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The Effect of Rhizosphere Bacterial Consortium on the Manifestation of Tobamoviral Infection Symptoms on Tomato

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Authors' contributions

This work was carried out in collaboration between all authors. Author OMA designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author AOA reviewed the first draft of the article. Authors SOO, WFO and AOF contributed to the in vivo biocontrol assay, yield assessment and publication process. All authors read and approved the final manuscript.

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ABSTRACT

Contrary to available reports on the prevalence of microbial diseases of tomato in Nigeria, the emergence of mosaic viral disease on tomato farms is gradually becoming a very significant additional threat to the production of this economically important vegetable crop. In addition, the resistance of Tobamoviral diseases to chemical control measures makes them particularly difficult to control, once established. Unlocking the specificity of rhizosphere microbiome towards the general health and performance of tomato is key to achieving a safer means of combating microbial diseases of tomato. In this study, the predominant rhizosphere bacteria associated with healthy and infected tomato were compared, and the effect of tomato seed treatment (with selected

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rhizobacterial consortium) on the incidence of Tobamoviral infection symptoms was determined. *Bacillus thuringiensis, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus macerans* and *Bacillus cereus* were isolated from the rhizosphere of healthy tomato plants using the pour plate isolation method, while *P. aeruginosa* and *B. subtilis* were not isolated from rhizosphere samples associated with infected tomato. Tomato seeds were treated with the predominant rhizobacterial consortium isolated from healthy tomato plants, while seedlings were mechanically inoculated with triturated tissue extracts from infected plants. Significant reduction in the incidence of Tobamoviral disease symptoms were recorded on tomato plants grown from treated seeds. Additional (new) Tobamoviral disease symptoms were not recorded at the second, fifth and sixth weeks after the transmission of the pathogen in treated plants while disease incidence values of 23.83, 3.03 and 3.33% were recorded on untreated plant set respectively. Average growth performance measures, including the fruit count, fruit weight, stem girth and the number of flower clusters per plant at 16.76, 43.80 g, 0.93 cm and 9.67 respectively were significantly higher in treated tomato plants than in plants grown without the seed treatment. Consequently, it could be inferred that the healthy-tomato associated rhizosphere bacterial consortium used in this study influenced the resistance of mechanically inoculated tomato to the manifestation of Tobamoviral infection symptoms and the overall performance of the plant.

Keywords: Tobamoviral; rhizosphere; bacteria; resistance; performance.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important fruit vegetables in Nigeria, it is a widely cultivated member of the nightshade family, Solanaceae. The cultivation and consumption of this fruit vegetable in Nigeria is largely due to its nutrient value as well as its low cost and short period of production [1]. Large scale production of tomato in the Northern agro-ecological zones of Nigeria under irrigation systems have been relatively successful compared with the southern states of the country, representing the Southern savanna and the Rainforest zones where the production is seasonal and erratic, largely due to high incidence of microbial diseases [2,3]. Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV) are the two closely related mosaic viruses in the genus Tobamovirus with high sequence homology, producing very similar symptoms and detrimental effects (distinctive of these two viruses) on infected tomato [4]. Tobamoviral diseases are among the least studied diseases of tomato in Nigeria, with a limited report on the prevalence and biological control on local varieties. Symptoms characteristic of mosaic viral disease of tomato was observed as a prevalent disease on tomato plots within the research farm of the Federal College of Agriculture, Moor Plantation, Ibadan (FCAI), Oyo State, Nigeria within the planting period of January to March, 2018. Specific disease symptoms observed on tomato plants, most of which were equally reported by Jones et al. [5], included mottled light and dark green leaves; stunted growth with chlorotic tissues; affected leaves were mostly curled, malformed and reduced in size; fruits of affected plants ripe unevenly and some ripe infected fruits bore yellow rings, while others showed internal browning (brown-wall) just under the skin.

