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A Combined Nutrient/Biocontrol Agent Mixture Improve Cassava Tuber Yield and Cassava Mosaic Disease

Kumar Neelakandan [1], Kalarani M. Karuppasami [2,*], Nageswari Karuppusamy [3], Kavitha P. Shanmugam [3], Pugalendhi Lakshmanan [1], Suganya Subramanian [3], Venkatachalam S. Ramasamy [3], Deivamani Mariyappan [1], Velmurugan Muthusamy [3] and Djanaguiraman Maduraimuthu [2]

[1] Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore 641003, India; kumarhort@yahoo.com (K.N.); deanhortcbe@tnau.ac.in (P.L.); deivamanimariyappan@gmail.com (D.M.)
[2] Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641003, India; jani@tnau.ac.in
[3] Tapioca and Castor Research Station, Tamil Nadu Agricultural University, Yethapur, Salem 636117, India; nageswarihort@yahoo.co.in (N.K.); oviya232@yahoo.com (K.P.S.); agri_sugan17@yahoo.com (S.S.); venkattnau@gmail.com (V.S.R.); hortmrvelu@yahoo.com (V.M.)
* Correspondence: kalarani.mk@tnau.ac.in

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Abstract: Cassava (*Manihot esculenta* Crantz) is an important tropical root crop and a major dietary energy source for more than 500 million people. The major production constraints in cassava are the occurrence of nutrient deficiency and cassava mosaic disease (CMD). Hence to increase the cassava yield, it is critical to develop a technology to overcome the problems associated with nutrient deficiencies and CMD. Series of field experiments were conducted to evaluate and validate a new mixture containing biocontrol agent and nutrients on different genotypes and locations. The result indicated that foliar spray of combined nutrient/biocontrol agent mixture at 21 d interval from one to five-month after planting (MAP) had significantly decreased the incidence of nutrient deficiency symptom and CMD incidence resulting in an increased tuber yield. There were significant differences among the cassava genotypes for CMD reaction and foliar spray of combined nutrient/biocontrol agent mixture at 21 d interval from 1 to 5 MAP. The genotype H226 had lower CMD incidence and higher tuber yield. The multilocation trial indicated that foliar spray of combined nutrient/biocontrol agent mixture at 21 d interval from 1 to 5 MAP significantly improved the tuber yield (24%) and decreased the CMD incidence (65%) than unsprayed control. Metabolomic study indicates that foliar spray of combined nutrient/biocontrol agent mixture has altered lipid biosynthesis and metabolism, as evidenced by increased accumulation of octadecatrienoic acid (2.28-fold) trilinolein (126.3-fold) in combined nutrient/biocontrol agent mixture sprayed plants over unsprayed control. Overall, it is evident that foliar spray of combined nutrient/biocontrol agent mixture from 1 to 5 MAP has decreased CMD incidence and increased the tuber yield.

Keywords: cassava; biocontrol agent; CMD; nutrient deficiency; tuber yield

1. Introduction

Cassava or tapioca (*Manihot esculenta* Crantz) is a tuber crop that plays an important role in food security for millions of people, especially in the developing countries of the globe, by an alternate source of energy. Among the starchy staples, cassava gives a carbohydrate production of about 40% higher than rice and 25% more than maize. Apart from this, cassava roots consist of essential micronutrients, such as vitamins A, B, and C, iron, and zinc [1]. Approximately 500 million people across the globe depend on cassava for carbohydrate sources [2], making it the third-largest source of carbohydrate for human food in the world [3]. The reason is that it is tolerant to poor soils, diseases, and drought [4]. In addition, cassava can produce more yield per unit area, capacity to withstand adverse biotic and abiotic stresses and adaptability to marginal lands [5]. In India, cassava has been cultivated for more than a century, and it is considered as an industrial crop because the tuber is used for starch and sago production. In India, 60–70 percent of the total cassava production is used commercially to produce sago, starch, dried chips, flour, etc. In addition, the starch from cassava is used in textile industries as a sizing agent in pharmaceutical industries, making adhesives, dextrin manufacturing, paper industry, laundry, and many fast-food preparations.

Unlike other crops, cassava is a drought-resistant crop grown in areas with poor soil fertility conditions and soil problems [6]. Furthermore, cassava has a strong competitive advantage in the tropical and subtropical cultivation systems because of its high efficiency in transforming solar energy into bioenergy (250 k cal ha^{-1} day^{-1}) with high yield potential, wide adaptability to different climates, and cropping systems [5,7]. In addition, the high starch content and its excellent physical and chemical characteristics have shifted the crop from being a small-scale subsistence crop to a large-scale commercial crop.

Cassava mosaic disease (CMD) is a serious disease caused by different species of cassava mosaic begomoviruses, which significantly limits cassava yield. To date, eleven distinct begomovirus species have been identified as the causal agents of CMD [8]. Begomoviruses are known to be vectored by the whitefly (*Bemisia tabaci*), a species complex consisting of more than 36 genetically distinct but morphologically indistinguishable cryptic species [9]. Cassava is a vegetatively propagated crop; due to this, CMDs and their DNA satellites are transmitted through infected stem cuttings [10]. Besides, the vegetative propagation of cassava also favours the propagule transmission of CMD. Unlike fungi and bacteria, viruses do not attack the structural integrity of their host tissues; instead, they subvert the synthetic machinery of the host cell, acting as molecular pirates [11]. Therefore, the management of plant viral diseases appears difficult at the field level. Nevertheless, the management strategies like prevention of propagule transmission and control of viral vectors are currently adopted in cassava.

