids were allowed a 24 h inoculation access period, after which insect proof mesh coverings were removed and the aphids were killed. Exposed plants were treated with fipronil granules to prevent further infections. Quantifying the progression of disease development was done by calculating the vulnerability index (VI) using a standardized score chart specific to BBrMV [3]. Where, the intensity was expressed as per the following equation [21].

$$VI = \frac{(0n1 + 1n2 + 2n3 + 3n4 + 4n5)}{nt(nc - 1)} \times 100$$

Whereas, VI -Vulnerability Index/Disease severity; The variables n0, n1, n2, n3, n4, and n5 correspond to the counts of plants in each category, while nt represents the total number of plants, and nc stands for the total number of categories present.

The presence of BBrMV in infected plants was confirmed using PCR with specific primers targeting the BBrMV coat protein. Actin (Actin F-CTGGTGATGGTGTGAGCCACACTGTTC

and Actin R- CACTGAGAACGATGTTGCCATACAGGTC) [22] is used as the housekeeping gene.

Table 1. Method of analysis of soil and fruit sample

Estimation	Method	References
Soil parameters		
pH	Potentiometry (Cyber scan PC510, EuTech Instruments, Singapore)	Jackson (1973) [23]
EC	Conductometry (EC-TDS Analyzer-CM 183, Elico, India)	Jackson (1973)
Available N	Alkaline potassium permanganate method	Subbiah and Asija (1956) [24]
Available Ca, Mg	Neutral 1N ammonium acetate extraction using Atomic Adsorption Spectrometry (An Analyst 400, Perkin Elmer Inc., USA)	Hesse (1971) [25]
Available S	Turbidometry- BaCL2 method (0.15% CaCl2 extraction) (UV-VIS spectrophotometer 2201, Systronics, India)	Massoumi and cornfield (1963) [26]
Available Fe, Mn, Zn, Cu, Na	0.1 M HCl extraction and Atomic Adsorption Spectrometry (A Analyst 400, Perkin Elmer Inc., USA)	Osiname et al. (1973) [27]
Available B	Hot water extraction and spectrophotometry (UV-VIS spectrophotometer 2201, Systronics, India)	Gupta (1967) [28]
Fruit parameters		
P	Vanadomolybdate yellow colour method (UV-VIS spectrophotometer 2201,Systronics, India)	Jackson (1973) [22]
K	Flame photometry (Digital flame photometer 130. Systronics, India)	Stanford and English (1949) [29]
Ca, Mg (Secondary nutrients)	Nitic-perchloric acid(9:4) acid digestion and EDTA titration method	Piper (1966) [30]
Zn, Fe, Mn, Na (Micronutrients)	Atomic Adsorption Spectrometry (An Analyst 400, Perkin Elmer Inc., USA)	Lindsay and Norvel (1978) [31]

2.6 Estimation of shoot and fruit biomass

Representative banana plants were uprooted and the various plant parts (leaf and pseudostem) were separately incised and weighed individually. The overall fresh weight of the whole plant was determined by adding the weights of its individual components. Similarly, the yield elements *viz*., bunch, hands and fingers were weighed individually and recorded.

2.7 Yield and nutrient estimation in fruits

The banana yield was accounted for control, virus alone infected plants, *P. indica* colonized plants and *P. indica* pre-colonized plants post infected with virus. This yield was correlated with nutrient uptake. The nutrient accumulation in fruits were estimated by segregating the representative samples. Samples were prepared and the P, K, Ca, S, Mg, Fe, Mn and Zn concentrations were quantified using standard protocols as specified in Table 1. The nutrient contents in fruits were calculated for 100 g of edible portion of fruit.

2.8 Soil nutrient estimation

The soil was sampled from the cultivated layer spanning a depth of 0–30 cm of healthy, BBrMV alone infected plants, *P. indica* colonized plants and pre-colonized plants post infected with virus. Eight samples were randomly collected and were combined to form one composite sample. The collected samples were air-dried and subsequently examined in the laboratory for the parameters in Table 1.

2.9 Estimation quality parameters of banana fruit

The total soluble solids were estimated with the help of a calibrated hand refractometer, which was calibrated at 20°C with 0 ^{0}Brix, with the assistance of a temperature correction correlation [32]. The titrable acidity was determined using a titration procedure [33]. For the analysis of moisture and ash content, the gravimetric method was employed [34].

2.10 Statistical analysis

Nutrient uptake in different treatments were compared using ANOVA for one-way classified data. Statistical analysis was done using GRAPES 2.0 [35].

3. RESULTS AND DISCUSSION

3.1 *P. indica* pre-colonization enhanced tolerance against BBrMV

P. indica exhibits a broad host range for colonization and this symbiotic association fosters remarkable improvements in plant growth and development. The present study revealed that *P. indica* spores germinate and colonize in the banana roots within 20 days. Later, pear-shaped chlamydospores were sequentially arranged in the intracellular region of the plant roots (Fig. 1B and 1D). Colonized plants exhibited distinct morphological alterations in roots and shoots compared to control plants (without *P. indica*) (Fig. 1A and 1C).