The control of viral diseases of tomato has been rather difficult than their bacterial and fungal counterparts. Till date, no efficient chemical control measure of Tobamoviral diseases of tomato had so far been reported [6,7]. Consequently, disease management in practice has been the major focus to reduce, or possibly eliminate the sources and vectors of viral pathogens on tomato [7]. Rhizosphere microorganisms can be defined as the diverse resident microbes occupying the soil layer adjacent to plant roots, whose presence and abundance are highly affected by root exudation. Plant rhizodeposits can be diverse within the soil; they include amino acids, organic acids, polysaccharides and proteins. These deposits constitute available food source to rhizosphere microbiome and this makes exudates important determinants of microbial composition in the rhizosphere. The interaction between plants and rhizosphere microorganisms could be beneficial (protective and growth promoting) or detrimental (pathogenic) [8]. The ability of rhizosphere microorganisms to suppress soil plant pathogens has been considered to be general (involving the totality of rhizosphere microbiome) or specific (involving specific or limited microbial consortium). [9]. Hu et al. [10] in their work; "Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression" proposed that the presence of protective rhizosphere bacteria could intensify resource competition between the plant root bacterial community and a potential pathogen, which could lead to a competitive exclusion of the pathogen and in such context, elevate the host plant protection. Similarly, they reported that the production of secondary metabolites by the predominant rhizosphere bacteria could also suppress pathogen growth. Consequent upon recent reports, as well as the observations recorded on FCAI research farm, this study was designed to determine the effect of the predominant (most abundant) tomato-associated rhizosphere bacterial consortium on the manifestation of Tobamoviral infection symptoms on tomato.

2. MATERIALS AND METHODS

2.1 Isolation of Bacteria from Tomato Rhizosphere

Tomato plants from infected and healthy plots of the research field of FCAI were carefully uprooted from the soil and the loosely adhering soil to each was gently removed from the roots by shaking the uprooted plants. Ten grams (10 g) of each group of soil samples (from healthy and infected plants) was dissolved in sterile distilled water (at 10^{-1} dilution) and vortexed vigorously. The samples were then further diluted to 10^{-7} in order to select for the predominant (culture dependent) rhizosphere bacteria and thereafter grown on Nutrient and Tryptone Soy Agar using the pour plate method. Plates were incubated at 30ºC for 48 hours. Colonies were purified and identified; while isolates were kept on agar slants and Nutrient Broth in sterile glycerol (30% v/v) at 2 and -80ºC respectively [11,12]. Preserved isolates were retrieved and subcultured as required.

2.2 Mechanical Transfer of Viral Pathogen

Nutrient Agar and Potato Dextrose Agar were used to investigate the possible presence of bacterial and fungal pathogen on infected tomato plant parts (tissues) respectively [13,14]. Pathogenicity was established through mechanical transfer of pathogen, this was done using a slightly modified method of Murphy et al. [15]. Five (5) mm^2 section of infected tomato plant part showing the symptoms of Tobamoviral infection was surface sterilised (1% NaOCl for two minutes) dried and subsequently triturated in 20mM sodium phosphate buffer (pH 7.0) containing 1% (wt. /vol.) celite. Collected tissue extracts were sprayed (5 mL/plant) on healthy carborundum pre-dusted tomato leaves to inoculate them. After 10 minutes, inoculated leaves were rinsed with sterile distilled water and held in screenhouse under natural light at screen house conditions, and observed for the development of symptoms.

2.3 Biocontrol Treatment (in vivo)

Pot experiment was used to determine the protective and growth promoting effects of the isolated healthy-tomato associated rhizosphere bacteria in a completely randomised design, maintaining intra and inter spacing of 0.5 m and 1 m within the pots. Soil sample (sandy loam) was collected at a depth of 0-15 cm; the collected sample was properly mixed and prepared for planting. Sterilisation (autoclaving) of soil was done for an hour at 121°C and 15 psi for two consecutive days. Three sets of plastic pots (20 cm in diameter, each set for treated seeds, untreated seeds and the control experiment) were washed and air dried, then sterilised with Clorox. The sterilised pots were filled with sterilised soil (5 kg/pot). Tomato seeds (local variety: Hausa Scissors) were surface sterilised (70% ethanol for a minute) and appropriately rinsed thrice; these seeds were thereafter drained on sterile blotting paper and treated with the isolated rhizosphere bacterial consortium. Seeds were placed in sterile Petri dish and sprayed with 2.5 mL mixture of culture suspension of bacteria (3×10^6 cfu/mL) or distilled water (for untreated and control seeds). Treated seeds were subsequently air dried for two hours prior to sowing [16]. Treated tomato seeds were sown in autoclaved potting preparation while watering and weeding were regularly observed. Growing seedlings were thereafter transplanted and thinned to one seedling per pot after 25 days.