A plant's nutrition determines the plant's health, and better nutrition might improve the resistance to insect and disease infestation [12]. Nutrients are the first line of defense against plant diseases and influence all parts of the disease "pyramid" [13]. All the essential nutrients can decrease disease severity by altering disease resistance or tolerance level [14]. Nutrients can reduce disease to an acceptable level or at least to a level that other practices further control [15].

A survey was conducted in cassava growing regions of Tamil Nadu, India, and the result indicated that CMD incidence was more than 90%. The disease severity ranged from 2.35 to 4 on a scale of 1–5 with an overall mean of 3 [16,17]. The higher incidence may be attributed to indiscriminate use of CMD infected planting material, which was significantly higher than whitefly transmitted infection [16,17]. In addition, yield loss of 18–25% was recorded due to Indian cassava mosaic virus (ICMV) infection in India by Dasgupta et al. [18], and this necessitates the development of a management strategy against CMD. Earlier study has indicated that upon fermentation of cow dung, the major and minor nutrients will be released which may be available to the plants. Also, the odour of fermented cow dung deters the insect vector in the field [19]. Similarly, the neem cake provides the macro- and micronutrients to the plants [20]. *Bacillus subtilis* has biopesticide properties against seed and soil-borne diseases, also enhances plant growth through the production of metabolites [21]. The nutrients are required to alleviate the nutrient deficiency symptoms. With this background, a combined nutrient/biocontrol agent mixture was prepared.

We hypothesize that a combination of better nutrition and biocontrol agent could help cassava to tolerate CMD, resulting in higher yield. With this background, the study was formulated with the following objectives to (i) optimize the cassava spray timing on yield, starch, and cassava mosaic disease incidence on cassava genotype, (ii) quantify the interaction of cassava spray timing and genotypes on yield, starch and cassava mosaic disease incidence on different cassava genotype, and (iii) validate the effects of combined nutrient/biocontrol agent mixture in multilocation trials.

2. Materials and Methods

2.1. Preparation of the Combined Nutrient/Biocontrol Agent Mixture

Combined nutrient/biocontrol agent mixture was prepared by adding 40 kg of fresh cow dung, mixed in 100 L of distilled water, and filtered. One kg of *Bacillus subtilis* and one kg of neem cake were added and mixed. Later, the container containing this mixture was tied with a gunny bag and allowed for fermentation (7–10 d) with intermittent stirring at an interval of 3 d. This fermented mixture is supplemented with 3 kg of a nutrient mixture containing 0.5% S as sulphate of potash, 0.9% K as potassium nitrate, 0.5% Mg as magnesium sulphate, 0.25% Zn as zinc sulphate, and 0.5% Fe as ferrous sulphate at the time of spraying and content volume was made up to the final spraying volume of 200 L with distilled water which was sufficient for one acre. The components of the combined nutrient/biocontrol agent mixture were presented in Table 1.

Table 1. The components of combined nutrient/biocontrol agent mixture and its special features.

Components	Special Features
Cow dung	Humic compounds and fertilizing bioelements are present in cow dung, which can promote plant growth. In addition, odor acts as a repellent for the insect vector.
Neem cake	Neem cake contains macro and micronutrients. Sulphur compound in neem cake has a negative effect on insects. All the components in it are needed for plant growth.
Bacillus subtilis	It has a biopesticide property against seed and soil-borne diseases and also produces metabolites related to growth-promoting and disease prevention.
Nutrient mixture	Improves plant growth, development, induces enzymes involved in disease resistance mechanisms. Spraying the nutrient mixture can alleviate the deficiency symptoms.

2.2. Effect of Timing of Combined Nutrient/Biocontrol Agent Mixture Foliar Spray on Growth, Yield, Starch, and CMD

The experiment was designed in a randomized block design with four replications with a plot size of 46 m^2. Cassava genotype H226 setts were planted with 90 × 90 cm spacing. To the experimental field, farmyard manure at the rate of 25 t ha^{-1} was applied at the time of planting. Fertilizers at the rate of 45:90:120 kg NPK ha^{-1} were applied as basal, and 45:0:120 kg NPK ha^{-1} was applied as a top dressing on 90 days after planting during earthing up. Intercultural operations *viz.*, weeding, and application of plant protection chemicals were made on a need basis as a common package of practices to all the experiments.

Foliar spray of combined nutrient/biocontrol agent mixture in 10 lit of spray fluid for one treatment with four replications (4 plots = 185 m^2) was sprayed through a knapsack sprayer. The treatments include foliar spray of combined nutrient/biocontrol agent mixture at 15-, 21- and 30-days interval and water sprayed control (without nutrient mixture spray) from one month after planting and to five months. The observations on plant growth parameters *viz.*, plant length, stem girth, and yield attributes *viz.*, number of tubers per plant, tuber length, tuber girth, tuber yield, starch, and CMD incidence were recorded in the middle of the field.