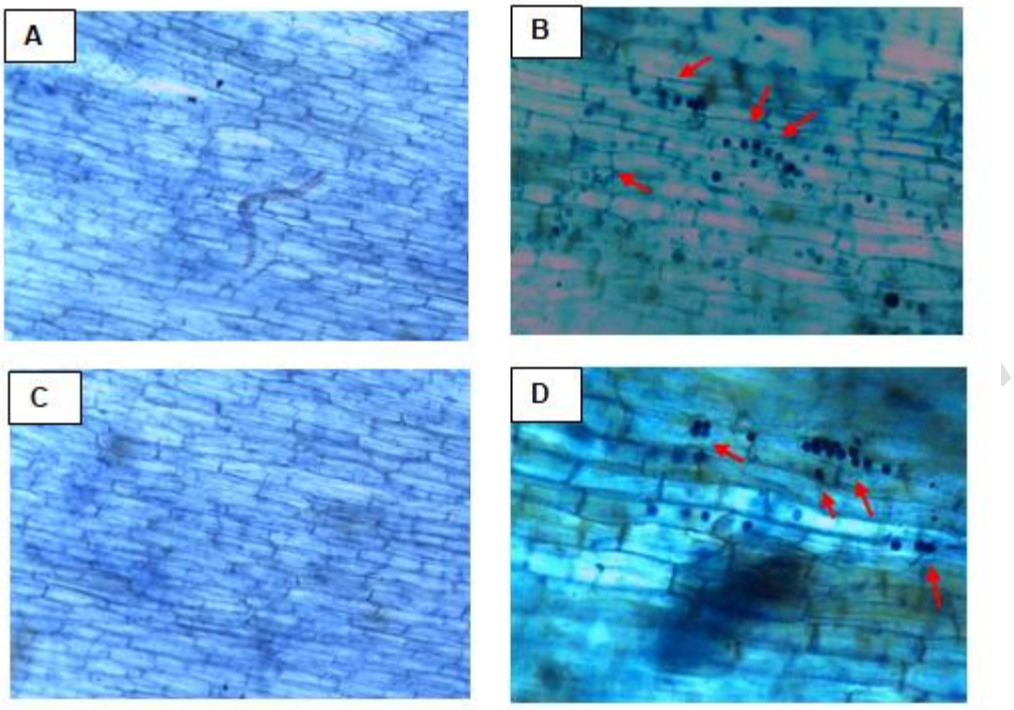

Fig. 1. Representative micrograph of the P. indica colonization in banana roots (at fruit development stage) of (A) uninoculated control, (B) colonized with P. indica (C) BBrMV alone and (D) P. indica colonized plant post infected with BBrMV. Colonized roots show the presence of chlamydospores spores (red arrows). Roots were stained with trypan blue (0.02 %).

In the present study, disease severity (VI) of BBrMV was observed (Fig. 2A-2D). *P. indica*-colonized plants recorded more tolerance to BBrMV infection. In the *P. indica* pre-colonized plants, the vulnerability index of BBrMV was reduced (72.5 %) drastically (Fig. 2E). Molecular confirmation of BBrMV was accomplished using specific primers targeting the Coat protein, while *P. indica* colonization was verified through the Pitef primer. Pitef amplification occurred exclusively in plants colonized with *P. indica*. Moreover, when *P. indica*-colonized plants were exposed to the virus, the amplification of BBrMV-specific coat protein exhibited reduced band intensity compared to BBrMV alone. These results strongly suggest that pre-colonization of banana plants with *P. indica* reduces virus titre, indicating its potential role in mitigating the effects of BBrMV (Fig. 2G). BBrMV infection resulted in a 50 per cent reduction in shoot fresh weight at the fruit development stage compared with control plants. Whereas shoot fresh weight was recorded an increase of 38 per cent in *P. indica*-colonized plants, compared with the BBrMV alone infected plant (Fig. 2F).

