https://dx.doi.org/10.4314/dujopas.v12i1a.23
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol.12 No. 1a March 2026*

Biocontrol Efficacy of *Pseudomonas Fluorescens* and *Bacillus Subtilis* On *Fusarium* Wilt and Yield Performance of Cowpea Varieties

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Abstract
Combined action of microbes for biological control may help improve the efficacy of these microbes action both in vitro and in vivo. This study evaluated the effects of different concentrations of Pseudomonas fluorescens and Bacillus subtilis on the growth and yield of three cowpea varieties (Aloka local, IT89KD-288 and Kanannado Brown) commonly grown in northern Nigeria under pathogen stress. The screen house experiment was carried out in a Randomized Complete Block Design (RCBD) containing 12 treatments (B1(10^9), B2 (10^8), B3 (10^7), B4 (10^6), P1(10^9), P2 (10^8), P3 (10^7), P4 (10^6), C1(10^9), C2 (10^8), C3 (10^7) and C4 (10^6) CFU/ml) with three replications. The pathogen (Fusarium oxysporum) was isolated from infected cowpea plant obtained from the field and identified based on morphological appearance. Pseudomonas fluorescens and Bacillus subtilis were isolated from healthy cowpea rhizosphere soil and were identified using biochemical test. The Screenhouse experiment confirmed the effectiveness of microbial treatments, C1 and C2 consistently enhanced plant height, leaf number, chlorophyll content, root length, flower and pod production, and yield, with soil amendment outperforming foliar spray and control. C1-treated plants achieved the highest yield (8.43 g) and root length (10.52 cm), while untreated controls recorded the poorest growth performance. Among cowpea varieties, Kanannado Brown and IT89KD-288 responded better to microbial inoculants than Aloka Local, although varietal differences were not always significant. However, high-concentration combinations of P. fluorescens and B. subtilis, especially when applied as soil amendments, were most effective in improving cowpea performance. The findings underscore the potential of these bioagents as eco-friendly alternatives to chemical control in cowpea cultivation.

Keywords: Biocontrol**,** *Fusarium* Wilt, *Pseudomonas flourescens*, *Bacillus subtilis*, Cowpea.

INTRODUCTION

Fusarium oxysporum is a globally distributed soil-borne fungus commonly found in various soil types. It typically inhabits the plant rhizosphere and displays saprophytic behavior, feeding on organic residues from a wide range of plant species (Steinberg *et al*., 2016; Das *et al*., 2021; Kutama *et al*., 2022). In addition to its saprophytic lifestyle, *F. oxysporum* is a known pathogen capable of infecting numerous plant species by entering the roots and colonizing

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the vascular system (Aliyu and Kutama, 2007). One of its most harmful effects is the induction of fusarium wilt, which can lead to severe crop damage and significant economic loss. In cowpea, fusarium wilt typically manifests through symptoms such as stunted growth, leaf chlorosis, early leaf drop, and brownish-purple discoloration on the foliage (Moses, 2008; Kumar *et al.*, 2019).

The agricultural productivity, essential for global food security, confronts ongoing challenges from diverse pathogens that can detrimentally impact crop health and yield (FAO, 2019). Among these challenges is *Fusarium oxysporum*, a notorious soil-borne pathogen recognized for causing wilt diseases in a broad spectrum of plant species, with cowpea (*Vigna unguiculata*) particularly vulnerable (Michielse and Rep, 2009; Jha *et al.,* 2020; Sampaio *et al*., 2020). Fusarium wilt poses a substantial threat to cowpea production, resulting in significant yield losses and economic hardships for farmers (Bhar *et al.,* 2021).

Responding to the escalating demand for sustainable and eco-friendly agricultural practices, researchers are increasingly exploring alternatives to conventional chemical interventions for disease control. This shift has prompted investigations into the potential of beneficial microorganisms as biocontrol agents, with *Pseudomonas fluorescens* and *Bacillus subtilis* emerging as promising candidates due to their documented capacity to suppress pathogenic activities and promote plant growth (Berg, 2009; Lugtenberg and Kamilova, 2009).

