GLOBAL JOURNAL OF BIO-SCIENCE & BIOTECHNOLOGY

www.scienceandnature.org

EVALUATION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA FOR THE CONTROL OF BACTERIAL WILT DISEASE OF TOMATO

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ABSTRACT
Two commercially formulated plant growth-promoting rhizobacteria (PGPR): equity and trichoshied at 2 concentration each (0.15%, 0.3%) and (0.5%, 1%) respectively were evaluated for the control of bacterial wilt disease of tomato caused by *Ralstonia solanacearum,* a soil borne pathogen using two varieties of tomato (Ibadan local and UC82B). Their possible effects on plant growth were equally tested. The effect of PGPR on the incidence of bacterial wilt and growth of tomato seedling were evaluated in the screen wase. Results showed that 0.3% equity and 1% trichoshield recorded the lowest incidence of 68.0%, 44.0%, 54.0%, and 48% on Ibadan local and UC82B respectively. Plant growth was also enhanced by 1% trichoshield recording the highest shoot, root and leaf weight of 5.28g, 3.52g and 2.38g respectively. Collectively, PGPR proffer a remedy of an environmentally sustainable approach to increase crop production and enhancement of public health.

KEYWORDS: Plant growth, Rhizobacteria, wilt disease, Ibadan local, trichoshield.

INTRODUCTION

The tomato (*Lycoperisicon esculentus* mill) belongs to a large family of plant called solanaceae, and is one of the most versatile and widely grown vegetables throughout the whole world (Hartmann *et al*., 1981). The crop origin dated from tropical parts of central and southern America and was domesticated in mexico and later taken to Europe and across the pacific by the early Spanish explorers and Portuguese traders (Villareal, 1990). Tomato in Nigeria probably descended from varieties brought from Europe (Villareal, 1990). It is cultivated throughout Nigeria and the most important area lie between latitude 7.5^0N and 13^oN mostly in the north and south western part of the country. Tomato is an important source of vitamin A and C, minerals, some dietary fibre, a little protein and much water (Pest Control manual, PCM 1983).The tomato fruit may be eaten raw, made into salads, cooked or processed into juice, paree, paste and sauces (Goose and Binsted, 1973).

Sustainable tomato production is constrained worldwide by pest and diseases and more than a hundred of them have been made known on tomato. Some of the major disease include bacterial wilt caused by *Ralstonia solanacearum*, bacterial canker caused by *xanthomonas vasicaforia* tomato leaf curl virus disease, Fusarium wilt caused by *Fusarium oxysporium*, early blight caused Alternaria solani and damping-off disease caused by organisms such as *pithium spp. Phyto-phthora spp* and *Botrytis spp* (Mc Collum, 1988).some major pest of tomatoes are birds and nematodes (Messiaen, 1982). Bacterial pathogens (*Ralstonia solanacearum* Smith) are endemic in many of the vegetable growing areas of the world including Nigeria (Erinle, 1979; Hayward and Hartmann 1994). The estimates of yield losses caused by this pathogen on tomato vary from 15-95% (Hayward and Hartmann, 1994; Kuku *et al*., 1996).

Effective methods of controlling external wilt disease have been developed but some have limitation, either site specific or socioeconomic condition (Hayward, 1991). Various strategies of controlling bacterial wilt disease e.g. intercropping, rotation and soil amendment against the pathogen has been reported (Sun and Hucaug, 1985; Michel *et al*., 1997; Sood *et al*., 1998) but result from these studies are variable and not always effective control measure against wilt diseases of tomato (Hayward, 1991). The prospect of manipulating crop rhizosphere microbial population by inoculation of beneficial bacteria to increase plant growth has shown considerable promise in laboratory and green oragence studies, but responses have been variable in the field (Bowen and Rovira, 1999). Plant growth promoting rhizobacterial (PGPR) offer an environmentally sustainable approach to increase crop production and health. It is therefore imperative to evaluate the plant growth-promoting rhizobacteria for the control of bacterial wilt disease of tomato in order to increase crop production in developing countries particularly in Africa and possibly for the enhancement of public health.

MATERIALS AND METHODS

Sources of materials

Seeds of tomato (co Ibadan local and UC82B) were obtained from the genetic resources unit of the National Horticultural Research Institute (NIHORT) Ibadan. Topmost soil, used in the nursery planting and screen house were collected at premises of NIHORT Ibadan. Plant growth-promoting rhizobacteria (PGPR) formulation used were equity and trichoshield (commercial product), collected from pathology Department of NIHORT Ibadan.

The strain of *R. solanacearum* used was obtained from the pathology laboratory (NIHORT). It was originally isolated from tomato plant.

Sterilization of hardwares

Pyrex petri dishes and other glass wares used were washed with detergent, rinsed in clean tap water, dried and sterilized in a Philip Harris hot air-oven(160^{o}C) for at least 3hours. Metals (inoculating needles, wire loops, forceps) were always sterilized before use by exposure to the blue part of burning flame till red hot.