The plant height of each plant was measured from the base of the shoot to the longest leaf. An average of five plants was worked out in each replication, and the value was expressed in cm. Stem girth was calculated by measuring the circumference at the mid-portion of the stem in five randomly selected plants from each replication and expressed in cm.

The length of each tuber was measured from the base to the tip of the tuber. The tuber length was recorded in 25 tubers in each replication, and the average was expressed in cm. Tuber girth was measured at the three places of tuber, one at the center and two at halfway between the center on both edges of the tuber, and the average of these three values was expressed in cm. The tuber yield $plant^{-1}$ was quantified as the fresh weight of the whole root of a single plant and expressed as kg $plant^{-1}$.

The starch content was quantified using the anthrone method [22]. In this method, 100 mg of tuber sample was macerated in 10 mL of 80% ethanol in a mortar and incubated for an hour. Later the macerate was centrifuged for 15 min at 3000 rpm, and the supernatant was discarded. The residue was stirred well with another 10 mL of perchloric acid mixture (6.5 mL of perchloric acid + 5 mL water) and incubated for three h. After the expiry of time, the mixture was filtered and made up to 50 mL with distilled water. An aliquot of 0.2 mL was taken in a test tube, and 4 mL of 0.2% anthrone reagent was added slowly. The mixture was placed in an ice bath for five min, well shaken, and placed in a boiling water bath for 10 min and then cooled. The green colour developed was measured in a spectrophotometer at 640 nm. The glucose content was referred from the standard curve and multiplied by 0.9 for measuring starch content and expressed in percentage on a fresh weight basis.

CMD incidence was assessed by the number of visibly diseased plants, usually in relation to the total number of plants assessed and expressed as the proportion or percentage of plants infected with CMD [23]. Approximately 50 samples were chosen for the observation of disease incidence and disease severity. Plants were selected from two sides and along a diagonal across the field in a "Z" configuration [24]. Disease severity was estimated based on the 1–5 scale as demonstrated by Hahn et al. [25] (Table 2).

Table 2. Scale followed to assess the CMD disease severity as explained by Hahn et al. [25].

Description of Damage Caused by Virus	Scale
No damage	1
Mild damage with mild chlorosis at base of leaves	2
Most of the leaves were affected and expressed mosaic pattern, Narrowing and distortion of the lower leaflets.	3
Severe damage with mosaic pattern, distortion of 2/3rd of leaves, reduction in leaf size and stunting of shoots	4
Very severe damage with mosaic pattern on all leaves and distorted leaves	5

2.3. Interaction Effect of Timing of Combined Nutrient/Biocontrol Agent Mixture Foliar Spray and Genotypes on Yield Attributes, Tuber Yield, Starch, and CMD Incidence

An experiment in a factorial randomized block design with four replications was conducted to quantify the interaction effect of timing of foliar spray of combined nutrient/biocontrol agent mixture and different cassava genotypes on the yield attributes, starch, and CMD incidence in farmer's field in one acre field. The first factor was the timing of foliar spray of combined nutrient/biocontrol agent mixture and the second factor was genotype. The first factor had four levels (15-, 21- 30-days interval, and water sprayed control). The second factor was genotype with three levels (Kungumarose, H226, and Mulluvadi 1). The crop husbandry was the same as experiment 1. The observations on yield attributes *viz.*, number of tubers per plant, tuber length, tuber girth, tuber yield, starch, and CMD incidence were recorded.

Apart from this large-scale field experiment as Frontline Demonstrations (FLDs) at farmers holding was conducted to assess the performance of different cassava varieties. The field experiment was conducted in a factorial randomized block design with four replications in a one-acre field. The experiment had two factors, namely foliar spray of combined nutrient/biocontrol agent mixture and genotypes. There were two levels in a foliar spray (combined nutrient/biocontrol agent mixture spray at 21 d from one month after planting to five months and water sprayed control) and four levels in cassava genotypes (MVD 1, YTP 1, Kungumarose, and H226). The crop husbandry was similar to experiment 1. At harvest, the observations like tuber yield, starch content, and CMD incidence were recorded as detailed elsewhere.

2.4. Multilocation Validation of Combined Nutrient/Biocontrol Agent Mixture Foliar Spray on Tuber Yield, Starch, and CMD

The field experiment was conducted in a randomized block design with three replications. The experiment was conducted at 20 locations. The cassava plants were either sprayed with combined nutrient/biocontrol agent mixture at 21 d interval from 1 month to 5 months after planting or water sprayed control. All the experiment was conducted at farmers field, in an area of one acre. The crop husbandry was the same as experiment 1.

The observation like tuber yield, starch content, and CMD incidence were recorded as described elsewhere.