Conventional disease control methods, reliant on chemical treatments, not only have potential environmental repercussions but also raise concerns about the development of resistance in pathogens. In contrast, biological control utilizing beneficial bacteria offers a sustainable and environmentally friendly alternative (Lugtenberg and Kamilova, 2009). *Pseudomonas fluorescens* and *Bacillus subtilis* are recognized for their versatile roles, including the production of antifungal metabolites, induction of systemic resistance, and promotion of plant growth (Berendsen *et al*., 2012).

The dynamic interaction between plant roots, pathogenic microbes, and beneficial bacteria underscores the complexity of plant-microbe relationships (Berendsen *et al*., 2018). *Pseudomonas fluorescens* and *Bacillus subtilis*, identified as plant growth-promoting rhizobacteria (PGPR), have shown potential in mitigating the adverse effects of *Fusarium oxysporum* (Berg, 2009). While these bacteria exhibit varying degrees of efficacy individually, research suggests that their combined application may result in synergistic effects surpassing the benefits observed in isolation (Lugtenberg and Kamilova, 2009).

This study evaluates the biocontrol efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* on *fusarium* wilt and yield performances of cowpea varieties (Alocal local, IT89KD-288 and Kanannado Brown) using three application methods (Control, Foliar spray and Soil Amendment).

MATERIALS AND METHODS

Study Area

The research was conducted at the Botanic Garden of Plant Biology Department and Microbiology Laboratory of Microbiology Department, Federal University Dutse, Jigawa State, Nigeria. Dutse is located in the Sudan savanna Agro-ecological zone at latitude 11.7024°N, longitude 9.3340° E, and altitude of 1500m above sea level (Kowal and Knabe, 1972; Kutama *et al*., 2009).

Sample collection

Seeds of three susceptible cowpea varieties (Kanannado Brown, Aloka local and IT89KD-288) were collected from IITA, Kano station. Cowpea Plants showing *Fusarium* wilt symptom were randomly uprooted from cowpea farm and placed in labeled sterile polythene bags to prevent dehydration. The diseased cowpeas were then taken to the laboratory for etiological studies. Soil from the rhizosphere were collected from around each diseased cowpea plant root zone using a soil auger, and were placed in sterile labeled envelopes for culture, isolation, and possible identification of pathogens (Baraka *et al*., 2006; Arogundade *et al*., 2007).

Isolation and Identification of *Fusarium oxysporum*

Fungal Isolation from plant parts was conducted following the procedure of Arogundade *et al*. (2007) while isolation from soil sample was conducted following the standard procedure of soil serial dilution technique (Taylor, 1997). Pure culture was obtained following the procedure of (Bem *et al*., 2010) and identification was conducted based on microscopic appearance, including growth pattern, pigmentation/color of hyphae, septation, shape, and type of asexual spores, with reference to Nelson *et al*. (1983); Bernett and Hunter (1992); Artson (2002); Leslie and Summerell (2006).

Preparation of Conidial Suspension and Inoculation

Conidial suspension was prepared from 7 days old culture of *Fusarium oxysporum*.10ml of sterilized distilled water was added in culture plate, rubbed with sterilized spatula gently to remove the conidia, the suspension was strained with double layered muslin cloth and collected in a sterilized glass beaker. The conidial density was adjusted to 10^5 conidia per milliliter with the help of Hemocytometer (Waller *et al*., 1998; Yang *et al*., 2011).

Pathogenecity Test

Pathogenecity test was conducted by artificially inoculating seeds of susceptible cowpea variety with *Fusarium oxysporum* by placing them in 100 mL inoculum suspension (10^5 conidia per milliliter). The seeds was then dried on the blotter paper and sown (3 seeds per pot) by direct seeding in sterilized plastic pots filled with sterilized mixture of 5kg soil (Sand-loam) with fertilizer (N:P:K 15:15:15). Un-inoculated pots were served as control and this was replicated 3 times. After germination plants was monitored for the development of disease symptoms.

