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SYSTEMIC BIOCONTROL OF FUSARIUM WILT IN TOMATO (*Solanum lycopersicum* L.) USING *Bacillus* ENDOPHYTES

Adedire, O. M.[1,2], Aduramigba-Modupe, A. O.[2,*] and Odeniyi, O. A.[3]
[1]Department of Microbiology, Federal College of Agriculture, Ibadan, Nigeria.
[2]Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria.
[3]Department of Microbiology, University of Ibadan, Nigeria.
[*]Corresponding Author's Email: fyaduramigba@yahoo.com
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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables consumed globally for its culinary and medicinal significance. However, fusarium wilt, caused by *Fusarium oxysporum* Schlechtendal, is a recalcitrant limitation to tomato production. This is due to the evasive pathogenesis and systemic proliferation of *F. oxysporum*. Therefore, this study attempts to develop a stable, effective, passive-systemic control measure against fusarium wilt in tomato. Tomato seeds were bioprimed with 10^8 CFU/mL of antifungal endophytes (*Bacillus amyloliquefaciens* Sa08 and *Bacillus subtilis* Og04). Biocontrol experiment was set up as a factorial experiment arranged in completely randomized design involving two tomato varieties (Alausa and Ibadan-local), endophytes and carbendazim. Endophyte colonization pattern, *in vivo* fusarium-biocontrol potential, and residual wilt control efficacy were determined. Both antifungal *Bacillus* species exhibited a systemic colonization pattern in treated tomato. Highest tissue colonization (13.25×10^2 CFU/g) was expressed by *Bacillus subtilis* Og04 in the root of Ibadan-local tomato variety. *Bacillus* endophytes also conferred systemic protection against fusarium wilt and improved yield properties in treated plants. *Bacillus amyloliquefaciens* Sa08 increased fruit production in infected Alausa tomato from 0% to 79%, while *Bacillus subtilis* Og04 retained 86.40% of its wilt control efficacy six months after seed treatment in Ibadan-local variety. Seed treatment with *Bacillus* endophytes will limit the laborious application process associated with previously recommended biocontrol materials against fusarium wilt. Based on their residual biocontrol potentials, the *Bacillus* species should be evaluated under field conditions, as a seed pre-treatment strategy, to reduce the need for scientific expertise by farmers and other end-users.

Keywords: Antimycotic bacteria, endophyte, fungal wilt, systemic inhibition, vegetable.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a globally cultivated vegetable crop. It is widely produced in Nigeria due to its multiple culinary applications and medicinal significance, associated with the presence of lycopene (Przybylska and Tokarczyk, 2022). Tomato is taken raw in salad, as fermented juice or processed into sweetened candies as well as ketchup. Unfortunately, the production of tomato is severely limited by both biotic and abiotic stressors. These include insect and microbial pests, as well as adverse environmental factors like nutrient limitation and drought (Rai *et al.,* 2023). From chronological investigation, fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen (FOL) has always been the most prevalent and destructive fungal disease of tomato plant (Glynn and Kavanagh, 1980; Du *et al.,* 2022). The pathogen (FOL) is an ascomycete belonging to the family Nectriaceae. *Fusarium oxysporum* causes extensive tissue damage, wilt, chlorosis and necrotic symptoms on infected tomato plants (Du *et al.,* 2022).

Despite extensive research over decades, effective and sustainable management of Fusarium wilt remains elusive. Traditional approaches, including soil fumigation, crop rotation, and resistant cultivars, have shown variable success (Ma *et al.,* 2013). Breeding for resistance through introgression of *I*, *I-2*, and *I-3* genes has been a primary strategy, yet pathogen races frequently overcome these resistances due to high genetic variability and the ability of FOL to evade host recognition systems (Pincot *et al.,* 2022; van der Does *et al.,* 2019; Ma *et al.,* 2013). Chemical fungicides are often ineffective against vascular pathogens once systemic infection occurs, and their repeated use raises concerns about environmental contamination, non-target effects, and the development of resistance (van der Does *et al.,* 2019). These limitations underscore the need for alternative, ecologically sound strategies that provide durable protection.