Mechanical transfer of viral pathogen was done as earlier described. Young leaves of tomato plants growing from both treated and untreated seeds were inoculated 2 weeks after planting. Healthy control plants were inoculated with sterile 0.1 M potassium phosphate buffer (pH 7.2). The inoculated leaves were rinsed with water and kept under screenhouse conditions (ambient temperature, 68-80% RH) and observed until symptoms appeared [15].

2.4 Determination of Growth and Yield of Tomato

The growth and postharvest parameters measured to determine the growth promoting effect of rhizosphere bacterial consortium used in this study on tomato included the plant height, number of leaves, number of branches, number of fruit clusters and fruit weight. Plant height was measured from the base of each plant (soil surface level) to the highest growing tip and the values were recorded in centimeters [17]. Similarly, leaves on each plant were counted and the mean value was determined and recorded. The mean number of fruits was determined for each treatment while the fruit weight for each treatment was measured and recorded at the time of harvest and expressed in gram [18].

Replicate measurements of each parameter were collected and analysed using the analysis of variance (ANOVA) and means were compared using the Duncan Multiple Range Test at 5% level of probability.

3. RESULTS AND DISCUSSION

3.1 Prevalence of Tobamoviral Infection

From the observations recorded on tomatoes planted at different plots (January to March, 2018) within FCAI farm (Table 1), prevalence of Tobamoviral disease symptoms on tomato was relatively high. This is contrary to the generally documented reports on the prevalence of viral infections of tomato [19], especially in Nigeria [2,3]. There was no record of the manifestation of Tobamoviral infection symptoms on tomato plants at sites 3 and 5. However, the prevalence of symptoms was relatively high on tomato plots in sites 1, 2 and 4 while the values of average deviation of these symptoms on individual site were low. This is a possible indication that once infected, the rate of transmission or mode of spread of this disease among different tomato varieties planted on different plots of each site was similar. Besides tomato as a potential host, first reports of mosaic viral diseases have been recently documented on a number of other economically valuable plants, including *Solanum macrocarpon* [20]. These reports, coupled with the observations recorded in Table 1 increase the significance of Tobamoviral infections as potential threat to tomato production in Nigeria (especially in the south western states where rainfall and humidity encourage the transfer, incubation and manifestation of phytopathogens).

Successful mechanical transmission of the virus from infected plant, using the collected tissue extracts (filtrate), generated the same disease symptoms characteristic of mosaic viral infection on healthy recipient tomato plants as observed in primary plants from which the infected tissue contents were extracted. No bacterial (using the Nutrient Agar) or fungal (using the Potato Dextrose Agar) pathogen was isolated from the tissues of infected plants using standard procedures [14].

3.2 Isolation of Bacteria from Tomato Rhizosphere

As indicated in Table 2, *Bacillus* species were the predominant organisms isolated from tomato rhizosphere in this study. The average number of strains of *B. thuringiensis* and *B. cereus* isolated from the rhizosphere of infected tomato plants exhibiting symptoms of mosaic infection at 10^{-7} dilution of soil samples were 6.3 and 8.2 respectively. Lower numbers of strains of these organisms (5.3 and 4.8 respectively) were isolated from healthy tomato rhizosphere. However, the diversity of rhizobacteria isolated from healthy tomato field was relatively higher than the species isolated from infected plant. *P. aeruginosa* and *B. subtilis* were absent in diluted rhizosphere samples associated with infected tomato plants used in this study. These species of bacteria might either be absent or present in lower units within such rhizosphere and they (*P. aeruginosa* and *B. subtilis*) may contribute to the resistance of tomato to microbial infection.