2.5. Impact of Combined Nutrient/Biocontrol Agent Mixture on Cassava Leaf Octadecatrienoic Acid and Trilinolein

Two sets of cassava plants were grown till the harvest stage under greenhouse conditions. One set was treated with combined nutrient/biocontrol agent mixture while the other with water from 1 to 5 months after planting at 21 d intervals. Leaf samples were harvested for metabolite extraction at 1 day after spraying at 5th month after planting. Metabolite extraction and sample derivatization were performed as described by Lisec et al. [26], and the metabolites were determined using GC-TOF-MS. 1 µL of the derivatized extract was injected into DB-5MS capillary (30 × 0.25 × 0.25 µm). The inlet temperature was set at 260 °C. After a solvent delay for six minutes, the initial GC oven temperature was set at 60 °C, after injection for 1 min, the GC oven temperature was raised to 280 °C with 15 °C min^{-1} and held at 280 °C for 15 min. The injection temperature was set to 280 °C, and the ion source temperature was matched. Helium was used as the carrier gas with a constant flow rate set at 1 mL min^{-1}. The measurement was performed with electron impact ionization (70 eV) in the full scan mode (m/z from 30 to 550). The metabolites were identified based on retention time index specific masses, via comparing with reference spectra in mass spectral libraries (NIST 2005, Wiley 7.0).

3. Statistical Analysis

Statistical analyses were performed using SAS programs version 9.4 (SAS Institute 2003, Cary, NC, USA). Experiment to understand the effect of timing of combined nutrient/biocontrol agent mixture foliar spray was set in randomized block design with five replications. The same experiment was repeated in the next year with the same treatment and crop husbandry. The data were combined because there was no significant difference between the years. Therefore, the mean of the two years was presented along with its significance level. The interaction effect of timing of combined nutrient/biocontrol agent mixture foliar spray and genotypes experiment was set in a factorial randomized block design with five replications. The same experiment was repeated in the next year with the same treatment, genotype, and crop husbandry. There was no significant difference between the years; therefore, the mean of the two years was presented with significance. A large-scale frontline demonstration experiment was set in a factorial randomized block design with five replications. The first factor being the foliar spray of combined nutrient/biocontrol agent mixture (4 levels), and the second factor was genotype (3 levels). The mean was presented with its significance level. The multilocation trial experiment was designed in a randomized block design and conducted in 20 locations. In this experiment, the location was considered as a random effect to get the overall effect of foliar spray of the nutrient mixture. Data were analyzed using an analysis of variance (ANOVA) with the GLM procedure, and Fisher's least significant difference (LSD) at 5% significance level was used to test differences between mean values. Regression analyses among cassava tuber yield, starch, and cassava mosaic disease incidence were carried out by using the data from all experiments using the PROC REG procedure of SAS.

4. Results

4.1. Effect of Timing of Combined Nutrient/Biocontrol Agent Mixture Spray on Growth, Yield Components, Tuber Yield, Starch, and CMD Incidence

The result indicated that foliar application of combined nutrient/biocontrol agent mixture has significantly ($p < 0.05$) improved morphological, yield and yield components, and starch content (Table 3). In contrast, the CMD incidence was decreased by foliar spray of the mixture. Among the treatments, foliar application of combined nutrient/biocontrol agent mixture at 15 d intervals has improved the plant height (48%), stem girth (29%), number of tubers $plant^{-1}$ (24%), tuber length (11%), tuber girth (10%), tuber yield (11%) and starch (4%) compared with control (Table 3). However, the CMD incidence was lower

(74%) in the foliar application of combined nutrient/biocontrol agent mixture at 15 d intervals than control, and there was no significant difference between foliar application of combined nutrient/biocontrol agent mixture for every 15 days and 21 days interval for stem girth, number of tubers plant^{-1}, tuber length (cm), starch (%), and tuber yield (kg). Overall, it is concluded that foliar application of combined nutrient/biocontrol agent mixture at 21 d interval from one month after planting to 5 months after planting was found to increase the yield and reduce the CMD incidence than control.

Table 3. Effect of combined nutrient/biocontrol agent mixture foliar spray on plant height (cm), stem girth (cm), number of tubers plant^{-1}, tuber length (cm), tuber girth (cm), tuber yield (kg), starch (%), and cassava mosaic disease incidence (CMD-%) of cassava. The data presented are mean of two experiments (n = 10). Means followed by the same letter are not statistically significant at $p < 0.05$.

Treatment	Traits							
	Plant Height (cm)	Stem Girth (cm)	Number of Tubers Plant^{-1}	Tuber Length (cm)	Tuber Girth (cm)	Tuber Yield (kg)	Starch (%)	CMD%
T_1	273 ^{a}	8.655 ^{a}	8.51 ^{a,b}	30.92 ^{a}	19.65 ^{b}	34.60 ^{a}	27.50 ^{a}	8.50 ^{c}
T_2	193 ^{b}	8.655 ^{a}	9.16 ^{a}	33.68 ^{a}	23.16 ^{a}	37.35 ^{a}	28.25 ^{a}	13.25 ^{b}
T_3	194 ^{b}	8.100 ^{a}	8.02 ^{b}	31.93 ^{a}	23.55 ^{a}	36.65 ^{a}	28.0 ^{a}	13.70 ^{b}
Control	184 ^{b}	6.715 ^{b}	6.84 ^{c}	27.97 ^{b}	17.88 ^{b}	31.25 ^{b}	26.5 ^{a}	33.45 ^{a}
LSD ($p < 0.05$)	18.5	0.77	0.84	2.89	2.05	3.23	NS	2.04

T_1: Foliar spray of combined nutrient/biocontrol agent mixture at 15 days interval; T_2: Foliar spray of combined nutrient/biocontrol agent mixture at 21 days interval; T_3: Foliar spray of combined nutrient/biocontrol agent mixture at 30 days interval, and Control -water spray.