Isolation and identification of *Pseudomonas fluorescens* and *Bacillus subtilis*

For isolation of Rhizobacteria, the tactic followed by (Manasa *et al*., 2017) was followed. During this procedure 10g of soil from every soil sample was taken in an exceedingly cone-shaped flask of 90ml water. The sample was shaked for fifteen minutes and serial dilutions of soil suspensions were done. One millilitre of several dilutions was unfold on sterilized Petri plates of NA medium. The petri plates were incubated at temperature (28 ± 2^{o}C) for 24h. The plates were examined daily up to three days for microorganism colonies. *Pseudomonas fluorescens* and *Bacillus subtilis* were identified based on biochemical criteria (Gram staining, Oxidase, Catalase, Growth on Tryptic soy Agar, Growth on Cetrimide Agar and Growth on NA after heat shock by (Fahy and Hayward 1983; Schaad *et al*., 2001) with some modifications.

Preparation of *Pseudomonas fluorescens* and *Bacillus subtilis* Concentrations

A stock solution of 10^9CFU/ml was prepared from the pure culture of *Pseudomonas fluorescens* and *Bacillus subtilis* isolated from the soil obtained from healthy cowpea field. Optical density (OD) was measured at 600 nm using a spectrophotometer to estimate the bacterial concentration. The culture was diluted with sterile distilled water to achieved the desired

concentration (10^9CFU/ml). From the stock solution, the following concentrations were formulated from each bacterium and their combination: 10^8 CFU/ml, 10^7 CFU/ml and 10^6 CFU/ml.

Effect of *Pseudomonas flourescens* and *Bacillus subtilis* on Growth and Yield of Cowpea Varieties (Aloka local, IT89KD-288 and Kanannado Brown) Infested with *Fusarium oxysporum*

The experiment was organized in a Randomized Completely Block Design (RCBD) containing 12 treatments (B1(10^9), B2 (10^8), B3 (10^7), B4 (10^6), P1(10^9), P2 (10^8), P3 (10^7), P4 (10^6), C1(10^9), C2 (10^8), C3 (10^7) and C4 (10^6) CFU/ml) with three replication, 5kg sterilized soil (Sand-loam) with fertilizer (N:P:K 15:15:15) was placed into sterilized nylon bag, seeds were surface sterilized/disinfected for 10 minutes in 1.5% sodium hypochlorite solution (NaOCl), rinsed with sterile distilled water before planting.

Treatments were applied using three application methods (Control, Foliar spray at 2, 4, 6 and 8 weeks after germination and Soil Amendment). Data on growth parameters (Plant height, Number of leaves, chlorophyll Content and Root length) and Yield Parameters (Number of Flowers, Number of Pod and Yield) were measured at two weeks interval for twelve weeks.

Statistical Analysis

Data collected was subjected to Analysis of Variance and significant difference within the means was separated using Fisher's least significant difference test at $p \leq 0.05$ using General statistics software (Genstat 17th edition). Simple correlation analysis between the growth and yield parameters for individual trials was carried out.

RESULTS

Table 1: Essential Morphological Features of *Fusarium oxysporum*

Features	Description
Colony colour	White colour
Microconidia	Oval shaped
Macroconidia	3-5 septate
Chlamydospores	Thick-walled, spherical, Slightly on chain

SOURCE: Summerell 2019

Table 2: Biochemical Identification of *Pseudomonas fluorescens and Bacillus subtilis*

Biochemical Test	*Bacillus subtilis*	*Pseudomonas fluorescens*
Growth on NA after heat shock	Growth (Spore former)	No growth (Non-spore former)
Growth on Cetrimide agar	No growth	Creamy Colonies (Selective for *Pseudomonas spp*)
Growth on NA	Circular, white, slightly raised, wrinkled and undulate	Circular, creamy with greenish pigment, smooth and convex
Growth on TSA	Irregular, creamy, dry, opaque and flat	Circular, yellowish with green pigment, smooth, shiny and flat
Gram stain	Gram-positive rods	Gram-negative rods
Oxidase	Negative	Positive