Table 1. Prevalence of Tobamoviral disease symptoms on tomato

Site designation	Number of sampled plots	Average number of infected plants	Average deviation (infected plants)	Prevalence of infection (%)
1	3	33a	1.3	55a
2	3	22.67c	2.3	37.8c
3	3	0d	0	0d
4	3	28.67b	1.7	47.8b
5	3	0d	0	0d

**Mean values with similar letter (s) along the column are not significantly different at 5 % level of probability by Duncan Multiple Range Test (DMRT)*

Table 2. Isolated tomato rhizosphere bacteria

Isolate	Average number of strains	
	Healthy tomato	Infected tomato
Bacillus thuringiensis	5.3	6.3
Pseudomonas aeruginosa	5.4	0.0
Bacillus subtilis	7.6	0.0
Bacillus macerans	6.1	5.7
Bacillus cereus	4.8	8.2

Depending on their root colonisation ability and affinity for exudates, diverse microbial communities have been reported for plant rhizosphere [10]. Biodiversity-ecosystem functioning experimental reports suggest that species diversity provides various community-level benefits related to plant productivity, decomposition rates, cycling of nutrients, resistance to environmental change, and resistance to pathogenic species invasion. It is equally reported that these types of relationships are present in all soil types, and the microbial community therein plays an important role in the health of host plants by ensuring efficient functioning and abundance of the host-associated endophytes as well as other beneficial rhizobiome. Antoniou et al. [8] also reported the predominance of Firmicutes, including several species of Bacillus, in the rhizosphere samples of tomato through rDNA sequence identification, using the 27F (5-AGAGTTTGATCMTGGCTCAG-3) and 907R (5-CCGTCAATTCMTTTRAGTTT-3) primers.

3.3 Incidence of Tobamoviral Infection

On overall weekly assessment of newly infected tomato plants as shown in Fig. 1, lower percentage incidence was reported for plants grown from treated seeds. Incidence of 3.03 and 3.33% were recorded on untreated tomato plants at both the 5th and 6th weeks after transmission respectively, while the treated set had 0.00%. All the plant sets recorded no additional manifestation of symptoms at the 7th and 8th weeks after mechanical transmission of pathogen. Symptoms, typical of Tobamoviral disease, observed on infected tomato plants included malformed, curled, mottled light or dark green leaves; stunted growth with chlorotic tissues; while the fruits of affected plants ripe unevenly and some ripe infected fruits bore several yellow rings. However, the control plants (with neither bacterial seed treatment nor the mechanical transmission of pathogen) showed no sign of infection. The diversity created by the introduced microbial consortium could have affected the establishment, survival, and functions of beneficial rhizosphere microbial community within the complex plant microbiome and could shape the ability of the community to induce disease suppression. Enhancing the species richness around the plant root (through seed treatment before planting) might improve community survival in the temporally and spatially fluctuating rhizosphere environment and ensure that at least one of the beneficial species will survive under the prevailing conditions [21]. The delayed manifestation of infection symptoms as observed on treated plants two weeks after transmission (0%) might have been as a result of the resistance conferred on the treated plants by the rhizobacterial consortium. This could have elevated the minimum infective dose of the virus and possibly prolonged the incubation period required by the transmitted load to establish the disease symptoms on tomato as reported by Adedire et al. [13].

As reported by Coyte et al. [21] and Hu et al. [10], wide community niche breadth could intensify resource use in general, which could help bacteria to better colonise and persist in the rhizosphere and by so doing, intensify the resource competition between the introduced bacterial community and a potential pathogen. This relationship could lead to the competitive exclusion of such pathogen and consequently elevate the host plant protection [10]. Rudrappa et al. [22] also reported that the exogenous application of *Bacillus subtilis*-derived elicitor, acetoin (3-hydroxy-2-butanone), triggered induced systemic resistance (ISR) and protection of tomato plants against pathogens.