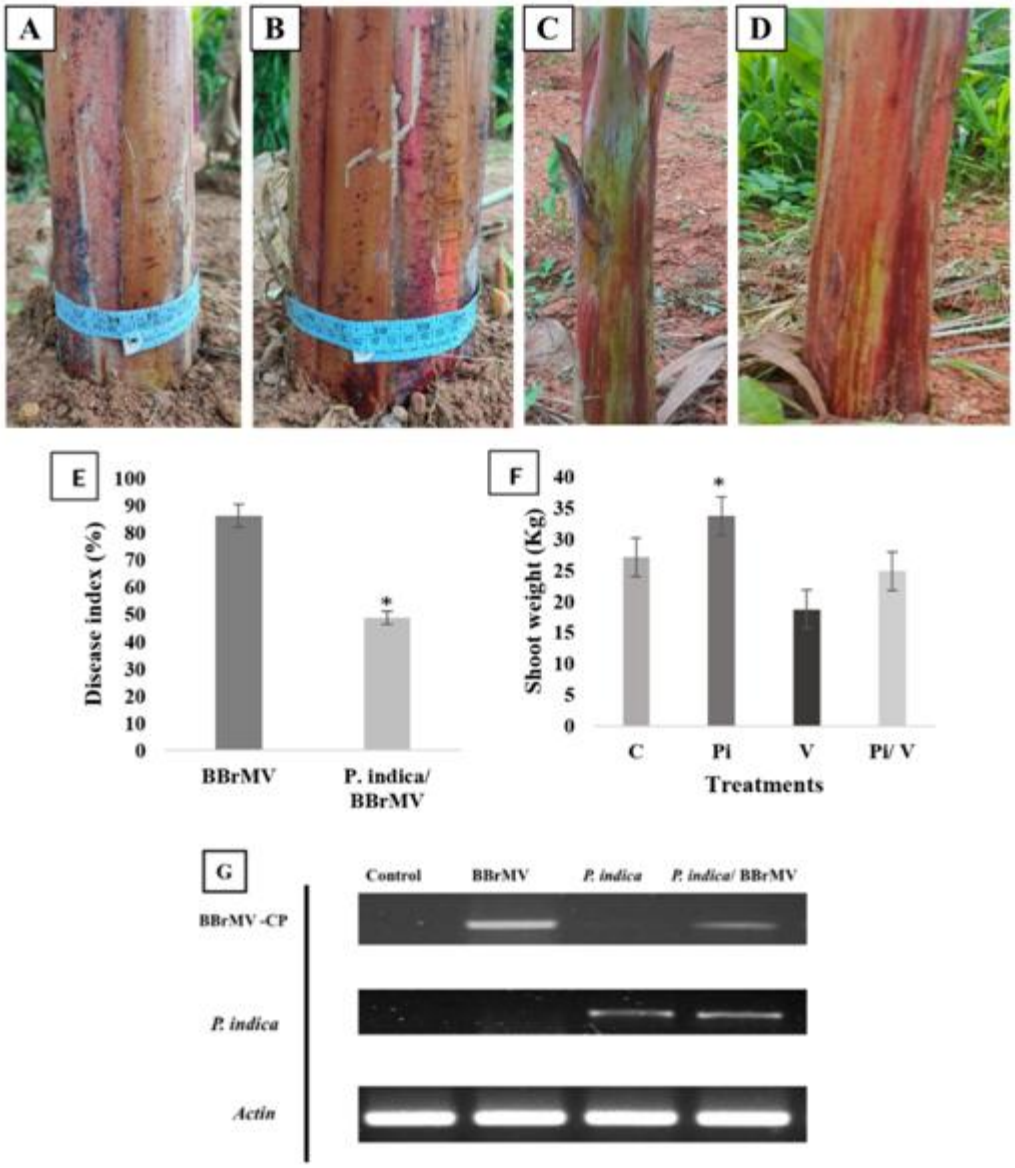

Fig. 2. *P. indica* conferred systemic disease tolerance. The severity of BBrMV infection (vulnerability index) was calculated based on the characteristic symptom (Spindle red colour streaks on the pseudostem) appeared on the pseudostem (A-Control; B- P. indica alone; C-BBrMV alone; D- *P. indica* pre-colonized plants infected with BBrMV).P. indica pre-colonized plants enhanced tolerance against BBrMV (6 MAP) (E). Fresh weight of banana shoot at 6-month-old plants subjected to different treatments (F). Where, C- uninoculated control; Pi- colonized with *P. indica*; V-BBrMV alone; and Pi/ V- *P. indica* colonized plant post infected with BBrMV. * Denotes statistically significant difference between the treatments (* P < 0.05). (G) Molecular confirmation of BBrMV infection and *P. indica* colonization in respective treatments

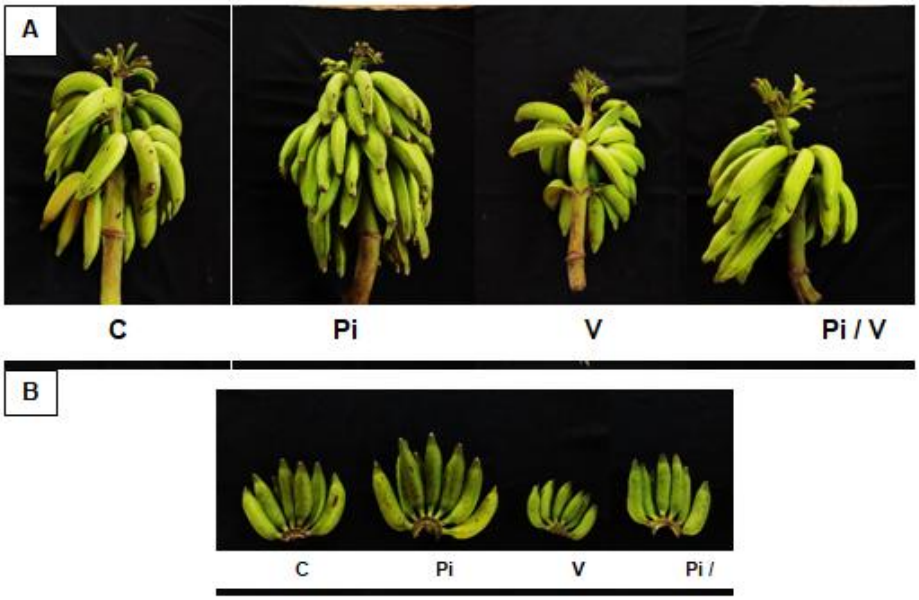

Fig. 3. ***Effect of P. indica colonization on size and shape of banana bunch (A) and fruit (B) subjected to BBrMV infection under natural conditions. Where, C- uninoculated control; Pi- Inoculated with P. indica; V-BBrMV alone; and Pi/ V- P. indica colonized plant post infected with BBrMV.***

3.2 *P. indica* enhanced plant and fruit biomass

The endophyte-colonized banana plants exhibited greater bunch weight, number of fruits per hand, hand weight, number of fingers per bunch and weight of finger at harvest compared with those of the control plant (Fig. 3). The bunch yielded from the inoculated (*P. indica*) banana plant exhibited greater fresh weight (10.30 ± 1.21 Kg) compared with the control (8.20 ± 2.90 Kg). *P. indica* colonized plants reduced days taken for bunch emergence (188.00 ± 5.33 days) compared to non-colonized control plants (208.75 ± 8.81 days). The BBrMV infected plant delayed bunch emergence (212.78 ± 6.78 days), whereas *P. indica* colonized plants challenged with the virus, reduced the days taken for bunch emergence (about 3 weeks). A similar trend was observed in case of days taken for maturity. *P. indica* reduced the days taken for maturity by ten days compared with the control plant. The fresh weight of harvested fruit bunch was higher in the *P. indica* colonized plant (10.30 ± 1.21 Kg) and *P. indica* colonized plant challenged with virus (7.85 ± 0.48 Kg) compared with the BBrMV alone infected plant (5.89 ± 3.47 Kg). The number of hands per bunch was on par, whereas the number of fingers per hand of the harvested bunch for the control plant was 8.94 ± 0.85 and 9.50 ± 0.52 for the *P. indica* colonized plant (Table 2).