Biological control using beneficial microorganisms, particularly endophytic bacteria,

has emerged as a promising approach. Endophytes colonize internal plant tissues without causing disease, offering advantages such as direct competition with pathogens, production of antimicrobial compounds, induction of systemic resistance, and promotion of plant growth (Andrić *et al.,* 2020). Species within the genus *Bacillus* are especially attractive due to their spore-forming ability, which confers resilience to adverse environmental conditions, and their capacity to produce a wide array of bioactive metabolites, including chitinases, glucanases, proteases, lipopeptides, and volatile organic compounds (Andrić *et al.,* 2020; Adedire *et al.,* 2023b). These metabolites directly antagonize fungal pathogens like FOL while enhancing host vigor through mechanisms such as phosphate solubilization, nitrogen fixation, and hormone production.

However, significant gaps persist in the development and deployment of endophyte-based biocontrol for Fusarium wilt. Conventional screening often relies on *in vitro* antagonism assays that may not translate to in planta efficacy (Adedire *et al.,* 2023a), overlooking critical factors such as host-endophyte compatibility, colonization efficiency, and persistence under stresses. Many candidate biocontrol agents fail to deliver consistent, residual disease suppression due to poor rhizosphere or endophytic establishment and sensitivity to environmental fluctuations (Wei *et al.,* 2019; Jaffar *et al.,* 2023). Moreover, limited attention has been given to seed-based delivery systems, passive-systemic colonization patterns across plant tissues, and the longevity of protective effects during seed storage. These are factors essential for efficacy and practical adoption by farmers in resource-limited settings (Jaffar *et al.,* 2023).

The present study aims to addresses these challenges within the framework of systemic biocontrol of Fusarium wilt in tomato using *Bacillus* endophytes. It evaluates the host colonization patterns of promising antifungal *Bacillus* isolates to confirm their endophytic establishment in roots, stems, and leaves. It further investigates the efficacy of seed treatment with these endophytes in providing systemic protection against FOL challenge, coupled with disease assessment and evaluation of agronomic performance in treated tomato plants. By integrating colonization studies, *in vivo* disease suppression trials, and seed storage assessments, this work aims to advance the selection and deployment of stable, effective *Bacillus* endophytes. Such an integrated approach is expected to contribute to sustainable tomato production by reducing reliance on synthetic inputs while enhancing plant resilience and productivity.

MATERIALS AND METHODS

Experimental sites

Screenhouse experiments (endophyte colonization and biocontrol assays) were conducted at the screenhouse of Institute of Agricultural Research and Training, Apata, Ibadan (7°22'32.0"N; 3°50'49.7"E). Microbial cultivation and generation of resistant *Bacillus* strains were done at the Seed Health Laboratory of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Apata, Ibadan.

Sources of soil, pathogen, antifungal tomato endophytes and tomato varieties

Biocontrol endophytes (*Bacillus* strains) used in this study were isolated from the tissues of healthy tomato plants, growing within close proximity to wilting plants on fusarium-infected fields. *Bacillus* strains (*Bacillus amyloliquefaciens* Sa08 and *Bacillus subtilis* Og04) were previously identified and screened through a modified dual-culture, *in vitro* assay (Adedire *et al.,* 2023b) and subjected to *in vivo* wilt control assay in this study. The selected *Bacillus* strains (Sa08 and Ogo4) expressed notable *in vitro* antifungal potential against *F. oxysporum*. *Fusarium oxysporum* IB3q, used in this study, was isolated from wilting tomato plant acquired from a selected Farm at Ibadan-Ibarapa agricultural zone of Oyo State, Nigeria (Adedire *et al.,* 2023b). The pathogen caused chlorosis, necrosis vascular discolouration and wilt in inoculated tomato varieties (Ibadan-local: IBL-57 and Alausa: NGB00709) (Adedire *et al.,* 2023b). These tomato varieties were acquired from the National Horticultural Research Institute, Idi-Ishin (IBL-57), and the Seed Bank, NACGRAB (NGB00709), Ibadan. Samples of soil used for the wilt biocontrol experiment and host colonization assay were collected from Moor Plantation Farm in Ibadan (7°22'N; 3°50'E). The soil samples were bulked, steam-sterilized and analysed for its physical and chemical properties.

Preparation of microbial cell suspensions

Spore suspension of *F. oxysporum* IB3q was prepared from the mycelial growth of a 10-day-old

culture of the pathogen. Fifty milliliters of sterile distilled water was used to flush the fungal spores, with a scalpel, into a cheesecloth to collect spores as a filtrate solution. Subsequently, collected spores were quantified with a Neubauer improved, bright-line hemocytometer (HBG-Germany) (Silveira *et al.,* 2009).