3.4 Performance of Tomato

As presented in Table 3 below, fruit per cluster, plant height, leaf and branch numbers were not significantly different in all the plant categories. However, fruit number (16.76), fruit weight (43.80 g), stem girth (0.93 cm) and the number of flower clusters per plant (9.67) were all significantly higher in tomato plants whose seeds were

Table 3. Performance of tomato

Category	Fruit number	Weight/Fruit (g)	Leaf number	Branch number	Stem girth (cm)	Height (cm)	Flower cluster/Plant	Fruit/Cluster
Treated	16.76±0.3a	43.80±0.1a	179.67±3.3	15.33±2.7	0.93±0.1a	68.33±4.4	9.67±0.3a	1.67±0.3
Untreated	8.33±0.3c	41.97±0.2b	133.33±12.9	12.00±0.6	0.67±0.0b	67.33±1.4	4.33±0.2b	1.33±0.3
Control	12.67±0.9b	42.967±0.6ab	164.67±38.9	15.00±3.8	0.83±0.1a	70.33±10.9	4.67±0.4b	1.67±0.7
Significance	*	*	ns	ns	*	ns	*	ns

**Mean values (± standard error) with similar letter (s) along the column are not significantly different at 5 % level of probability by Duncan Multiple Range Test (DMRT).*

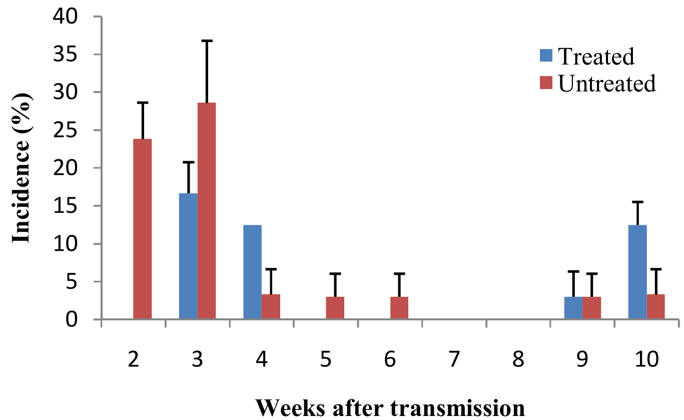

Fig. 1. Incidence of Tobamoviral infection symptoms
%Incidence: Mean values ± Standard error of means

treated with bacterial consortium isolated from healthy tomato rhizosphere than values recorded for untreated plants. As previously reported by Chaparro et al. [23], a variety of soil factors have been documented to increase nutrient availability, growth promotion and overall plant productivity.

The most influential soil factor affecting nutrient availability in the soil might be the bacteria comprising the soil microbial community of the rhizosphere. These complex groups of microorganisms, generally classified as the plant growth promoting rhizobacteria (PGPRs), have been demonstrated to produce several growth promoting enzymes and other biostimulating extracellular metabolites. Several genera of bacteria, including *Bacillus* and *Pseudomonas* are widely studied as PGPR due to their incredible root colonisation ability and stability. Inoculation of rhizosphere bacteria into the soil as plant growth promoting microbial consortium could solubilise fixed soil nutrients and increase inorganic elements availability to plants, thereby resulting in higher crop yields. Many strains of *B. subtilis*, *B. amyloliquefaciens*, *B. cereus* and *Pseudomonas aeruginosa* have been found to interact with plants and produce beneficial effects, including disease suppression and growth promotion [24].

4. CONCLUSION

It could be concluded from the observations recorded in this study, that *Bacillus thuringiensis, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus macerans* and *Bacillus cereus* added as bacterial consortium to seeds prior to planting, suppressed the manifestation of infection symptoms characteristic of Tobamoviral disease of tomato and also improved some yield parameters of tomato grown from treated seeds significantly.

5. RECOMMENDATION

The interaction, independent niche contribution and specific molecular qualities of healthy plant associated rhizobacterial consortium (as well as their fungal counterparts) should be further investigated to better unravel the factors that could possibly affect their presence and activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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