4.2. Effect of Timing of Combined Nutrient/Biocontrol Agent Mixture Spray and Genotypes on Yield, Starch, and CMD Incidence in Different Genotypes of Cassava

The trial indicated significant ($p < 0.05$) differences among the timing of foliar application of combined nutrient/biocontrol agent mixture for the number of tuber plant^{-1}, tuber length (cm), tuber girth (cm), tuber yield (kg plant^{-1}), and CMD incidence (%) (Table 4). Similarly, the genotypes varied significantly ($p < 0.05$) for the number of tuber plant^{-1}, tuber length (cm), tuber yield (kg plant^{-1}), and CMD incidence (%) (Table 4). The interaction of timing of foliar application of combined nutrient/biocontrol agent mixture and genotype indicate tuber length, and CMD incidence were significantly ($p < 0.05$) influenced (Table 5).

Table 4. Main effect of timing of combined nutrient/biocontrol agent mixture spray and genotypes on the number of tuber plant^{-1}, tuber girth (cm), tuber yield (kg plant^{-1}), and starch (%) in cassava (n = 10). Means followed by the same letter are not statistically significant at $p < 0.05$.

Main Effects	Number of Tubers Plant^{-1}	Tuber Girth (cm)	Tuber Yield (kg Plant^{-1})	Starch (%)
Time of combined nutrient/biocontrol agent mixture spray				
T_1	5.84 ^{b}	18.80 ^{b}	34.8 ^{b}	27.00 ^{a,b}
T_2	6.37 ^{a}	23.89 ^{a}	39.0 ^{a}	28.16 ^{a}
T_3	4.86 ^{c}	18.52 ^{b}	36.0 ^{a,b}	27.16 ^{a,b}
Control	4.52 ^{c}	17.78 ^{b}	31.5 ^{c}	26.00 ^{b}
LSD	0.40 ***	1.44 ***	2.52 ***	NS
Genotypes				
Kungumarose	4.14 ^{b}	20.55 ^{a}	34.35 ^{b}	26.25 ^{a}
H226	7.84 ^{a}	19.34 ^{a,b}	38.80 ^{a}	27.75 ^{a}
Mulluvadi	4.21 ^{b}	19.29 ^{b}	32.80 ^{b}	27.25 ^{a}
LSD	0.35 ***	NS	2.18 ***	NS

T_1: Foliar spray of combined nutrient/biocontrol agent mixture at 15 days interval; T_2: Foliar spray of combined nutrient/biocontrol agent mixture at 21 days interval; T_3: Foliar spray of combined nutrient/biocontrol agent mixture at 30 days interval, and Control—water spray. ***—significant at $p < 0.001$.

Table 5. Interaction of time of combined nutrient/biocontrol agent mixture spray and genotypes on tuber length (cm), and CMD (%) in cassava (n = 10). Means followed by the same letter are not statistically significant at $p < 0.05$.

Foliar Spray	Tuber Length (cm)				CMD (%)			
	Genotypes							
	Kungumarose	H226	Mulluvadi	Mean	Kungumarose	H226	Mulluvadi	Mean
T_1	23.97 ^{f,g}	31.25 ^{b,c}	22.90 ^{g,h}	26.04 ^{b}	9.3 ^{f}	10.0 ^{e,f}	9.0 ^{f}	9.43 ^{d}
T_2	36.38 ^{a}	32.95 ^{a,b}	29.45 ^{c,d,e}	32.92 ^{a}	9.6 ^{e,f}	12.3 ^{d}	11.4 ^{d,e}	11.10 ^{c}
T_3	26.85 ^{e,f}	27.45 ^{d,e}	20.12 ^{h,i}	24.80 ^{b}	12.4 ^{d}	14.7 ^{c}	12.3 ^{d}	13.13 ^{b}
Control	30.55 ^{b,c,d}	27.30 ^{d,e,f}	17.84 ^{i}	25.23 ^{b}	29.3 ^{b}	38.5 ^{a}	27.6 ^{d}	31.80 ^{a}
Mean	29.43 ^{a}	29.73 ^{a}	22.57 ^{b}		15.15 ^{b}	18.87 ^{a}	15.07 ^{b}	
LSD	T = 2.008 ***; G = 1.73 ***; T × G = 1.73 ***				T = 1.17 ***; G = 1.01 ***; T × G = 2.03 ***			

T_1: Foliar spray of combined nutrient/biocontrol agent mixture at 15 days interval; T_2: Foliar spray of combined nutrient/biocontrol agent mixture at 21 days interval; T_3: Foliar spray of combined nutrient/biocontrol agent mixture at 30 days interval, and Control—water spray. ***—Significant at $p < 0.001$.