Strains of antifungal *Bacillus* endophytes were harvested from a Nutrient Broth culture (24-hour-old) through centrifugation for 10 min at 5,000 rpm. Concentrated microbial cells were subsequently washed in 10 mM sodium phosphate buffer (pH 6.0) and quantified in colony forming units using spectrophotometer (OD_{600}) and corresponding spread-plate count (Cao *et al*., 2018).

Host colonization assay by endophytes

Streptomycin resistant strains of each *Bacillus* endophytes (*Bacillus* Sa08 and *Bacillus* Og04) were generated using Nutrient Agar (NA), amended with an increasing concentration (50, 100 and 150 µg/mL) of streptomycin sulphate (SS) (Abdallah *et al.,* 2020). Cultures of the endophytes were inoculated on NA amended with 50 µg/mL SS, and incubated at 30°C for 48 hours. Growing colonies were subsequently cultured on NA amended with 100 µg/mL SS. *Bacillus* strains were thereafter subcultured on NA amended with 150 µg/mL SS. Seeds of tomato (Ibadan-local and Alausa) were surface sterilized with 1.5% NaOCl for 2 min and rinsed thrice with sterile distilled water. Thereafter, seeds were bioprimed (soaked in 10^8 CFU/mL microbial cell suspension for 30 minutes) with resistant *Bacillus* endophytes (O'Callaghan, 2016) generated through successive cultivation in NA amended with streptomycin sulphate. Bioprimed seeds were sown in pots containing 5 kg pre-sterilized soil (Haddad *et al*., 2013). The study was set up as a 2-factor (tomato varieties and endophytes) factorial experiment with 6 replications. Ibadan-local and Alausa seeds treated with sterile distilled water served as control. Four weeks after sowing, rhizosphere, root, stem and leave samples were taken from treated plants for the re-isolation of resistant *Bacillus* endophytes on NA amended with 150 µg/mL SS (Singh *et al.,* 2022; Yin *et al*., 2022). The SS-resistant *Bacillus* strains isolated from each sample represented the abundance of the strain and an indication of the rate of colonization.

Systemic fusarium wilt control by antifungal *Bacillus* endophytes

An *in vivo* fusarium wilt control experiment, using endophytic *Bacillus* strains, was carried out for four months. Surface sterilized tomato seeds were bioprimed with *Bacillus* strains (Og04 and Sa08) as described for host colonization assay (O'Callaghan, 2016). Seeds were also soaked in sterile distilled water as positive control. Subsequently, treated seeds were sown (0.5 cm deep) in pre-sterilized soil at the rate of three seeds per pot. Seedlings were later thinned to one plant per pot 10 days after sowing (Borisade *et al.,* 2017). Chemical fungicide (1 mL of 0.001 g/mL carbendazim-WP50) was applied to the rhizosphere of an untreated plant set, 24 hours before pathogen inoculation (Omar *et al*., 2006). Ten-day-old tomato seedlings were inoculated with *Fusarium oxysporum* IB3q (1 mL of 10^6 conidia mL^{-1}) (Omar *et al*., 2006; Borisade *et al.,* 2017). The screenhouse study was set up as a 2 × 5 factorial experiment arranged in CRD and replicated six times. Treatments used included infected tomato treated with *Bacillus* Sa08, *Bacillus* Og04 or carbendazim, untreated infected plant set (negative control), and uninfected control plants for both tomato varieties (Ibadan-local and Alausa).

Disease assessment and performance of treated tomato plants

Disease incidence and severity in treated tomato plants were determined 5 weeks after inoculation. Disease severity index (using a wilt scale of 0-9), and control efficacy of each treatment were calculated using the modified Jaiswal *et al.* (2015) and Ganiyu, *et al.* (2016) equations, respectively, as described below:

$$\text{Disease severity index (\%)} = \sum \left(\frac{\text{Severity score} \times \text{Number of plants}}{\text{Highest severity score} \times \text{total number of plants}} \right) \times 100$$

$$\text{Percentage control efficacy} = \frac{\text{SIC} - \text{SIE}}{\text{SIC}} \times 100$$

Where SIC = Severity index of control plant (negative control); SIE = Severity index of treated plant.