P. indica pre-colonization enhanced 45 per cent greater number of fingers per bunch in BBrMV infected plant compared to virus alone infected plant. While comparing virus infected bunch and *P. indica* alone infected plant, the average fresh weight of fingers (150 g and 366 g respectively), average length of the fingers (11 cm and 25 cm respectively) and average girth of the fingers (7 cm and 13 cm respectively) were showed almost double the value in *P. indica* colonized plants compared to the BBrMV infected plant. The duration for ripening is

more or less similar in case of BBrMV infected plants and control, albite the fruits from *P. indica* colonized plants delayed ripening by one week.

Table 2. *P. indica* enhanced fruit metrics of banana var. Nendran challenged with BBrMV under open field condition

Physical parameter	Control	*P. indica*	BBrMV	*P. indica*-followed by BBrMV
Days taken for bunch emergence	208.75± 8.81	188.00 ± 5.33	212.78 ± 6.78*	190.45 ± 6.34
Days taken for maturity	89.00 ± 4.59	79.20 ± 3.27	85.78 ± 5.89*	80.32 ± 1.45
Weight of bunch (Kg)	8.20 ± 2.90	10.30 ± 1.21*	5.89 ± 3.47	7.85 ± 0.48
Number of hands per bunch	4.13 ± 0.50	4.63 ± 0.50*	3.97 ± 0.45	4.09 ± 0.97
Number of fingers per hand	8.94 ± 0.85	9.50 ± 0.52*	7.94 ± 0.78	8.13 ± 0.67
Total number of fingers per bunch	43.31 ± 4.21	54.01 ± 1.20*	26.56 ± 1.89	38.69 ± 2.06
Weigh of D hand of a bunch (Kg)	1.63 ±0.30	2.98 ± 0.28*	0.65 ± 0.12	1.10 ± 0.45
Average weight of fingers (g)	256.21± 39.12	366.40 ± 18.62*	150. 09 ± 9.08	223.78 ± 12.32
Average length of fingers (cm)	21.66 ± 1.06	25.88 ± 1.11*	11.89 ± 1.45	18.98 ± 2.09
Average girth of fingers (cm)	10.45 ± 0.41	13.46 ± 0.59*	7.13 ± 0.72	8.78 ± 0.74
Days taken for ripening	5.25 ± 0.68	7.81 ± 0.83*	5.34 ± 0.97	6.56 ± 0.45

*Values are the mean of five replications ± standard deviation of two independent experiments. *indicate significant difference of the values compared to P. indica and BBrMV treatments, as determined by One-way ANOVA analysis (* P ≤ 0. 005)*

3.3 *P. indica* enhanced total soluble sugar and titrable acidity of banana fruit

In the study of fruit quality attributes, the total soluble solids and titrable acidity were significantly higher in *P. indica* colonized plants. Fruits obtained from BBrMV infected plants were curved and poorly developed (Fig. 3). Even though, the fruits were observed with poor physical parameters, the quality parameters *viz*., total soluble solids (Fig. 4A) and titrable acidity (Fig. 4B) were recorded a slight enhancement in comparison with control fruit. TSS was 11.7 per cent higher in fruits of both virus infected and *P. indica* colonized plants compared to control plants. Similarly, the titrable acidity recorded 26.1 and 37.7 per cent increase in *P. indica* colonized plant and *P. indica* pre-colonized plants challenged with virus in comparison to virus alone infected plant. The percentage of ash (Fig. 4C) in the sample provides insight into the inorganic composition of a sample from where the mineral content may have been obtained. Fruits from *P. indica* colonized plants showed more ash content (1.62 %) compared to virus infected plants (0.67 %). This increase in ash content can be

presumed to be concomitant with the mineral element composition. Fruits from *P. indica* colonized plants observed with low moisture percentage (64.34 %) compared to control (66. 78 %) (Fig. 4D). Whereas fruit from *P. indica* colonized plant challenged with BBrMV (65.08 %) exhibited a slight increase in moisture percentage in comparison to virus alone infected plant (61.81 %).