Catalase	Positive	Positive

NA= Nutrient Agar; TSA= Tryptic Soy Agar

Effect of Different Concentrations of *Pseudomonas fluorescens* and *Bacillus subtilis* on Plant Height of Cowpea Varieties (Aloka local, IT89KD-288 and Kanannado Brown) Infested with *Fusarium oxysporum* using different application methods

The combined treatments of *Pseudomonas fluorescens* and *Bacillus subtilis* (particularly C1 and C2) consistently produced the tallest cowpea plants across all time points, with C1 (10^9 CFU/ml) showing significantly higher plant heights at 4, 8, 10, and 12 weeks after treatment (WAT). This demonstrates a strong synergistic effect at higher concentrations. Among individual treatments, B1 and P2 performed better than other single inoculations but were still less effective than the combinations. Lower concentrations (B4, P4) consistently resulted in the shortest plants, indicating a dose-dependent response.

Application method significantly influenced plant height, with soil amendment consistently out performing foliar spray and control, especially at 12WAT where it recorded the highest plant height (25.03 cm). This underscores the effectiveness of root-zone interaction for microbial activity under *Fusarium* stress. Among cowpea varieties, Kanannado Brown exhibited the highest plant height, followed by IT89KD-288 and Aloka Local, the latter showing the lowest growth likely due to higher disease susceptibility or lower treatment responsiveness.

Significant interaction effects were observed between treatment and application method from 4WAT onwards, indicating application method influences treatment effectiveness. However, treatment efficacy was generally stable across varieties, though application method and variety interactions were significant, suggesting variety-specific responses to different application techniques.

Table 3: Effect of Different Concentrations of *Pseudomonas fluorescens* and *Bacillus subtilis* on plant Height(cm) of Cowpea Varieties Infested with *Fusarium oxysporum* using Different Application Methods.

Factors	2WAT	4WAT	6WAT	8WAT	10WAT	12WAT
Treatments (CFU/ml)						
B1 (10^9)	13.37^{ab}	15.69^{bc}	18.76^{ab}	20.33^{bc}	21.67^{bc}	22.25^{abc}
B2 (10^8)	12.91^{b}	15.26^{cd}	18.13^{bc}	20.01^{c}	20.96^{cd}	21.32^{bc}
B3 (10^7)	12.54^{b}	15.03^{cd}	16.70^{de}	17.77^{f}	18.87^{ef}	19.12^{de}
B4 (10^6)	12.50^{b}	14.46^{de}	15.81^{ef}	16.61^{g}	17.64^{f}	17.82^{ef}
C1 (10^9)	13.66^{ab}	17.43^{a}	19.16^{a}	21.83^{a}	23.98 ^{a}	24.26^{a}
C2 (10^8)	13.83^{ab}	17.09^{a}	18.46^{ab}	21.13^{ab}	22.97^{ab}	23.20^{ab}
C3 (10^7)	13.70^{ab}	16.13^{b}	17.43^{cd}	18.74^{e}	20.14^{de}	20.19^{cd}
C4 (10^6)	13.61^{ab}	15.55^{bc}	17.08^{d}	18.01^{ef}	18.60^{f}	19.13^{de}
P1 (10^9)	12.33^{b}	14.49^{de}	17.23^{cd}	19.76^{cd}	20.98^{cd}	20.73^{cd}
P2 (10^8)	16.96^{a}	14.11^{ef}	16.99^{d}	18.81^{de}	20.46^{cd}	20.83^{cd}
P3 (10^7)	11.79^{b}	13.44^{fg}	15.40^{f}	16.39^{gh}	17.56^{fg}	16.90^{f}
P4 (10^6)	11.21^{b}	12.62^{g}	14.31^{g}	15.55^{h}	16.19^{g}	16.08^{f}
Application Methods						
Control	11.25^{b}	12.63^{c}	13.71^{c}	14.69^{c}	14.71^{c}	13.09^{c}
Foliar spray	13.69^{a}	15.12^{b}	17.68^{b}	19.67^{b}	21.31^{b}	22.34^{b}
Soil amendment	14.66^{a}	17.57^{a}	19.98^{a}	21.87^{a}	23.98^{a}	25.03^{a}
Varieties						
Aloka Local	10.94^{c}	14.10^{b}	15.83^{c}	17.27^{c}	18.59^{b}	19.24^{a}
IT89KD-288	12.95^{b}	14.75^{b}	16.84^{b}	18.72^{b}	20.40^{a}	20.29^{a}