The effect of seed treatment on tomato flowering, fruit number per plant and weight per fruit (g) were also determined (Azad *et al.*, 2019; Ddamulira *et al.,* 2022; Feng *et al.,* 2023).

Effect of tomato seed storage on wilt control efficacy of endophytes

The residual biocontrol effect of seed treatment with strains of antifungal endophytes (*Bacillus* Og04 and Sa08) was determined 3 and 6 months after seed treatment. Tomato seeds were bioprimed with *Bacillus* endophytes (10^8 CFU/mL) and treated seeds were dried to 12% moisture content, at 35°C with a seed-dryer (NIF/64/014) (Olosunde *et al.,* 2018). Dried seeds were subsequently stored in paper bags at 25±3.92°C. Stored seeds were retrieved at 3 or 6 months to investigate the residual biocontrol effect of seed treatment against fusarium wilt disease caused by *F. oxysporum* IB3q. The wilt-control experiment was set up as a Completely Randomized Design with 10 treatment combinations (2 tomato varieties and 5 wilt-control treatments).

Data collected were subjected to two-way analysis of variance using the statistical analysis system (SAS software, version 9.2), and means were subsequently separated according to Duncan's Multiple Range Test at 5% level of significance.

RESULTS

Bulked soil sample used for the screenhouse experiment was sufficient in essential nutritional requirements for tomato growth (Table 1). The sandy loam soil was slightly acidic, with a pH value of 6.89, available organic carbon content of 43.00 g/kg, while the nitrogen, phosphorus and potassium contents were 3.5 g/kg, 8.50 mg/kg and 0.81 Cmol/kg, respectively.

Tomato colonization pattern by antifungal *Bacillus* endophytes

The *Bacillus* species (Sa08 and Og04) successfully colonized the rhizosphere, roots, stems and leaves of both Alausa and Ibadan-local tomato varieties through seed biopriming. Therefore, colonization pattern of the selected endophytes in these tomato varieties was systemic. The endophytes however expressed different colonization levels in the rhizosphere, and tissues of treated plants (Table 2). Similar colonization trend (by endophytes) was observed in the rhizosphere and leaves of both tomato varieties. The extent of rhizosphere colonization around Alausa tomato variety by both *Bacillus* species was not significantly different. However, *Bacillus* Sa08 was more abundant (71.99×10^2 CFU/g) within the rhizosphere of Ibadan-local tomato variety. The best tissue colonization, by *Bacillus* strain Og04 (13.25×10^2 CFU/g), was observed in treated Ibadan-local tomato variety.

Systemic fusarium wilt control in tomato by antifungal *Bacillus* endophytes

The variety of tomato notably influenced the activities of wilt-control materials (*Bacillus* endophytes and carbendazim) with respect to days to flowering of tomato host (Table 3). Also, applied treatments significantly affected the flower cluster and fruit production of infected tomato plants. Seed treatment with *B. subtilis* Og04 had the highest effect on days to flowering (45.00) of *F. oxysporum* IB3q-infected Alausa tomato variety used in this study.

Table 1: Physical and chemical properties of experimental soil

Parameter	Unit	Value
pH		6.89
Available phosphorus	mg/kg	8.50
Organic carbon	g/kg	43.00
Organic matter	g/kg	39.02
Total Nitrogen	g/kg	3.50
Exchangeable bases		
Sodium	Cmol/kg	0.27
Potassium	Cmol/kg	0.81
Calcium	Cmol/kg	16.25
Magnesium	Cmol/kg	2.02
CEC	Cmol/kg	19.42
Particle size		

Sand		649.80
Silt	g/kg	184.01
Clay	g/kg	166.22
Textural class		Sandy loam

Infected, untreated Alausa plant set (negative control) produced the latest set of flower clusters (50.67 days), while flowering was slightly delayed (43.00 days) in infected, *B. subtilis* Og04-treated Ibadan-local plants. Endophyte treatment competed favourably with carbendazim ($P < 0.05$) at improving flower clusters per plant and the number of fruits produced by fungal-infected tomato plants (Table 3). Flower abscission was observed in infected, flowering tomato plants at the 7th week after sowing, especially, on *F. oxysporum* IB3q-infected, negative control plants.

