UK Journal of Pharmaceutical and Biosciences Vol. 6(5), 41-47, 2018 RESEARCH ARTICLE

UK Journal of Pharmaceutical and Biosciences

Available at www.ukjpb.com

ISSN: 2347-9442

Rhizobacteria Suppress Fusarium wilt and Early Blight Diseases in Tomato Grown with Organic Fertilizers

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Article Information
Received 5 August 2018
Received in revised form 14 Oct 2018
Accepted 15 Oct 2018

Keywords:
Rhizobacterial consortium,
Fusarium oxysporum,
Alternaria solani

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Abstract

Due to the established toxic effects of agrochemical accumulation in humans, there is an increasing demand for organic agricultural production in Nigeria. The production of highly nutritious and daily consumed crops like tomato through organic farming (using biosupplements) would go a long way in reducing the risks associated with the consumption of inorganic pesticides and fertilizers. In order to investigate the effects of rhizobacterial biosupplement on the resistance and yield of tomato grown with organic fertilizers, the predominant rhizosphere bacteria associated with healthy tomato isolated in this study (*Bacillus thuringiensis, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus macerans* and *Bacillus cereus*) were added as a consortium to improve the availability of organic nutrients to plants. Performance measures including the numbers of fruit, branches, leaves, plant height, days to first flowering and days to 50% flowering at 20.00, 17.67, 176.33, 73.33 cm, 70.43 and 78.00 were all better (higher or lower) in treated tomato plants than untreated set. A significant reduction in percentage prevalence was recorded for Fusarium wilt, as well as Early blight diseases on treated plants (seed treatment with rhizobacterial consortium) compared to plants grown from untreated seeds. However, seed treatment appeared to be less effective in the treatment of Bacterial wilt disease of tomato, with percentage prevalence of 41.70 and 42.90 recorded for treated and untreated plants at nine weeks after planting respectively. It could be concluded that the application of rhizobacterial consortium (as seed treatment) improved the yield and resistance of tomato to Fusarium wilt and Early blight diseases caused by *Fusarium oxysporum* and *Alternaria solani* respectively.

1 Introduction

Tomato (*Solanum lycopersicum* L.) is so far one of the most important vegetable crops cultivated worldwide, its short cultivation period, nutritional properties and overall economic significance make it an agricultural crop of choice to many small and large scale farmers[1]. Microbial diseases remain the major limitation to the production of tomato in Nigeria and the world at large[2]; these include Fusarium wilt (*Fusarium oxysporum*), Late blight (*Phytophtora infestans*), Powdery mildew (*Leveillula taurica*), Early blight (*Alternaria solani*), *Alternaria* stem canker (*A. alternaria* Keissl. F.sp. *lycopersici*), Anthracnose (*Collentotrichum acutatum*), Septoria leaf spot (*Septoria lycopersici*), Leaf mold (*Fulvia fulva*), Grey leaf spot (*Stemophylium solani*), Phytophthora root rot (*Phytophthora parasitica*), Verticillium wilt (Verticillium *alboratrum*), Fusarium, crown and root rot (*Fusarium oxysporum*), White mold (*Sclerotinia sclerotiorum*), Pythium damping-off (*Pythium* species), Rhizoctonia damping-off (*Rhizoctonia solani*), Corky root rot (*Pyrenochaeta lycopersici*), Bacterial spot (*Xanthomonas euvesicatoria*), Bacterial speck (*Pseudomonas syringae*), Bacterial canker (*Corynebacterium michiganensis*), Bacterial wilt (*Ralstonia solanacearum*), Pith necrosis (*Psuedomonas corrugata*), Alfalfa mosaic (Alfalfa mosaic virus), Curly top (Beet curly top virus), Tobamoviral mosaic diseases (Tomato and tobacco mosaic viruses), Tomato spotted wilt (Tomato Spotted Wilt Virus).

An unbalanced soil microecosystem has been attributed to high incidence and overall prevalence of microbial disease of plants[1]. Most of these pathogens need to grow saprophytically in the rhizosphere to eventually access their host or basically to achieve sufficient numbers (minimum infective dose) on their host before they can infect such host tissue[3].

Organic Farming is a system which avoids or largely excludes the use of synthetic inputs (such as chemical fertilizers, pesticides and synthetic feed additives) and rely mainly upon agricultural practices like crop rotations, use of crop residues, animal manures, off-farm organic waste, mineral grade rock additives and biological system of nutrient mobilization and plant protection. This therefore suggests organic agriculture as a unique production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles and soil biological activity[4]. This is however essentially important to reduce the accumulation of toxic chemicals, especially, the broad range of chemical products used for microbial pest control. Also, large quantities of organic wastes such as poultry manure are readily available and very affordable, both in rural and urban centers, and are effective sources of nutrients for plants.