Sterilization of soil

Topsoil obtained from the premises of NIHORT was loaded into a trough watered and steamed for six hours. The soil was allowed to cool before transferring it to the screen house

Preparation of media

Tripheny tetrazotion chloride Agar (TTCA). 1.5 litres of tripheny tetrazotion chloride Agar was formulated using 5g peptone, 0.5g casein-hydrolysate, 2.5g glucose, 8.5g Agar into 1.5 litres of distilled water.

30ml of Tripheny tetrazotion chloride (TCZ) solution was pipette into 1.5 litres of prepared media, stirred thoroughly in a conical flask. The mouth of the conical flask was then plugged with cotton wool and wrapped up to the temperature of 121^{o}C for 15 minutes. The flask was removed and allowed to cool to a temperature of 40-45^{o}C. The cooled agar was dispensed aseptically into sterile glass Petri-dishes inside the inoculating chamber and allowed to cool during which is solidified.

Preparation of innoculum

Suspensions of bacterial cells were made by washing 48 hours old colonies of plates containing TTCA into 100ml of sterile distilled water. This stove solution was used to prepare dilution of 1:1, 1:2, 1:4, 1:8, and 1:16. Percentage transmittances of each dilution were determined using a Bosch photocolorimeter at a wavelength of 600nm. Thereafter 0.1ml of each dilution was pipetted aseptically into sterile Petri dishes and 0.1ml of sterile TTCA was added. Plates were rotated gently to get adequate mixing of medium and bacterial cells. Plates were incubated at 30^{o}C for 48 hours colonies were counted and used to estimate the number of organizing per ml of each dilution. Infestation of the seedling 500ml of inoculums suspension was prepared, 250ml each for each variety. The tomato plant root (seedling) was soaked (dipping method) for 30 minutes inside *Ralstonia solanacearum* suspension.5 seedling each were planted on each pot, 10ml of *Ralstonia solanacearum* suspension were added to each planted seedlings and transferred into screen house. The trial was a 2X6 factorial arranged in completely randomized design and replicated five times.

Preparation of the treatment

Equity I

1.5ml of equity solution (commercial product) was prepared in 1 litre of distilled water making (0.15%).

2.6.2 Equity II

3ml of equity solution was prepared in 1 litre of distilled water making (0.5%).

2.6.3 Trichoshield I

10g of trichoshield was dissolved in 1 litre of distilled water making (1%).

Application of treatment

100ml of each treatment was applied to each pot across the row (replicate) water (distilled) was applied to the control experiment once a week.

Data recording and termination of trials

The data recorded include disease incidence, leave number, fresh shoot weight, and percentage disease incidence. The fresh weight was determined using analytical balance (SARTORIUS). Termination of trial was at 21days after transplanting. Statistical analysis of data was conducted using SAS: mean separation was accomplished using Duncan's Multiple Range Test.

Disease incidence

The percentage incidence of wilt disease was estimated as follows:

% incidence = n/N X100/1

Where n= number of plants showing wilt symptoms with at least one leaf (Michel *et al*., 1996).

N = Total number of sample plant.

RESULTS

The effect of Plant growth-promoting rhizobacteria (PGPR) on the incidence of bacterial wilt disease was evaluated as shown in table 1. The treatments had a significant effect on the incidence of bacterial wilt disease at each sampling dates. At IWAT, UC82B that received equity at 0.3% recorded the lowest wilt incidence of 44.0% (as shown in table 1). The wilt incidence of UC82B at trichoshield 1% and Ibadan local local at trichoshield 1% were similar and also significantly lower. Higher wilt disease incidences were recorded in Ibadan local and UC82B seedlings that did not receive any PGPRT treatment. At 2WAT, UC82B that received equity at 0.3% recorded the lowest wilt incidence of 60.0% as shown in table 1.The wilt incidence of Ibadan local at equity 0.15%, trichoshield 0.5%, trichoshield 1% and UC82B at trichoshield 0.5% trichoshield 1% are also similar and significantly lower as the results are presented in table 1.Higher wilt disease incidences were recorded in Ibadan local and UC82B seedlings that did not receive any PGPR treatment as shown in table 1.At 3WAT, Ibadan local that received equity at 0.15% and UC82B that received equity at 0.3%, trichoshield at 1% recorded the lowest wilt incidence 0f 80% respectively. The wilt incidence of UC82B at trichoshield 0.5% Ibadan local at trichoshield 0.5%, 1% were similar and significantly lower as presented in table 1.At 3WAT, seedlings that do not received any PGPR treatment as shown in table 1 depicted 100% wilt incidence. Furthermore, the effect of PGPR on the growth of tomato seedling was evaluated. The treatment recorded a significant effect on the growth of tomato seedlings in term of shoot, root and leaf weight. At 3WAT, Ibadan local and UC82B that received trichoshield at 1% showed the highest growth weight of 5.28g, 3.52g and 2.38g in terms of shoot, root and leaves respectively as shown in table 2.Furthermore, UC82B and Ibadan local that received trichoshield at 0.5% significantly higher with 1.87g, 1.60g and 1.68g in terms of shoot, root and leaves weight respectively. At 3WAT, seedlings that do not received any PGPR treatment recorded lowest growth weight of 1.43g, 1.66g and 1.35g in terms of shoot, root and leaves weight respectively as presented in table 2.