Table 2: Tomato-colonization pattern of selected *Bacillus* endophytes

		Colony forming units per gram of soil/tissue (CFU/g)			
Variety	Treatment	Rhizosphere ($\times10^2$)	Root ($\times10^2$)	Stem ($\times10^1$)	Leaf ($\times10^1$)
Alausa	B1	59.88 ± 1.75^{ab}	4.22 ± 0.11^{d}	7.33 ± 0.29^{d}	6.23 ± 0.20^{b}
	B2	52.88 ± 4.70^{b}	10.10 ± 0.47^{b}	18.50 ± 0.36^{b}	14.93 ± 0.23^{a}
	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}
Ibadan-local	B1	71.99 ± 9.94^{a}	5.60 ± 0.21^{c}	9.90 ± 0.11^{c}	6.63 ± 0.15^{b}
	B2	51.69 ± 1.75^{b}	13.25 ± 0.70^{a}	27.00 ± 1.58^{a}	17.30 ± 4.43^{a}
	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}
Variety × Treatment		ns	*	*	ns

B1: *Bacillus amyloliquefaciens* Sa08; B2: *Bacillus subtilis* Og04; *: Significant; ns: not significant.
Mean values (± standard error) with similar letter(s) down the column are not significantly different at 5% level of significance according to Duncan's Multiple Range Test.

This loss of flower cuts across both wilting and some asymptomatic, infected plants (Figure 1). The most severe effect of *F. oxysporum* IB3q was, therefore, observed on fruit production by infected tomato plants. In addition to its effects on flower production and fruit development, *F. oxysporum* IB3q caused severe chlorotic and necrotic symptoms on the leaves of both Alausa and Ibadan-local tomato varieties. However, wilt control treatments (endophytes and carbendazim) significantly improved the severity of wilt disease observed in treated plants (Table 4). Disease severity index of inoculated plants ranged between 0.00% (healthy) and 43.67% (severely infected plants) on Ibadan-local variety and 0.00% to 36.65% on Alausa tomato variety. At the fifth week after sowing, only the *F. oxysporum* IB3q-infected, untreated tomato plants (negative controls) manifested significant wilt symptoms, with disease severity index of 7.32% and 6.33%, respectively.

Table 3: Effect of seed treatment with *Bacillus* species on flowering and tomato yield

Variety	Treatment	Days to flowering	Flower clusters/plant	Number of fruits/plant	Weight/fruit (g)
Alausa	B1F	46.00 ± 0.58^{cd}	15.67 ± 1.33^{ab}	25.67 ± 4.91^{ab}	18.10 ± 3.09^{ab}
	B2F	45.00 ± 1.73^{d}	12.33 ± 2.67^{ab}	19.67 ± 5.17^{b}	17.80 ± 1.90^{ab}
	CF	47.67 ± 0.33^{bc}	8.67 ± 1.33^{b}	24.33 ± 48^{ab}	17.83 ± 2.46^{ab}
	WF	50.67 ± 0.33^{a}	11.33 ± 3.67^{ab}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
	WW	48.33 ± 0.40^{b}	16.33 ± 0.67^{a}	32.67 ± 1.33^{a}	16.83 ± 2.68^{ab}
Ibadan-local	B1F	43.00 ± 0.58^{a}	13.00 ± 1.00^{ab}	26.01 ± 2.00^{ab}	24.50 ± 0.38^{a}
	B2F	41.33 ± 0.33^{ab}	14.33 ± 2.19^{ab}	25.83 ± 3.47^{ab}	24.63 ± 0.91^{a}
	CF	42.33 ± 0.41^{ab}	11.00 ± 1.00^{ab}	22.00 ± 2.06^{ab}	23.73 ± 0.22^{a}
	WF	40.67 ± 0.32^{b}	8.67 ± 3.71^{b}	2.30 ± 2.01^{c}	22.53 ± 1.53^{a}
	WW	40.33 ± 0.34^{b}	15.33 ± 1.67^{ab}	29.57 ± 5.46^{ab}	23.67 ± 0.87^{a}
Variety × Treatment		*	ns	ns	ns