Several soil factors are known to improve nutrient availability and plant productivity. The most influential of these factors had been reported to be the organisms comprising the soil microbial community of the rhizosphere, which is the soil environment around each plant root where the production of root exudates serve to nourish and to a large extent, determine the group of rhizobacteria associated with the roots of different plants. This structure initiates a complex interaction within the rhizosphere of each type of plant. The microbial community, once artificially added or naturally signaled by exudates form a potentially dynamic and self sustaining rhizobacteria, reduce the requirement for repeated application and could possibly influence the growth and resistance of host plants to pathogens, especially those evolving resistance to available control measures[5]. The beneficial community of bacteria occupying the rhizosphere can readily thrive by assimilating the available nutrients characteristically adjusted by exudodeposits. Consequently, the establishment of rhizobacteria depletes the space, as well as resources availability to invading pathogens and as such, achieving sustained niche occupancy.

This research work is therefore designed to investigate the protective and yield improving effects of rhizosphere bacterial consortium associated with healthy tomato plant roots on tomato grown with properly cured poultry and rabbit waste (droppings).

2 Materials and Methods

2.1 Isolation of rhizosphere bacteria

Healthy tomato plants were carefully uprooted from the soil and the loosely adhering soil attached to each root was gently removed by shaking the uprooted plant. Ten grams (10g) of the soil sample was then dissolved in 100 mL sterile distilled water and vortexed vigorously. The preparation was then serially diluted 10^7 folds (to select for the culture-dependent, most abundant rhizosphere bacteria) and thereafter plated on Nutrient and Tryptone Soy Agar using the pour plate method. Plates were incubated at 30°C for 48 hours. Growing colonies were purified and identified; isolates were kept in freezer in Nutrient Broth with sterile glycerol (30% v/v)[6, 7].

2.2 Seed treatment

Low nutrient soil samples were taken from tomato plots with records of microbial diseases; these samples were bulked, properly mixed and used as stock potting soil (with a sandy loam precropping textural class). Three sets of plastic pots (20 cm in diameter, used for the replicates of each treatment) were washed and air dried. Two sets of pots (labeled treated and untreated) were filled with the prepared soil (5 kg/pot each), while the last set of pots, used for the control plants, was filled with soil presterilized (autoclaved) for an hour at 121°C and 15psi for two consecutive days. Tomato seeds (Hausa Scissors) were surface sterilized (70% ethanol for a minute), appropriately rinsed thrice with sterile distilled water and placed on sterile blotting paper to remove excess moisture; these seeds were thereafter treated with isolated rhizosphere bacterial consortium. Seeds were placed in sterile petri dish and uniformly sprayed with 2mL mixture of culture suspension of bacteria (3×10^6 cfu/mL) or sterile distilled water (for both untreated and control seeds). Treated seeds were subsequently air dried for two hours prior to sowing[8]. Growing seedlings were thereafter transplanted at the rate of one seedling per pot.

2.3 Application of manure

With the possibility of an enhanced mineralization rate, fertilization of soil for tomato cultivation was done two weeks before planting, two weeks after transplanting and at the onset of fruits at a cumulative rate of 10 t/ha. Although, the manures used in this study were properly composted and cured, poultry manure (excellent growth promoting manure for vegetables) was added to soil samples in both treated and untreated labeled pots two weeks before planting to allow proper mineralization of nutrients, as well as to reduce the effect of other undesirable compounds associated with poultry waste[9]. The (poultry) manure was added at an equivalent rate of 6 t/ha (conversions per pot were made to g/cm^2), while 4 t/ha rabbit manure was split and applied twice, three weeks after transplanting and at the onset of fruits[10,11].

2.4 Microbial isolation from symptomatic plants

Disease manifestation on plants developing from treated and untreated seeds were preliminarily identified through their symptoms, followed by the isolation of pathogens from symptomatic plants, identification of isolates and subsequent pathogenicity test.