Collectively, as the week progress, the wilt incidences in all treatment increased significantly.

TABLE 1: Effect of PGPR on the incidence of bacterial wilt disease of tomato.1, 2, and 3 depicted weeks after transplanting. Mean followed by the same letter are not significantly difference ($p<0.05$) using DMRT.

Treatment	1	2	3
Control (Ib. local)	80.0^{a}	92.0^{a}	100.0a
Equity (0.15%) (Ib. local)	68.0^{ab}	80.0^{b}	80.0^{ab}
Equity (0.3%) (Ib. local)	68.0^{ab}	82.0^{b}	82.0^{a}
Trichoshield (0.5%) (Ib. local)	56.0^{b}	80.0^{b}	88.0^{ab}
Trichoshield (1%) (Ib. local)	54.0^{ab}	80.0^{b}	92.0^{a}
Control (UC82B)	80.0^{a}	92.0^{a}	100.0^{a}
Equity (0.15%) (UC82B)	58.0^{b}	96.0^{a}	100.0^{a}
Equity (0.3%) (UC82B)	44.0^{b}	60.0^{c}	80.0^{ab}
Trichoshield (0.5%) (UC82B)	60.0^{ab}	80.0^{b}	92.0^{a}
Trichoshield (1%) (UC82B)	48.0^{b}	68.0^{c}	80.0^{ab}

TABLE 2: Effect of PGPR on the growth of tomato seedling. Mean followed by the same letter are not significantly difference ($p<0.05$) using DMRT.

Treatment	Shoot	Root	Leaves
Control	1.43bc	1.66b	1.35bc
Equity (0.15%)	1.30c	1.15b	1.10c
Equity (0.3%)	0.70d	1.23b	1.68b
Trichoshield (0.5%)	1.87b	1.60b	1.68b
Trichoshield (1%)	5.28a	3.52a	2.38a

DISCUSSION

The study demonstrates the potentials of PGPR in the control of *Ralstonia solanacearum*, a soil borne pathogen of the tomato crop. The two commercially formulated PGPR has shown tremendous potentials in the control of bacterial wilt disease of tomato. Trichoshield has *Trichoderma harzianum* as its major constituent; equity has *Baccilus subtilis* as its major constituent. The most promising treatment under screen house condition in the control of bacterial wilt of tomato is trichoshield. Our present data are in agreement with an earlier study where trichoshield was reported to control rot induced by *Rhizotonia solani* (Hadar *et al*, 1979). The reduction of wilt incidence may be due to microbial antagonism against the pathogen as reported in other studies thereby resulting in reduced tomato plant infections (Lemanceau and Alabouvette, 1993).Similarly, other study showed that Trichoderma viride significantly reduced fusarium with incidence and root population of pathogen but did not promote plant growth. This may be linked as a result of production of chitinolytic enzymes by T.viride with lytic activity against fusarium spp (Cherif and Benhamou, 1990; Lorito *et al*, 1993). Although, T.harzianum has earlier been reported to control fusarium wilt of tomato (Adebayo, 2005). Our present results showed a moderate protection of tomato seedling by trichoshield against bacterial wilt disease. PGPR had been reported to directly enhance plant growth by variety of mechanism: fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilisation of minerals such as phosphorus and synthesis of phytohormones (Glick, 1995).In addition, the disease control potential of equity may be due to the constituent which is *B. subtilis. Bacillus subtilis* though earlier reported by (Ghonim, 1999) to be antagonistic to *R. solanaceanum* exhibited only one week antagonistic properties in term of plant growth demonstrated in our present study. However, *B. subtilis* in accordance with the report of (Ghonim, 1999) improved growth parameters of the tomato plant. These organisms can be used in combination with other organisms showing better disease reduction for the control of bacterial wilt of tomato. Furthermore, our present study showed reduced disease incidence and increase root growth when tomato seedlings was treated with *Bacillus subtilis.* Culture filtrate of *B. subtilis* was found to contain some fraction (Tr-c) behaving like an auxin precursor, indole-3-pyruvic acid, including systematic resistance and stimulation of root growth of tomato seedling (Gupta *et al*,2000).Better disease control may be achieved when treatment concentration were increased as revealed in our present study. Lower disease incidences were recorded on seedlings treated with 0.3% equity and 1% trichshield. The incidence of wilt on the two tomato varieties did not show significantly difference. Although UC82B is an improved introduced variety which is still susceptible to wilt as Ibadan local variety. This may be linked to continuous planting of UC82B adapting the variety to biological environment under which tomato is cultivated in the country. Similarly, our data revealed high pathogenicity of *R. solanacearum* on tomato as seedlings without PGPR treatment recorded 100% wilt incidence at 3WAT.

CONCLUSION

Collectively, PGPR proffer a remedy of an environmentally sustainable approach to increase crop production and enhancement of public health.

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