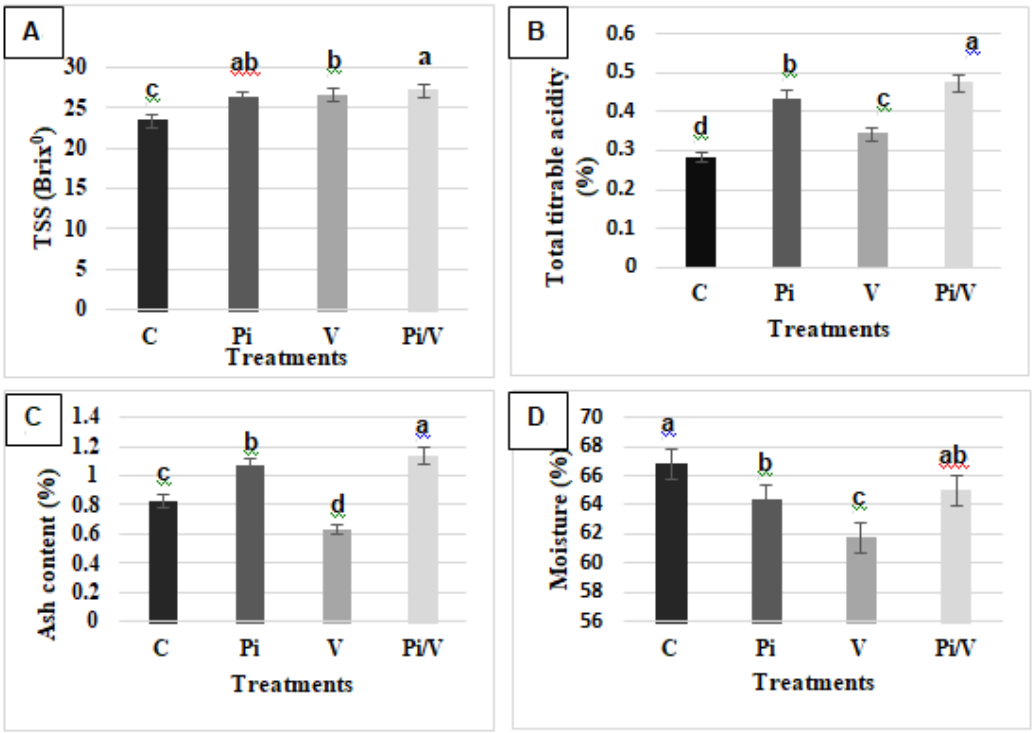

Fig. 4. ***P. indica* colonization enhanced biochemical properties viz., TSS (A) and titratable acidity (B); proximate values such as ash content (C) and moisture percentage (D) of banana fruits. One-way -ANOVA is performed and means marked by the same lower-case letter are not significantly different (P < 0.05) according to LSD. Where, C- uninoculated control; Pi- inoculated with P. indica; V-BBrMV alone; and Pi/ V- P. indica colonized plant post infected with BBrMV. Values are mean of five replications ± standard deviation of two independent experiments; Error bars represent standard deviation of the means.**

3.4 Endophyte improved soil nutrient mobilization

To develop an effective nutrient management strategy, it is imperative to comprehend the extent of nutrient availability in the root zone of the banana plant. The total uptake of both macro and micronutrients *viz*., N, P, K, Mg, Ca, S, Fe, Mn, Na, Zn and B as influenced by the BBrMV infection and endophyte colonization. Since the changes in nutrient mobilization between the root zone of virus infected plant and *P. indica* pre-colonized plant further infected with the virus was significantly different, the average nutrient availability of three treatments was computed and compared with control. Soil pH is influenced by various factors *viz*., type of plant, microbiome association and environmental factors. The rhizosphere of *P. indica* colonized plants recorded reduced soil pH (6.55 ± 0.205) in comparison to the control (6.90 ± 0.189). Root colonization, germination and spore number of endophytes were influenced by the soil pH and varies depending on the species [36;37]. *P. indica* slightly acidify the rhizosphere and enhance the assimilation of micronutrient *viz*.,

Fe, Mn, Zn etc. to the plants. Among the macronutrients, the concentration of N, P, K in the rhizosphere of *P. indica* colonized plants (160.02, 107.2 and 603.75 Kg ha^{-1} respectively) was 59.2, 75.3 and 89.8 per cent higher compared to the rhizosphere of BBrMV infected banana plants (100.47, 61.16 and 318.0 Kg ha^{-1}). Secondary nutrient Mg (113 ppm) recorded no significant difference in both the rhizosphere of BBrMV alone infected plant and *P. indica* pre-colonized plant challenged with BBrMV (Table 3). Micronutrients *viz*., B, Mn, Fe concentration in the rhizosphere of *P. indica* colonized plants (0.61, 25.38 and 28.20 ppm) was significantly 60.5, 28.4 and 73.1 per cent higher compared to the rhizosphere of control plants (0.38, 19.75 and 16.35 ppm).

Table 3. ***Endophyte colonization enhanced the banana rhizosphere nutrient composition of banana***

Soil properties and nutrient status	Control	*P. indica*	BBrMV	*P. indica*-followed by BBrMV
Ph	6.90 ± 0.189^{a}	6.55 ± 0.205^{b}	5.88 ± 0.203^{c}	6.75 ± 0.163^{ab}
EC (dSm^{-1})	0.06 ± 0.008^{b}	0.11 ± 0.013^{a}	0.03 ± 0.008^{c}	0.03 ± 0.010^{c}
N (Kg ha^{-1})	112.34 ±1.612^{c}	160.02 ± 2.472^{a}	100.47 ± 1.992^{d}	149.46 ± 2.606^{b}
P (Kg ha^{-1})	30.12 ± 1.159^{d}	107.21 ± 1.148^{a}	61.16 ± 1.146^{c}	65.86 ± 1.678^{b}
K (Kg ha^{-1})	318.0 ± 2.708^{b}	603.75 ± 3.403^{a}	218.05 ± 5.140^{c}	603.50 ± 5.066^{a}
Ca (ppm)	103.63 ± 6.238^{d}	140.49 ± 1.568^{c}	159.20 ± 1.577^{b}	221.10 ± 1.707^{a}
Mg (ppm)	96.90 ± 0.841^{c}	108.68 ± 0.624^{b}	113.90 ± 0.577^{a}	113.80 ±1.934^{a}
S (ppm)	17.13 ± 0.386^{a}	11.68 ± 0.330^{d}	16.23 ± 0.512^{b}	12.41 ± 0.368^{c}
B (ppm)	0.38 ± 0.017^{c}	0.61 ± 0.014^{a}	0.30 ± 0.013^{d}	0.43 ± 0.014^{b}
Mn (ppm)	19.75 ± 0.387^{b}	25.38 ± 0.512^{a}	7.43 ± 0.411^{d}	18.20 ± 0.804^{c}
Zn (ppm)	6.43 ± 0.263^{a}	4.50 ± 0.245^{b}	0.58 ± 0.096^{d}	1.24 ± 0.072^{c}
Fe (ppm)	16.35 ± 0.370^{b}	28.20 ± 0.497^{a}	11.33 ± 0.369^{d}	12.60 ± 0.698^{c}
Na (Kg ha^{-1})	44.13 ± 0.189^{a}	11.00 ± 0.408^{b}	11.03 ± 0.126^{b}	11.05 ± 0.173^{b}

The means of responses are provided with the standard deviation of the means. Means marked by the same lower-case letter are not significantly different (P < 0.05) according to LSD (C- uninoculated control; V- BBrMV alone infected; Pi- P. indica alone colonized; Pi/V- P. indica-colonized plants challenged with BBrMV).Values are the mean of five replications ± standard deviation of two independent experiments.