Tomato plant parts showing typical infection symptoms were collected and 5mm^2 segments were thereafter taken from the edge of infected samples, surface sterilized with 1% NaOCl for two minutes, this preparation was then rinsed twice with sterile distilled water and then dried on sterile filter papers. Each sample was thereafter macerated in 2 mL sterile distilled water using a sterile spatula, and the resulting suspension was stirred in a vortex mixer to obtain a turbid microbial suspension. A loopful of the resulting aqueous suspension in each case was streaked onto Nutrient Agar (for bacterial pathogen) or Potato Dextrose Agar (to isolate fungal pathogen) and incubated at 30˚C for 48 hours or 37˚C for 5-7 days respectively. Distinct colonies were thereafter subcultured and identified[3,12]. Identification of organisms was done using microscopic, biochemical, carbon utilization, as well as chemical sensitivity assays with a Biolog GENIII 96 microplate (Biolog, Omnilog, US).

2.5 Pathogenicity test

Pathogenicity test was done to ascertain the causative organism of each disease symptom (as stipulated by Koch's postulate) and relate to confirmed etiology of the disease. This was carried out using the isolated strains of organism from infected plant tissues to infect young, healthy tomato plants. Suspensions of pure microbial cells were used for the standardization of each organism for tomato infection. Tomato leaves and roots were inoculated with 2mL spray of each isolate (1 x 10^8 CFU/mL). Control plants were sprayed with sterile distilled water. Inoculated plants were thereafter kept humid for 48 hrs and left in the screen house for observation. The manifestation of symptoms associated with each disease was observed on properly labeled tomato plants 1-3 weeks post-inoculation. Microbial cells were then isolated from diseased areas of inoculated plants as described above[12].

2.6 Severity

The severity of each of the diseases observed, Fusarium wilt, caused by *Fusarium oxysporum*[13], Bacterial wilt, caused by *Ralstonia solanacearum*[14] and Early blight, caused by *Alternaria solani*[15,16] was recorded weekly from the third to the ninth week after planting.

2.7 Determination of performance

The performance parameters measured to determine the effect of rhizosphere bacterial consortium on treated tomato included the fruit number, fruit weight[17], number of leaves, number of branches, plant height, stem girth[18], days to first flowering, days to 50% flowering and seed germination percentage[17].

3 Results and Discussion

3.1 Isolation of rhizosphere bacteria

The culturable, predominant bacteria isolated from healthy tomato rhizosphere included *B. thuringiensis, Pseudomanos aeruginosa, B. subtilis, B. macerans* and *B. cereus*; these bacteria were added as a soil conditioning consortium (suspension of bacteria at 3 × 10^6 cfu/mL) towards the plant growth promotion and resistance of tomato to microbial diseases. Chaparro *et al.*[5] also reported the presence of several gram positive and negative bacteria, including *Bacillus* and *Pseudomonas* species, in the rhizosphere of tomato.

3.2 Application of manure

As presented in table 1 the percentage Nitrogen (N), Phosphorous (P) and Potassium (K) contents of rabbit waste were 1.85, 1.1 and 1.31 respectively, while poultry manure had 3.21, 2.07 and 2.54 percent of these nutrients respectively. However, organic carbon content was higher in rabbit waste while magnesium (Mg) was higher in poultry manure.

Table1: Chemical properties of poultry and rabbit manure

Manure	Organic C (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Poultry	15.61	3.21	2.07	2.54	1.56	0.63
Rabbit	17.02	1.85	1.1	1.31	0.72	0.32

This set of organic fertilizers were added to enhance the supply of nutrients to tomato plant roots within the soil[10,19,11]. As reported by Feijuan and Cheng[20], the application of organic fertilizers (before planting) at rates beyond 350 kg N/ha did not translate to a significant increment in tomato yield. However, as deduced from the recommendations of Oyewole *et al.*[21], the combination of mineral fertilizer (MF) with the best poultry manure rate (150-300 kg N/ha) yielded a better result, possibly due to the initial utilization of the readily available minerals (from MF) by soil microbiome, thus extending the availability and effect of poultry manure beyond the yield plateau initially observed with the sole application of poultry manure. Consequently, 4 t/ha (74.0 kg N/ha, 44.0 kg P/ha, 52.4 kg K/ha) rabbit manure was split and added two (2) weeks after transplanting and at the onset of fruits, in addition to 6 t/ha (192.6 kg N/ha, 124.2 kg P/ha, 152.4 kg K/ha) poultry manure added before planting.

3.3 Prevalence of diseases on tomato

Most of the infected plants possess parts affected by at least two of the observed diseases; there was a reduction in the prevalence of Fusarium wilt (Fig. 1), as well as Early blight (Fig.

3) diseases on treated plants (seed treatment with rhizobacterial consortium) compared to plants grown from untreated seeds.