Among the timing of spray, foliar spray at 21 d interval improved the number of tuber plant^{-1}, tuber length (cm), tuber girth (cm), and tuber yield (kg plant^{-1}) than other foliar spray timing treatments (Table 4). In contrast, the CMD incidence was significantly decreased by the foliar application of combined nutrient/biocontrol agent mixture at 15 d intervals (Table 5).

Among the genotypes, H226 has recorded a higher number of tuber plant^{-1}, tuber length (cm), tuber girth (cm), tuber yield (kg plant^{-1}) than other genotypes with a considerable reduction in CMD incidence (Tables 4 and 5).

The interaction of timing of foliar application of combined nutrient/biocontrol agent mixture and genotypes indicated that the foliar application of combined nutrient/biocontrol agent mixture at 15 d interval to genotype H226 had significantly higher tuber length (cm) (Table 5). However, the CMD incidence was drastically decreased in H226 (Table 5).

4.3. Frontline Demonstration to Confirm the Effect of Combined Nutrient/Biocontrol Agent Mixture Spray and Genotypes on Yield, Starch, and CMD Incidence

There were significant ($p < 0.05$) differences between the treatment for tuber yield (kg plant^{-1}) and CMD incidence (%) (Tables 6 and 7). Similarly, the genotypes varied significantly ($p < 0.05$) for tuber yield, starch (%), and CMD incidence (Tables 6 and 7). The interaction of treatment and genotype was significant for CMD incidence (Table 7).

Table 6. Main effects of combined nutrient/biocontrol agent mixture foliar spray and genotypes on tuber yield (kg) and starch (%) of cassava. The data presented are the mean of two seasons (n = 10). Means followed by the same letter are not statistically significant at $p < 0.05$.

Main Effects	Tuber Yield (kg Plant^{-1})	Starch (%)
	Foliar spray	
T_1	30.5 ^{b}	25.75 ^{a}
Control	36.5 ^{a}	26.62 ^{a}
LSD	2.22 ***	NS
	Genotypes	
Mulluvadi	29.75 ^{c}	27.5 ^{a}
YTP 1	40.75 ^{a}	24.75 ^{b}
Kungumarose	29.50 ^{c}	25.0 ^{b}
H226	34.00 ^{b}	27.5 ^{a}
LSD	3.14 ***	2.26 *

T_1: Foliar spray of combined nutrient/biocontrol agent mixture at 21 days interval and Control—water spray. ***—significant at $p < 0.001$; and *—significant at $p < 0.05$.

Foliar application of combined nutrient/biocontrol agent mixture increased the tuber yield (20%) and starch (3%) and decreased the CMD incidence (55%) (Tables 6 and 7). Overall, among the genotypes, a higher yield was observed in YTP 1 and a lower CMD incidence. The genotype H226 had the next higher tuber yield (38.0 kg $plant^{-1}$) and highest starch content (28.0%), and the highest CMD incidence (15%). The interaction of genotype and treatment indicates that the genotype YTP1 and H226 had a higher yield and lower CMD incidence due to foliar spray of combined nutrient/biocontrol agent mixture (Tables 6 and 7).

Table 7. Interactive effects of combined nutrient/biocontrol agent mixture foliar spray and genotypes on CMD (%) of cassava. The data presented are the mean of two seasons (n = 10). Means followed by the same letter are not statistically significant at $p < 0.05$.

Foliar Spray	CMD Incidence (%)				
	Genotypes				
	Mulluvadi	YTP 1	Kungumarose	H226	Mean
T_1	13.5 ^{d,e}	11.2 ^{e}	14.5 ^{d}	15.0 ^{d}	13.55 ^{b}
Control	32.0 ^{b}	23.0 ^{c}	31.0 ^{b}	35.0 ^{a}	30.25 ^{a}
Mean	22.75 ^{b}	17.10 ^{c}	22.75 ^{b}	25.00 ^{a}	
LSD	T = 1.39 ***; G = 1.97 ***; T × G = 2.78 ***				

T_1: Foliar spray of combined nutrient/biocontrol agent mixture at 21 days interval and Control—water spray. ***—significant at $p < 0.001$.

4.4. Effect of Combined Nutrient/Biocontrol Agent Mixture Spray at Multilocation Trial

The result indicated that foliar application of combined nutrient/biocontrol agent mixture at 21 d interval from one month of planting had significantly ($p < 0.05$) improved the tuber yield (24%), starch (9%), and decreased the CMD incidence (65%) (Table 8). The combined nutrient/biocontrol agent mixture-treated plants had higher levels of octadecatrienoic acid (2.28-fold) and trilinolein (126-fold) than control plants (Figure 1).

Table 8. Effect of combined nutrient/biocontrol agent mixture spray on tuber yield (kg $plant^{-1}$), starch (%), and CMD (%) of cassava. Each data point is an average of 20 independent trials conducted at different locations (n = 60). Means followed by the same letter are not statistically significant at $p < 0.05$.