To deliver better conclusion, further nutritional ratios were calculated to comprehend the interplay of nutrients influencing the disease severity. The study revealed that the available P:K ratio is high in the rhizosphere soil with respect to virus infected banana plants in comparison to healthy plants resulted due to the underdeveloped root system limited nutrient assimilation in the virus infected plant. The primary reason for the reduction in the absorption of P and K in BBrMV infected plants, resulting in a higher ratio compared to that observed in healthy and *P. indica* colonized plants. Similarly, it has been shown that healthy plants have greater K:Ca and K:Mg ratios than diseased ones. Current study demonstrated that K absorption is significantly decreased in BBrMV infected plants; Moreover, the uptake of Ca and Mg was also reduced, though not to the same extent as K in those plants, thereby confirming the vital role of potassium in disease resistance. Phosphorus, a critical macronutrient for the plant development, is required in large amounts to perform a variety of tasks, including regulatory, structural and energy transfer responsibilities. *P. indica* serves as a proficient facilitator of phosphate mobilization that enhances the P concentration at the rhizosphere of the *P. indica* colonized plants (107.21 ± 1.148 %) compared to control (30.12 ± 1.159 %).

Table 4. ***Fruit nutrient composition of banana var. Nendran influenced by virus-endophyte interaction***

Treatments	P(mg $100g^{-1}$)	K(mg $100g^{-1}$)	Ca(mg $100g^{-1}$)	Mg(mg $100g^{-1}$)	Fe(mg $100g^{-1}$)	Mn(mg $100g^{-1}$)	Zn(mg $100g^{-1}$)	Na(mg $100g^{-1}$)
Control	53.88 ± 3.39^{c}	534.00 ± 8.64^{b}	17.18 ± 0.35^{d}	44.50 ± 3.28^{c}	0.35 ± 0.03^{c}	0.070 ± 0.010^{b}	0.175 ± 0.013^{b}	12.43 ± 0.29^{c}
P. indica	74.63 ± 1.25^{a}	565.20 ± 5.56^{a}	28.10 ± 0.57^{a}	64.05 ± 2.90^{a}	0.54 ± 0.03^{a}	0.110 ± 0.013^{a}	0.230 ± 0.022^{a}	18.30 ± 0.62^{a}
BBrMV	47.90 ± 3.33^{d}	301.30 ± 14.10^{d}	19.45 ± 0.51^{c}	35.58 ± 4.61^{d}	0.28 ± 0.02^{d}	0.060 ± 0.008^{c}	0.160 ± 0.016^{b}	11.18 ± 0.46^{d}
P. indica followed by BBrMV	63.43 ± 0.96^{b}	515.50 ± 6.25^{c}	24.03 ± 0.51^{b}	51.38 ± 2.54^{b}	0.42 ± 0.04^{b}	0.090 ± 0.010^{b}	0.230 ± 0.026^{a}	16 00 ± 0.34^{b}
CD	3.352	12.27	0.780	6. 160	0.050	0.018	0.028	0. 812
SE(m)	1.048	3.835	0.244	1.93	0.016	0.006	0.009	0.254

The responses are provided with mean ± standard deviation. Means marked by the same lower-case letter are not significantly different (P < 0.05) according to LSD. Mineral nutrient content represented in mg 100 g^{-1} edible portion of fruit. Values are the mean of five replications ± standard deviation of two independent experiments.

3.5 *P. indica* increased nutrient accumulation in fruits

Fruits are enriched source of various mineral content. Bananas are known for its richness in potassium. Fruits from *P. indica* colonized plants recorded 30 per cent more potassium accumulation compared to control and a similar trend was observed in case of other plant nutrients *viz*., nitrogen, phosphorus, boron, manganese and iron. Whereas a reduction in the level of calcium, magnesium, zinc and sulphur; and no significant difference could be observed in sodium level compared with untreated fruits. Potassium helps in the synthesis and storage of carbohydrates by activating the sugar-synthesizing enzyme, which results in an increase in the amount of sugar in fruit. Additionally, it enhances the transfer of sucrose, aids in the conveyance and production of proteins (amino acids) and functions to neutralize organic acids [38].

The overall uptake of all the nutrients was more in the *P. indica* colonized healthy banana plants in comparison to the BBrMV infected banana plants. The percentage increase of nutrients *viz*., P, K, Ca, Fe, Mg and Zn in the fruit biomass of *P. indica* pre-colonized plants challenged with virus in comparison with fruits from virus infected plants was 34.0, 71.1, 26.3, 50.0, 45.7 and 43.7 per cent respectively. This demonstrated that the *P. indica* pre-colonization significantly enhanced the nutrient accumulation in the fruits obtained from BBrMV infected plant followed the order K > Fe> Mg> Zn> P > Ca (Table 4).