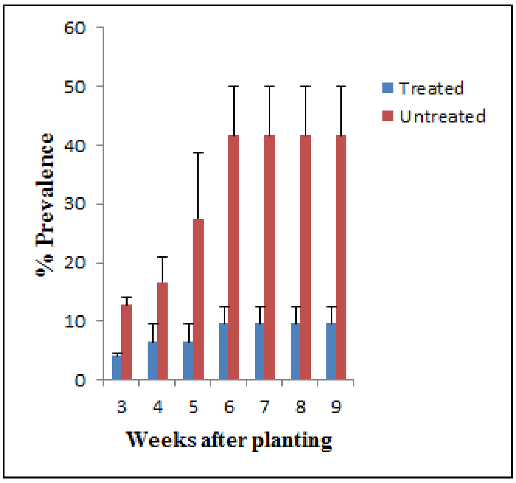

Fig. 1: Prevalence of Fusarium wilt

However, seed treatment (as used in this study) appeared to be less effective on the spread of Bacterial wilt disease of tomato (Fig. 2), especially, beyond the fifth week after planting.

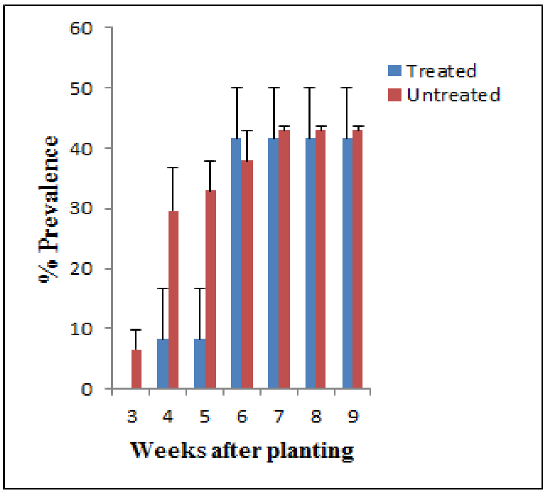

Fig. 2: Prevalence of Bacterial wilt

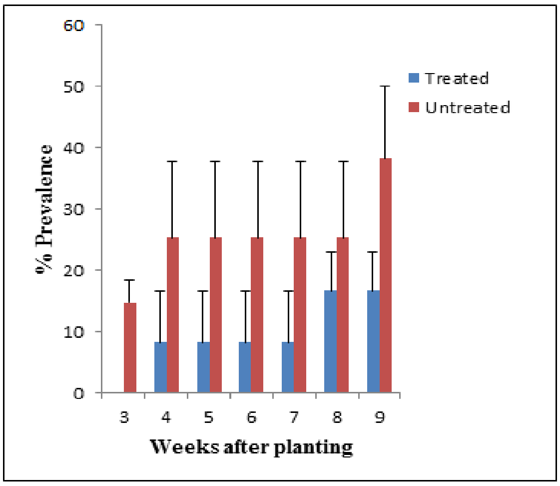

Fig. 3: Prevalence of Early blight

In addition to blocking open or vacant ecological niches, production of inhibitory extracellular metabolites and increasing the soil's resistance to pathogen invasion, plant growth promoting rhizobacteria (PGPR) tend to increase the yield of host plants, they aid in nutrient acquisition, stress tolerance and a reduction in disease incidence[22]. Direct effects of PGPR also include the provision of plants with fixed nitrogen and phytohormones, as well as increasing the availability of nitrogen, soluble phosphate and minerals in the soil[23].

As presented in table 2, seed treatment with rhizobacterial consortium appeared to have reduced the severity of infections observed on tomato. Plants grown from treated seeds showed no sign of Early blight and Bacterial wilt infections at the third week after planting. Fusarium infected plants (treated) showed slight recovery in terms of leaf chlorosis at the sixth and seventh weeks after planting but the severity increased slightly at the eighth and ninth weeks after planting. Although lower percentage prevalence of Early blight infection was recorded for tomato treated with rhizobacterial consortium, the severity of this disease was relatively high at the eighth and ninth weeks after planting. The observed reduction in severity associated with treated tomato plants might have been as a result of the accumulation of protective metabolites produced by *Bacillus* and *Pseudomonas* species added (as a consortium) to the seeds of these plants. Plant growth promoting and protective rhizobacteria have been documented to directly or indirectly enhance the resistance of host plants through a wide variety of mechanisms including the abiotic stress tolerance in plants, the production of siderophores, secretion of volatile organic compounds, as well as the production of protective enzymes such as chitinase, glucanase, and ACC-deaminase for the prevention of plant diseases[24].