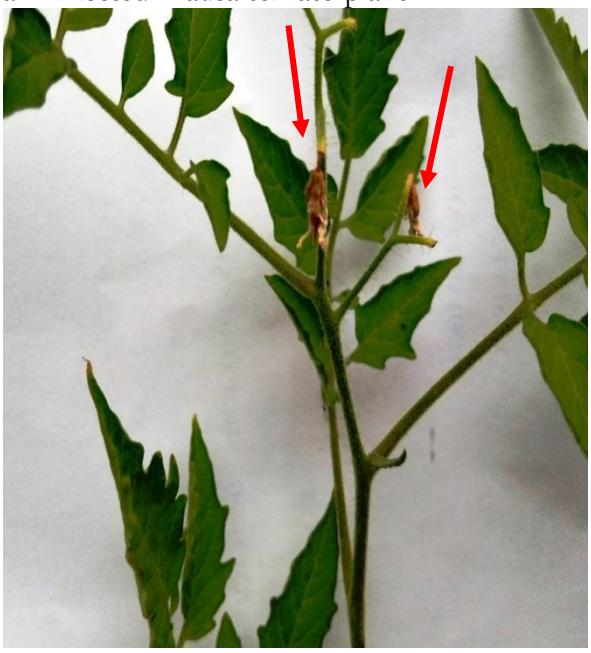
B1: *Bacillus amyloliquefaciens* Sa08; B2: *Bacillus subtilis* Og04; F: *Fusarium oxysporum* IB3q; C: carbendazim; W: sterile distilled water; *: Significant; ns: not significant.
Mean values (± standard error) with similar letter(s) down the column are not significantly different at 5% level of significance according to Duncan's Multiple Range Test.

However, chemical fungicide (carbendazim) and applied endophytes expressed similar wilt reduction potentials in treated tomato plants.

ai: Healthy Alausa tomato plant

aii: Infected Alausa tomato plant

bi: Healthy Ibadan-local tomato plant



bii: Infected Ibadan-Local tomato plant

Figure 1: Flower abscission caused by *F. oxysporum* f. sp. *lycopersici* IB3q on Alausa and Ibadan-Local tomato varieties

Table 4: Effect of seed treatment with *Bacillus* species on severity of fusarium wilt in tomato

Variety	Treatment	Disease severity index (%)			
		5WAS	7WAS	9WAS	11WAS
Alausa	B1F	0.00±0.00^{b}	13.33±0.67^{b}	13.33±0.67^{ab}	15.00±7.64^{b}
	B2F	0.00±0.00^{b}	10.00±5.77^{b}	10.00±5.77^{ab}	11.67±6.01^{b}
	CF	0.00±0.00^{b}	13.33±0.67^{b}	8.33±0.60^{ab}	8.33±0.60^{b}
	WF	7.32±6.36^{a}	36.65±8.82^{a}	26.67±6.67^{a}	35.00±2.89^{a}
	WW	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
Ibadan-local	B1F	0.00±0.00^{b}	14.00±7.02^{b}	15.00±5.00^{bc}	15.33±4.67^{b}
	B2F	0.00±0.00^{b}	13.00±6.51^{b}	16.67±3.33^{bc}	17.00±3.00^{b}
	CF	0.00±0.00^{b}	6.67±0.67^{b}	12.33±6.36^{bc}	12.67±0.62^{b}
	WF	6.33±4.91a	40.00±1.15^{a}	38.33±1.30^{a}	43.67±9.14^{a}
	WW	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
Variety × Treatment		ns	ns	ns	ns

B1: *Bacillus amyloliquefaciens* Sa08; B2: *Bacillus subtilis* Og04; F: *Fusarium oxysporum* IB3q; C: carbendazim; W: sterile distilled water; WAS: weeks after sowing; ns: not significant.
Mean values (± standard error) with similar letter(s) down the column are not significantly different at 5% level of significance according to Duncan's Multiple Range Test.

Effect of storage duration on the residual control efficacy of endophytes on treated seed

Storage duration of treated seeds significantly affected the wilt-control efficacy of antifungal endophytes used in this study (Table 5).