Treatment	Traits		
	Tuber Yield (kg $Plant^{-1}$)	Starch (%)	CMD (%)
T_1	33.01 ^{a}	27.41 ^{a}	12.416 ^{b}
Control	26.525 ^{b}	25.12 ^{b}	35.491 ^{a}
LSD ($p < 0.05$)	0.93 ***	0.69 **	2.09 ***

T_1: Foliar spray of combined nutrient/biocontrol agent mixture at 21 days interval and Control—water spray. ***—significant at $p < 0.001$; **—significant at $p < 0.01$.

4.5. Relationship among Cassava Tuber Yield, Starch, and Cassava Mosaic Disease Incidence

The result indicated that there was a significant ($p < 0.05$) positive linear relationship between tuber yield and starch content (r^2 = 0.36). However, there was a significant ($p < 0.05$) negative linear relationship between tuber yield and CMD incidence (r^2 = 0.26). There was no relationship between starch content and CMD incidence (r^2 = 0.03). (Figure 2).

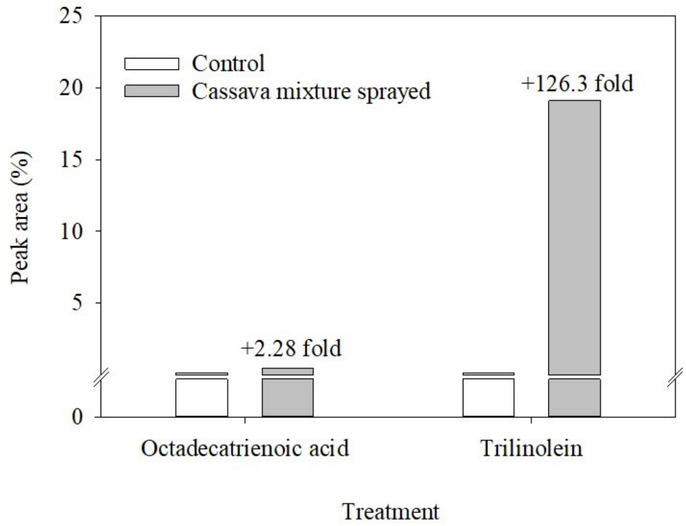

Figure 1. Effect of foliar spray of combined nutrient/biocontrol agent mixture on octadecatrienoic acid and trilinolein relative content in combined nutrient/biocontrol agent mixture sprayed and water sprayed plants.

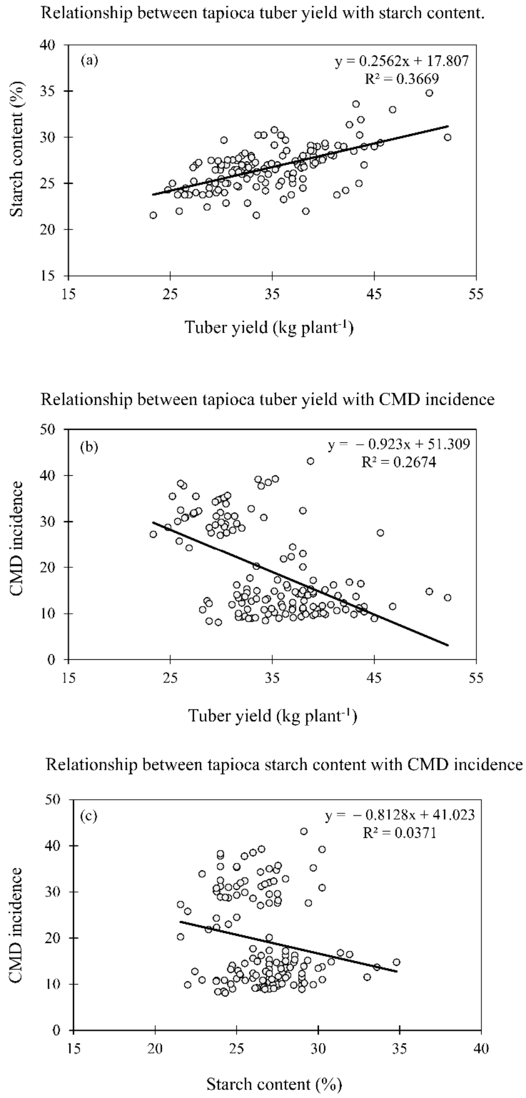

Figure 2. Relationship among cassava tuber yield, starch, and CMD incidence. (**a**) tuber yield vs. starch content, (**b**) tuber yield vs. CMD incidence, and (**c**) starch content vs. CMD incidence.

5. Discussion

The major findings of this study are (i) foliar application of combined nutrient/biocontrol agent mixture at 21 d interval from one to five months after planting had improved the tuber yield, and its associated traits along with decreased CMD incidence compared to water sprayed control plants, (ii) foliar application of combined nutrient/biocontrol agent mixture increased the defense metabolite namely octadecatrienoic acid and trilinolein content than water sprayed control plants and (iii) multilocation field experiment indicates that foliar application of combined nutrient/biocontrol agent mixture had improved the tuber yield along with decreased CMD incidence.