3.6 Endophyte conferred tolerance against BBrMV through enhanced vegetative and reproductive growth in banana plants

In the present study, *P. indica* colonized plants enhanced the shoot biomass 52.7 per cent compared to the BBrMV infected plants. In general, the systemic infection of virus reduced

the biometric characteristics of plants by altering the physiology of the plant system [39]. Similar results were found in the previous studies; the winter wheat infected by Triticum mosaic virus and Wheat streak mosaic virus significantly reduced the number of tillers per plant, shoot and root biomass [40]. Similarly, Tobacco mosaic virus infection reduced both vegetative growth parameters and the development of generative organs in the pepper plant [41]. Banana plants are prone to many viruses [42]. Vector borne plant viruses could be partially eradicated by the chemicals that could create harmful effects on the environment and living organisms, necessitating the development of a sustainable strategy to tackle this problem. Beneficial endophytes act as the bioprotectant, provides improved tolerance against virus along with increased assimilation of mineral nutrition led to the plant fitness [43]. In the current study, pseudostem girth was measured just prior to destructive sampling. Control plants exhibited an average width of 35.5 cm, whereas *P. indica* colonized plants displayed a greater width of 48.7 cm. These results highlighted capability of *P. indica* to enhance the strength and robustness of the plants. *P. indica* is a beneficial fungal root endophyte colonize in wide range of host plants enhanced plant growth by increased root and shoot biomass, enhanced reproductive growth, early emergence of flowers and early fruit set [44,45]. Which is significantly influenced by various phytohormones [46]. Fungus induced growth promotion is achieved by modulating phytohormones involved in the development of plants [47,48]. Production and management of indole-3-acetic acid (IAA) affect shoot development [49]. Li *et al.* corroborated with the results and confirmed the role of auxin and cytokinin in the development of banana plants colonized with *P. indica*. Present study assumes that endophytic plant hormone production may influence not only shoot biomass but also involved in the root development thus leads to the enhanced nutrient acquisition of the plant [50].

The root system acts as the connecting link between a plant and soil. In general, physiological responses like mass flow, transpiration, capillary pressure etc. were involved in the process of nutrient uptake in plants [51], hence the ability of plants to absorb nutrients could be influenced by modifications in its shoot and root biomass [52]. In the presence of endophytic fungi, plants developed good root architecture, proliferation of feeder roots and enhanced mass flow, which leads to increased nutrient uptake [53]. This is well exemplified with the study conducted in Lolium perenne with Epichloë endophytes, resulted an increased concentration of soil nutrients *viz*., N, Ca, Mg, S, Mn and Mo due to the endophyte-plant interaction [54]. Plant-endophyte interactions can lead to the modification of rhizosphere conditions, impacting the proliferation, survival and development of the rhizosphere microbiome. Consequently, this symbiotic relationship may enhance nutrient uptake capabilities in the plant [55, 56]. *P. indica* colonized rhizosphere soil of banana plants exhibited a reduction in soil pH. This result supported by Clark observed in his study that high soil pH generally reduced the root colonization by AMF [57].

3.7 *P. indica* enhanced banana fruit quality through increased nutrient accumulation in fruits

The size, shape and quality of fruit can also be affected by viruses, which manifest apparent symptoms that impair the aesthetic appeal and/or organoleptic qualities of fruit [58]. Leaf parameters *viz*., number of leaves, leaf length and leaf width were enhanced in *P. indica* colonized plants [50]. The enhanced leaf area facilitates increased photosynthetic activity leading to better photosynthate accumulation which is essential for overall plant growth. Potassium (K) and magnesium (Mg) exhibit significant impacts on activities closely associated with photosynthesis and translocation of photosynthates. In order to sustain plant growth and metabolism, it is important to make it easier for carbohydrates to move from photosynthetically active tissues to sink organs *viz*., roots, fruits and seeds. The amounts of

K and Mg nutrition have a considerable impact on the movement of sucrose, which is the primary carbohydrate transport form in plants [59]. It is important to note that plant virus infection lower photosynthesis activity by reduced nutrient assimilation and has been implicated in the decline in plant vigour, productivity and fruit quality. In contrast, At the physiological level, viral infections can disrupt the translocation, accumulation and distribution of nutrients, potentially leading to incomplete ripening or a diminished flavour in fruits [60]. This is well exemplified in case of grapes infected with Grapevine leaf associated virus 2 developed low quality taste with under-ripened fruit. In contrast, the fruits were observed with a high level of total soluble solids [61, 62]. This was corroborated with our results showed that fruits obtained from BBrMV infected banana plants recorded high TSS compared to control (without virus) plants. In addition, the systemic infection of viruses manipulates the nutrient concentrations in the infected plants. This is well demonstrated by the study of *Squash vein yellowing virus* (SqVYV) infection in the melon plants resulted in alterations in the concentration of nutrients *viz*., Mg, B, Zn and K in fruits [63].