Table 5: Fusarium wilt control efficacy of seed treatment with *Bacillus* species on tomato plant

Variety	Treatment	Control Efficacy (%)		
		B1F	B2F	CF
Alausa	24 Hours	73.33±13.33^{a}	80.00±11.55^{a}	73.33±13.33^{ab}
	3 Months	66.62±7.99^{b}	80.51±11.61^{a}	78.79±10.91^{a}
	6 Months	61.10±8.90^{c}	72.20±9.99^{b}	76.67±6.29^{c}
Ibadan-local	24 Hours	76.78±11.66^{a}	78.44±10.79^{a}	89.00±11.00^{a}
	3 Months	70.30±5.32^{b}	69.69±7.35^{b}	89.65±5.41^{a}
	6 Months	65.53±6.38^{c}	67.77±10.58^{c}	83.87±0.57^{b}
Variety × Treatment		*	*	*

B1: *Bacillus amyloliquefaciens* Sa08; B2: *Bacillus subtilis* Og04; F: *Fusarium oxysporum* IB3q; C: carbendazim; *: Significant
Mean values (± standard error) with similar letter(s) down the column are not significantly different at 5% level of significance according to Duncan's Multiple Range Test.

Although, strains of *Bacillus* endophytes and carbendazim retained significant proportion of their wilt control potentials six months after application, different retention patterns ($P < 0.05$) were observed in Alausa and Ibadan-local tomato varieties. *Bacillus* Og04 retained 90.25% of its wilt control efficacy six months after seed treatment on Alausa tomato, while *Bacillus* strain Sa08 expressed a lower residual control efficacy. Both *Bacillus* endophytes and chemical fungicide expressed gradual reduction in wilt control potential over a period of 3-6 months after application, however, carbendazim retained over 94% of its initial control efficacy, 6 months after application.

DISCUSSION

The selected strains of antifungal endophytes used in this study, exhibited a systemic colonization pattern in host tomato varieties (Alausa and Ibadan-local). This could improve their ability to

control systemic phytopathogens like *Fusarium oxysporum*. They also proliferated within the rhizosphere of treated plants and such replication could recondition the plant-health-promoting microbial community of tomato rhizosphere (Bo *et al.,* 2022). The observed endophyte colonization pattern could be subject to availability of soil nutrients, expression of root exudates and the production of extracellular bioactive metabolites by the *Bacillus* species. Bo *et al.* (2022) reported *Bacillus* species as biocontrol bacteria, widely distributed in the rhizospheres of plants. Host plants are known to produce root exudates, rich in complex combinations of nutrients and stimulants that could influence the rhizosphere microbial community. In addition, *Bacillus* species produce spores that are resistant to environmental stress (such as drought, extreme temperature and nutrient depletion), these aid in their ability to readily colonize the soil (Bo *et al.,* 2022). *Bacillus* species have equally been reported to produce root-penetrating enzymes, as well as flagellin-masking lantibiotics, a metabolite that cloaks potential endophytes from host's defense mechanisms, thereby enhancing plant-tissue invasion (Deng *et al.,* 2019).

Due to its latent phase of pathogenesis, *F. oxysporum* could exhibit mild effect on the growth properties of infected host. This could be associated with the evasive pathogenesis of *F. oxysporum,* whereby specific virulence factors are only produced after the pathogen successfully penetrates the xylem of host plants (Ma *et al*., 2013; van der Does *et al*., 2019). Such evasive penetration (through the epidermis, cortex, casparian strip and vascular bundles) requires the expression of limited concentration of tissue-depolymerising enzymes, thereby prolonging the onset of wilt symptoms manifestation in infected hosts.
However, yield-associated parameters in tomato plants were significantly affected by *F. oxysporum* IB3q, particularly, in Alausa. After the penetration of xylem, mycelial growth of *F. oxysporum* IB3q could have severely limited water and mineral nutrient transportation within the vascular tissue, creating water stress in the host, which could have resulted in flower abortion. da Silva *et al.* (2021), in their investigation on the effect of irrigation on tomato flowering and yield, reported water stress as a significant contributor to blossom drop in tomato plants. However, fruit development was significantly improved by the application of selected strains of antifungal *Bacillus* endophytes in this study. *Bacillus* species, either as endophytes or rhizobacteria, have been reported as promising biocontrol organisms, with potentials to improve flower production, fruit setting, fruit quality and yield of tomato plants (Balderas-Ruíz *et al*., 2021). These would, in turn, improve the yield and performance of tomato in the presence biotic stress.