3.4 Performance of tomato

The average seed germination percentage of rhizobacterial treated, untreated and control seeds of tomato (Hausa Scissors) used in this study were 73.33, 86.67 and 83.33 respectively (Table 3). The first set of leaves on sprouting treated seeds emerged as early as the third day after planting while the seeds of other treatment sets (untreated and control) bore their first pair of leaves five to seven days after sowing.

Both the fruit number and number of branches at 20.0 and 17.7 respectively were significantly higher in treated tomato plants (Table 3). Unlike the treated (inoculated) tomato set, the untreated (inoculated) tomato plants had significantly higher days to first flowering and days to 50% flowering when compared to control plants. However, the number of leaves, stem girth, height and seed germination percentage were not significant different among the plant sets. In organic production, the organic fertilizers and other forms of non-toxic biofortification of soil can be utilized to enrich the soil with nitrogen, phosphorus, potassium and other nutrients required by plants.

However, the mineralization of these manures could be slow in the absence of appropriate microbial community[19]; consequently, the release of plant-accessible nutrient to the soil would be problematic. This might have been one of the factors responsible for the improved yield observed in tomato plants grown from treated seeds.

Table 2: Average severity scores of diseases

Weeks after planting	Fusarium wilt		Bacterial wilt		Early blight	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
3	2.0	2.6	0.0	1.0	0.0	2.0
4	3.2	2.3	1.0	3.3	1.0	2.0
5	3.3	3.0	1.0	3.1	1.5	2.0
6	2.2	3.0	1.0	3.2	2.0	3.5
7	2.6	3.6	2.3	3.4	2.1	3.5
8	3.0	4.0	2.6	3.4	2.2	4.5
9	3.0	4.3	2.6	3.4	2.1	4.8

Table 3: Performance of treated and untreated tomato sets

Category	Fruit number	Weight/ Fruit	No. of leaves	No. of branches	Stem girth (cm)	Plant height (cm)	Seed germination (%)	Days to 1st flowering	Days to 50% flowering
Treated	20.00±0.5a	42.30±0.3b	176.33±3.3	17.67±0.7a	0.83±0.1	73.33±1.7	73.33±3.3	70.43±3.5ab	78.00±0.5ab
Untreated	14.33±0.6b	42.63±0.3ab	119.01±11	11.33±0.3b	0.63±0.0	66.67±1.6	86.67±6.7	82.00±4.0a	86.01±4.0a
Control	10.00±1.2c	43.53±0.2a	124.00±37	12.33±1.6b	0.77±0.0	67.67±9.3	83.33±3.3	63.33±3.3b	76.00±0.5b
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)

Jamal *et al*.[25] in their work on the effect of plant growth-promoting bacterium, Bacillus amylliquefaciensY1 on soil properties, pepper seedling growth, rhizosphere bacterial flora and soil enzymes also reported that PGPR indirectly promoted plant growth by producing the fungal cell wall degrading enzyme to protect plants against pathogens. They equally found that cellulase activity, including chitinase and dehydrogenase enzyme activity, increased within the rhizosphere of plant soil due to the inoculation of rhizosphere bacteria; this could increase the availability of nutrients locked in organic materials within the soil.

4 Conclusion

From the results recorded in this study, it appeared tomato seed treatment with bacterial consortium (*B. thuringiensis, Pseudomanos aeruginosa, B. subtilis, B. macerans* and *B. cereus*) improved the availability of nutrients within the applied organic manures (poultry droppings and rabbit waste) for plant utilization. This can be deduced from the improved growth characteristics and overall economic yield of fertilized plants grown from treated seeds. With the improvement of tomato resistance to diseases, as indicated by the reduction in incidence, it could be concluded that the use of predominant tomato rhizosphere bacteria alongside animal manure, improved the yield and resistance of tomato plant to Fusarium wilt and Early blight diseases.

5 Recommendation

Due to the documented variation in the distribution of rhizosphere bacteria, as largely conditioned by the root exudates of each plant species, carefully isolated rhizosphere bacterial consortium from healthy tomato plants could be applied alongside compost or cured agricultural waste (with prerecorded history) to protect and promote the growth of tomato, as well as to improve its economic yield. However, extending investigations to predominant rhizobacteria associated with other economically important plants and establishing the interaction between the rate of manure application and the population of rhizobacterial treatment would significantly contribute to achieving a better and stable organic farming system in Nigeria.

6 Conflict of interest

The authors declare that there are no conflicts of interest.

7 Author's contributions

AOM carried out the isolation and identification of rhizobacteria, and wrote the first draft of manuscript. IDO and OAO reviewed the first draft of the manuscript while PAA and FAK reviewed the research design as well as the result presentation.

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