Kanannado Brown	15.72[a]	16.47[a]	18.70[a]	20.24[a]	21.02[a]	20.93[a]
Interactions						
Trt x app methd	NS	<.001	<.001	<.001	<.001	0.002
Trt x Var	NS	NS	NS	NS	NS	NS
App methd x Var	NS	<.001	0.027	0.029	<.001	<.001

Means along column with different superscripts for each factor are significantly different at P<0.05 using Fitcher's LSD; NS = Not significant; WAT = Weeks after treatmet; B = *Bacillus subtilis*; P = *Pseudomonas fluorescens*; C = Combination of *Bacillus subtilis* and *Pseudomonas fluorescens;* Var = Varieties

Effect of Different Concentrations of *Pseudomonas fluorescens* and *Bacillus subtilis* on Number of Leaves of Cowpea Varieties Infested with *Fusarium oxysporum* using different application methods

Combined microbial treatments (C1 and C2) significantly enhanced leaf production across all growth stages, with C1 (10^9 CFU/ml) showing the highest leaf counts at 6, 10, and 12 weeks after treatment (WAT), indicating a strong synergistic effect between *Pseudomonas fluorescens* and *Bacillus subtilis*. C2 (10^8 CFU/ml) performed similarly well. Among single inoculants, B1 and P1 (both 10^9 CFU/ml) showed relatively high leaf numbers, especially at later stages, while the lowest concentrations (B4 and P4) consistently resulted in the fewest leaves, with P4 performing poorest.

Soil amendment was the most effective application method, producing the highest number of leaves across all time points (28.63 leaves at 12WAT), followed by foliar spray and control, suggesting that root-based application supports better microbial activity and nutrient uptake. Among cowpea varieties, IT89KD-288 had the highest leaf counts from early to late stages, followed by Kanannado Brown and Aloka Local. Although Kanannado slightly surpassed others at 12WAT, Aloka consistently had the lowest leaf numbers, indicating higher susceptibility to *Fusarium oxysporum* or weaker microbial response.

Significant interaction between treatment and application method at 6, 10, and 12WAT showed that treatment effectiveness depended on how microbes were applied. However, no significant interaction occurred between treatment and variety, suggesting all varieties responded similarly to microbial treatments. A significant interaction between application method and variety at 4, 6, and 8WAT suggests some early-stage varietal preference for specific application methods.

Table 4: Effect of Different Concentrations of *Pseudomonas fluorescens* and *Bacillus subtilis* on Number of Leaves of Cowpea Varieties Infested with *Fusarium oxysporum* using Different Application Methods