Fusarium oxysporum IB3q caused severe chlorotic, necrotic and wilt symptoms on Alausa and Ibadan-local tomato plants, especially, on infected, untreated plants (negative control). However, *Bacillus* species (Sa08 and Og04) expressed effective biocontrol potentials (similar to carbendazim) against fusarium wilt in tomato. *Bacillus* species produce antifungal, volatile metabolites, such as hentriacontane and 2,4-di-tert-butylphenol, which could reduce wilt severity caused by *F. oxysporum* and equally promote seedling growth in treated host (Ramírez *et al*., 2022). The reduction of wilt severity in tomato plants bioprimed with *Bacillus* Sa08 and *Bacillus* Og04 could also be as a result of mycelial inhibition and augmentation of host defense mechanisms. Antifungal, *Bacillus* endophytes produce cell wall-degrading enzymes (such as chitinases, glucanases and proteases) (Adedire *et al*., 2023b), initiate competitive exclusion of pathogens and elicit differential expression of resistance genes (Choub *et al.,* 2021; Zhou *et al.,* 2021). Such biocontrol properties could mask the effect of biotic stress on treated tomato plants, and improve the tolerance of susceptible varieties to mycopathogens. Infection severity increased up to 7 WAS in Alausa for both negative control and carbendazim treatment, and subsequently reduced at 9 WAS. This may be due to initial activation of systemic defense-related genes and enzymes in host, as well as natural drop in pathogen density within surviving, more resilient plant tissues (Tiwari *et al*., 2024; Ye *et al*., 2022).

Bacillus strains Sa08 and Og04 retained significant biocontrol potentials on treated tomato seeds over a period of six months. The endophytes were previously isolated from healthy tomato plants growing within close proximity to wilting plants; while preliminary antifungal screening was done through an improved *in vitro* assay (Adedire *et al*., 2023b). These could have contributed to the selection of endophytes with *in vivo* host-

compatibility, effective anti-fusarium activity and stability in tomato varieties, as expressed by *Bacillus* strains Sa08 and Og04.

The retention of biocontrol potential or viability of *Bacillus* species on treated seeds could also be attributed to their ability to produce spores resistant to adverse environmental conditions, initiated by seed drying before storage. In addition, the composition and diversity of spore coat structure could have encouraged the attachment of *Bacillus* spores to seed surface for an extended period. *Bacillus* species, under unfavorable environmental conditions, produce spores which are characterised by an elevated level of dipicolinic acid, low water content, thick proteinaceous coats, peptidoglycan cortex, and a balloon-like exosporium (Bressuire-Isoard *et al.*, 2018). Features like the exosporium, spore appendages and spore coat structure could contribute to spore attachment to abiotic and biotic surfaces. *Bacillus* spores germinate from viable dormancy into vegetative cells when conditions are favorable (Bressuire-Isoard *et al.*, 2018). With soil being a universal medium for microbial growth, spores on treated Alausa and Ibadan-local seeds could readily germinate (when such seeds are sown), colonize the growing tomato seedlings and confer a systemic protection against *F. oxysporum*. In addition to the systemic protection, rhizosphere colonization by the selected *Bacillus* endophytes could also suppress the ability of *F. oxysporum* to proliferate within the rhizosphere and penetrate the host roots. This multidimensional protective strategy by *Bacillus* endophytes could be responsible for the protection conferred on tomato plants (through seed biopriming) after storage.

CONCLUSION

The endophytic *Bacillus* strain used in this study colonized tomato rhizosphere and proliferated within the tissues of host plants through seed biopriming. They (endophytes) conferred systemic protection against fusarium wilt and improved the yield properties of treated plants. However, conventional determination of wilt severity, 6 weeks after sowing may not be sufficient to establish the virulence of *F. oxysporum* in tomato, as the most prominent effect of wilt infection was observed on flower development and fruit production, 8-10 weeks after sowing. Seed treatment with endophytic *Bacillus* strains could limit the laborious and time-consuming application processes (such as soil-drench, root-dip and crown-spray) associated with previously recommended biocontrol materials against fusarium wilt in tomato. Residual biocontrol potentials of the endophytes should be evaluated under field conditions and adopted to pre-treat seeds before packaging for end users.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s).

AUTHORS' CONTRIBUTIONS

All the authors were actively involved in this study. Aduramigba-Modupe, A.O. and Odeniyi, O.A. conceptualized the research work and reviewed the first draft of Manuscript. Adedire, O.M. conducted the *in vivo* antifungal assessment of *Bacillus* endophytes, systemic colonization assay and developed the first draft of manuscript. All authors read and approved the final manuscript.

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