Fruits are generally enriched with vitamins and minerals, however, certain micronutrients such as Fe and Zn were deficient in fruits [64] even though they are essential for birds, animals and human beings [65]. The application of endophytes may increase productivity along with improved nutritional quality of fruits, vegetables and seeds by boosting the concentration of certain minerals in the edible portions [66]. *P. indica* enhanced the development of plant vegetative growth in addition to encouraging reproductive growth. The increase in banana bunch weight was remarkably high. The notable increase in banana bunch weight was not attributed to a rise in the number of hands per bunch. Instead, it was observed that the fruit number and weight per fruit were higher in comparison to the control plants. This indicates that better nutrient availability and its translocation might have favoured the translocation of photosynthate to generative organs (fruits) during the harvest stage of the plants helps to gain more bunch weight. Both vegetative (Shoot biomass- 38 %) and reproductive phase (Bunch weight- 74 %) were enhanced in *P. indica* colonized plants could be due to enhanced N, P, K accumulation. In addition, nutritional levels of fruit pulp expressed wide variation among the four treatments. Compared with virus infected plants, the fruits obtained from *P. indica* colonized banana plants considerably elevated the concentration of P (74.63 ± 1.25 mg 100g $^{-1}$), Zn (0.230 ± 0.022 mg 100g $^{-1}$), Mn (0.110 ± 0.013 mg 100g $^{-1}$) and Fe (0.54 ± 0.03 mg 100g $^{-1}$) in fruit pulp (Table 4). Similarly, *P. indica*-colonized plants later challenged with the virus also dramatically increased P (63.43 ± 0.96 mg 100g $^{-1}$), K (515.50 ± 6.25 mg 100g $^{-1}$), Mn (0.090 ± 0.010 mg 100g $^{-1}$), Fe (0.42 ± 0.04 mg 100g $^{-1}$) and Zn (0.230 ± 0.026 mg 100g $^{-1}$) contents in fruit pulp. One time application of *P. indica* in banana plants (colonization till harvest) conferred tolerance against BBrMV but also had a beneficial impact on fruit quality. This finding introduced a novel approach to improving fruit quality in bananas. Thus, the enhanced nutrient status in *P. indica* colonized banana plant is the result of the cumulative effect of plant root morphology, nutrient translocation and defense mechanism. These results were also concurrence with a study conducted on tomato with AMF interaction enhanced shoot and fruit biomass resulted in an elevated N accumulation in adverse conditions [67]. Along with the biometric parameters endophytes also influenced the quality parameters. *P. indica* improves the nutritional value of food-grade fruits and vegetables [45]. It could be observed that *P. indica* colonized banana fruits had higher TSS, Titrable acidity (TA) and ash content for var. Nendran, whereas reduced moisture per cent were observed. Total soluble salts enhanced the sugar content of the fruit and enlightened the sweetness. This is affirmed by the study that sugarcane crops colonized with *P. indica* resulted in elevated sugar content in canes compared to the control plant [68]. As previously mentioned, *P. indica* colonized banana var. Nendran was nutritionally rich by expanding the sink capacity of roots, *P. indica* colonized banana var. Nendran exhibited a stronger nutrient sink compared to non-colonized plants. This resulted in a more effective diversion of nutrients towards the fruits. The fruit moisture

percentage serves as an indicator of water activity, reflecting both stability and susceptibility to microbial infection [69]. Based on this, fruits obtained from *P. indica* colonized banana var. Nendran yielded less moisture. The fruit ripening process in *P. indica* colonized banana plants was comparatively slower than in un-colonized plants, which could potentially result in an extended shelf life, thereby inhibiting microbial growth compared to the control plant.

Vazquez-Hernandez *et al.* reported lower weight loss in papaya fruits colonized with *Funneliformis mosseae* than in non-mycorrhizal plants [70]. It indicates that the fruits from endophyte colonized plants may exhibit an excellent postharvest quality.

3.8 Relationship among endophyte-soil-fruit nutrients and diseases severity

In this study, the yield from *P. indica* colonized plants was significantly superior to the yield of bananas from BBrMV infected plants. *P. indica* pre-colonized plants recorded high tolerance to BBrMV and relatively high yield. Hence, the prospect of planting *P. indica* colonized plants has demonstrated a significant potential. The findings clearly shows that high diseases severity, low fruit yield and total soluble sugar levels were observed in BBrMV infected fruits than those of *P. indica* colonized plants; The outcome might be explained by the exchangeable concentrations of K and P in the soil. The negative correlation observed between disease severity and potassium (K) levels in plants is consistent with findings from prior investigations using tomato fruit. When tomato plants were exposed to TMV [71], those treated with potassium (K) exhibited significantly less internal browning compared to other virus-exposed treatments, including phosphorus (P) and phosphorus plus potassium (P+K) [72].

Under the perspective of nutrient transfer in plant-endophyte symbioses, the uptake of soil nutrients facilitated by plant hosts, whereas the developing fungus receives space and energy from the host [73]. In this context, root-associated endophytes play a crucial role in mobilizing soil nutrients, thereby assisting plants in nutrient assimilation [74]. *P. indica*, as a root-colonizing endophyte, has the capacity to enhance plant growth regardless of the P concentrations present in the soil. [75]. Moreover, studies reported that *P. indica* could facilitate the elevation of gene expression related to nitrate reductase, resulting in the activation of trace element uptake (including Mo, B, Fe, Zn, and Mn) within the rhizosphere [76].

In the present study, the Ca concentration was comparatively high in fruits of *P. indica* colonized plants. Studies show the role of Ca in delaying ripening and senescence processes in some fruits [77, 78]. Moreover, the P, K, Ca, Mg, Cu, Zn and Mn levels in the fruit of *P. indica* colonized plants were significantly higher and could provide better dietary minerals than control and virus infected banana plants. The mutualistic colonization strategy of *P. indica* has been partially unveiled, demonstrating that nutrient mobilization is harnessed to foster plant growth and effectively reduce virus symptom development in banana plants.

4. CONCLUSION

The present study primarily revealed that *P. indica* colonized banana plants reduced symptoms of BBrMV in addition to enhanced predominant intrinsic and extrinsic fruit quality parameters compared to non-colonized plants under field conditions. These results pave the way for using *P. indica* as a biofertilizer for sustainable banana production by reducing the extensive usage of inorganic fertilizer. However, intense research needed to identify the relationships between the endophytic microenvironment and nutrient mobilization in the rhizosphere.

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