Factors	2WAT	4WAT	6WAT	8WAT	10WAT	12WAT
Treatments (CFU/ml)						
B1 (10^9)	7.556[abc]	10.85[a]	14.59[abc]	16.59[a]	20.41[ab]	21.59[a]
B2 (10^8)	7.296[abc]	10.48[ab]	13.30[b-e]	15.93[ab]	20.26[ab]	21.33[ab]
B3 (10^7)	7.037[c]	10.26[ab]	12.93[de]	15.22[abc]	16.37[d]	18.78[cd]
B4 (10^6)	7.407[abc]	9.33[b]	12.74[de]	14.26[bc]	14.85[de]	16.07[e]
C1 (10^9)	8.259[a]	11.04[a]	15.19 [a]	16.89[a]	21.52[a]	22.07[a]
C2 (10^8)	8.148[ab]	11.19[a]	14.74[ab]	17.00[a]	21.30[a]	21.89[a]
C3 (10^7)	7.852[abc]	10.30[ab]	13.52[bcd]	15.81[abc]	20.00[ab]	19.67[bcd]
C4 (10^6)	7.593[abc]	9.93[ab]	13.37[b-e]	15.30[abc]	16.44[cd]	17.78[de]
P1 (10^9)	7.111[c]	10.37[ab]	13.37[b-e]	15.96[ab]	20.00[ab]	20.48[abc]
P2 (10^8)	7.185[bc]	10.07[ab]	13.19[cde]	15.96[ab]	18.59[bc]	19.56[bcd]
P3 (10^7)	6.926[c]	9.48[b]	12.93[de]	14.30[bc]	15.93[d]	16.63[e]
P4 (10^6)	6.889[c]	10.07[ab]	11.93[e]	13.89[c]	13.22[e]	13.70[f]

Application Methods						
Control	6.519^{c}	8.70^{c}	9.98^{c}	8.58^{b}	5.93^{c}	3.93^{c}
Foliar spray	7.463^{b}	10.39^{b}	14.53^{b}	18.60^{a}	22.29^{b}	24.83^{b}
Soil amendment	8.333^{a}	11.75^{a}	15.94^{a}	19.59^{a}	26.51^{a}	28.63^{a}
Varieties						
Aloka Local	6.204^{c}	8.65^{c}	11.61^{c}	13.77^{b}	16.95^{b}	19.41^{a}
IT89KD-288	6.954^{b}	12.69^{a}	16.19^{a}	19.20^{a}	18.61^{a}	18.46^{a}
Kanannado Brown	9.157^{a}	9.51^{b}	12.64^{b}	13.81^{b}	19.16^{a}	19.52^{a}
Interactions						
Trt x app methd	NS	NS	0.035	NS	<.001	<.001
Trt x Var	NS	NS	NS	NS	NS	NS
App methd x Var	NS	0.002	<.001	<.001	NS	NS

Means along column with different superscripts for each factor are significantly different at P<0.05 using Fitcher's LSD; NS = Not significant; WAT = Weeks after treatment; B = *Bacillus subtilis*; P = *Pseudomonas fluorescens*; C = Combination of *Bacillus subtilis* and *Pseudomonas fluorescens*; Var = Varieties

Effect of Different Concentrations of Pseudomonas fluorescens and Bacillus subtilis on Chlorophyll Content (SPAD) of Cowpea Varieties Infested with Fusarium oxysporum using different application method

Combined treatments of *Pseudomonas fluorescens* and *Bacillus subtilis* at higher concentrations (C1 and C2) led to increased chlorophyll content throughout the growth period, with C2 (10^{8} CFU/ml) consistently showing high SPAD values at 4, 6, 8, and 12 weeks after treatment (WAT). This highlights a potential synergistic effect in maintaining chlorophyll levels under *Fusarium* stress.

Among single inoculants, higher concentrations of *P. fluorescens* (P1 and P2) showed favorable chlorophyll levels at early to mid-stages, with P1 peaking at 2WAT (26.31) and P2 recording the highest at 6WAT (21.04). In contrast, lower concentrations (B4 and P4) consistently showed the lowest SPAD values, indicating reduced microbial effectiveness at suboptimal doses.

Application methods significantly influenced chlorophyll content, with soil amendment consistently producing the highest SPAD values across all stages (peaking at 2WAT with 26.09), followed by foliar spray. Controls showed the lowest and steadily declining chlorophyll levels, confirming the effectiveness of microbial treatments, particularly via soil application, in sustaining photosynthetic activity under pathogen pressure.