The combined nutrient/biocontrol agent mixture contains fermented cow dung, neem cake, *Bacillus subtilis*, and nutrients like K, Mg. Fe, Zn, and S. Study indicated a strong positive relationship between K and cassava yield [27]. Long-term fertilizer experiment indicated that due to the monoculture of cassava, the soil will be deficient in K, and will be the most limiting nutritional constraint for crop production [28]. Also, K is required in a significant amount (343 kg ha^{-1}) to attain a tuber yield of 45 mt ha^{-1} [28]. Hence, it may be inferred that K in the combined nutrient/biocontrol agent mixture might help in achieving a higher yield than water sprayed control plants.

The increased yield in combined nutrient/biocontrol agent mixture treated plants may be associated with more translocation of carbohydrate from leaf to the developing tuber in the presence of K [29]. This was evidenced by increased starch content in the combined nutrient/biocontrol agent mixture sprayed plants (Tables 3, 4 and 8). Malavolta et al. [30] showed that K deficient plant tuber had a lower starch content than K sufficient plants. In the present study, we observed K deficiency in most of the places. Also, the present study showed a positive linear relationship between cassava tuber yield and starch content (Figure 2). Potassium causes thicker outer walls in epidermal cells, thus preventing disease attack, and hardened tissue is inversely related to infestation intensity [31]. In the present study, foliar application of K decreased the CMD incidence. Similarly, the application of K decreased the infestation of the Tobacco mosaic virus [32]. Increased tuber yield results from the increased number of tubers per plant, tuber length, and tuber girth. Hence, we conclude that foliar application of K might positively impact any one of the yield components, resulting in increased yield.

Fe, Zn, and Mg were added to the combined nutrient/biocontrol agent mixture to reduce the chlorosis symptom, which is caused by either soil factor or CMD. Our soil analysis data indicates the study area soils are also deficient in Fe, Zn, and Mg (data not shown). We hypothesize that the combined application of K, Fe, Zn, and Mg may improve the tuber yield, like in mungbean [33]. Micronutrients are able to reduce the severity by inducing the resistance within the plant known as Systemic Acquired Resistance (SAR) [15]. Iron is an essential micronutrient reported by most living organisms and pathogens [34]. Iron can catalyze the formation of deleterious reactive oxygen species, and hosts may use iron to increase local oxidative stress in defense responses against pathogens. Zinc plays an important role in activating enzymes involved in various metabolic pathways, especially in protein and starch synthesis. A balanced zinc application was found to increase the phenol contents to reduce the severity of rice sheath blight [35].

Wang [36] reported that *Bacillus subtilis* is a growth promoter and antagonistic to a variety of pathogens as a result of multiple mechanisms, including plant growth promotion (PGP), antibiosis, lysis of pathogen hyphae, and induced systemic resistance (ISR). *B. subtilis* has a positive effect on disease suppression by altering the composition and function of soil microbial communities [37]. *Bacillus* association stimulates plant immunity by ISR, leading to enhanced plant growth during cucumber mosaic virus infection [38]. Similarly, Wang et al. [39] reported that *Bacillus* spp. induced systemic resistance against viral disease caused by tobacco mosaic virus by inhibiting viral coat protein synthesis. Esawy et al. [40] and Radhakrishnan et al. [21] observed that, some of the *Bacillus spp*. produce the antiviral compounds against the pathogen. In the present study, due to foliar application of combined nutrient/biocontrol agent mixture had increased octadecatrienoic acid (2.28-fold) and trilinolein (126-fold) than control plants (Figure 1). Chan et al. [41] isolated trilinolein, an antioxidant from *Panax pseudoginseng* and found that trilinolein had a synergistic action with antioxidant defense systems. In the present study, the 126-fold increase in trilinolein indicates a strong antioxidant defense system in combined nutrient/biocontrol agent mixture sprayed plants. Octadecadienoic acid i.e., linoleic acid, is increased under combined nutrient/biocontrol agent mixture sprayed plants (Figure 1), and it is the precursor for jasmonate biosynthesis [42]. Jasmonic acid is known to be involved as a signaling compound in multiple aspects of plant responses to their biotic and abiotic environment and is also involved in the production of oxylipin or its derivatives [43,44]. Overall, it can be concluded that *Bacillus subtilis* can induce SAR as evidenced by higher levels of jasmonic acid and trilinolein, which might decrease the CMD severity in combined nutrient/biocontrol agent mixture sprayed plants.

The multilocation trial indicates that irrespective of the varieties and location, combined nutrient/biocontrol agent mixture sprayed plants recorded a significant reduction in CMD incidence (65%) as compared to water sprayed plants. Also, the yield was increased by 26% over unsprayed control. The increased yield could be achieved through increased starch mobilization (9%) and decreased CMD incidence (Table 8). The present study also indicated a negative relationship between CMD incidence and tuber yield (Figure 2).

6. Conclusions

This study proved that foliar application of combined nutrient/biocontrol agent mixture from 1 to 5 MAP has decreased CMD incidence and increased the tuber yield. The increased yield is due to an increase in yield components, namely tuber length, tuber girth, and the number of tubers, along with increased starch mobilization. The same result was obtained in three field experiments at the research station, large-scale frontline demonstrations, and 20 multilocation experiments. Therefore, it is evident that foliar spray of combined nutrient/biocontrol agent mixture can improve the cassava yield.

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