Varietal responses varied slightly. IT89KD-288 showed the highest SPAD value at 8WAT (17.78), Kanannado Brown peaked at 4WAT (24.22) and 6WAT (20.74), while Aloka Local had its highest value at 2WAT (25.47) and maintained steady levels afterward. However, differences among varieties were mostly not statistically significant at later stages, suggesting a generally uniform treatment response.

No significant interaction was observed between treatment and either application method or variety, indicating consistent microbial effects across treatments. However, a significant interaction between application method and variety at 4, 6, 8, and 12WAT revealed that cowpea varieties responded differently to application methods during vegetative and maturity phases.

Table 5: Effect of Different Concentrations of *Pseudomonas fluorescens* and *Bacillus subtilis* on Chlorophyll Content (SPAD) of Cowpea Varieties Infested with *Fusarium oxysporum* using Different Application Methods.

Factors	2WAT	4WAT	6WAT	8WAT	10WAT	12WAT
Treatments (CFU/ml)						
B1 (10^9)	25.47^{ab}	22.96^{bc}	19.37^{abc}	15.69^{ab}	13.65^{a}	11.35^{bcd}
B2 (10^8)	25.61^{ab}	23.75^{ab}	19.92^{ab}	15.35^{ab}	12.38^{ab}	12.33^{abc}
B3 (10^7)	25.33^{ab}	23.79^{ab}	18.71^{bc}	15.60^{ab}	13.17^{ab}	11.75^{a-d}
B4 (10^6)	24.80^{ab}	21.75^{c}	19.01^{abc}	14.44^{b}	11.47^{b}	10.31^{d}
C1 (10^9)	24.97^{ab}	24.66^{a}	20.56^{ab}	16.20^{ab}	12.15^{ab}	12.74^{ab}
C2 (10^8)	24.98^{ab}	24.01^{ab}	20.38^{ab}	16.91^{a}	14.03^{a}	13.21^{a}
C3 (10^7)	24.49^{ab}	24.31^{ab}	19.87^{ab}	15.64^{ab}	13.16^{ab}	12.89^{ab}
C4 (10^6)	24.26^{b}	23.21^{abc}	18.77^{bc}	15.03^{b}	12.73^{ab}	12.07^{a-d}
P1 (10^9)	26.31^{a}	23.06^{bc}	19.90^{ab}	16.16^{ab}	12.38^{ab}	10.68^{cd}
P2 (10^8)	25.63^{ab}	22.81^{bc}	21.04^{a}	15.53^{ab}	12.19^{ab}	11.76^{a-d}
P3 (10^7)	25.81^{ab}	22.97^{bc}	19.13^{abc}	14.63^{b}	12.70^{ab}	10.85^{cd}
P4 (10^6)	25.43^{ab}	23.56^{ab}	17.54^{c}	14.60^{b}	12.12^{ab}	10.63^{cd}
Application Methods						
Control	24.60^{b}	22.05^{c}	17.74^{b}	13.00^{b}	7.64^{b}	5.52^{b}
Foliar spray	25.09^{b}	23.14^{b}	20.43^{a}	16.33^{a}	15.05^{a}	14.77^{a}
Soil amend	26.09^{a}	25.01^{a}	20.39^{a}	17.12^{a}	15.34^{a}	14.86^{a}
Varieties						
Aloka Local	25.47^{a}	23.29^{b}	21.84^{a}	14.07^{b}	12.60^{a}	11.62^{a}
IT89KD-288	24.04^{b}	22.70^{b}	15.97^{c}	17.78^{a}	12.64^{a}	11.93^{a}
Kanannado Brown	26.27^{a}	24.22^{a}	20.74^{b}	14.59^{b}	12.79^{a}	11.60^{a}
Interactions						
Trt x app methd	NS	NS	NS	NS	NS	NS
Trt x Var	NS	NS	NS	NS	NS	NS
App methd x Var	NS	<.001	0.035	<.001	NS	0.049

Means along column with different superscripts for each factor are significantly different at P<0.05 using Fitcher's LSD; NS = Not significant; WAT = Weeks after treatment; B = *Bacillus subtilis*; P = *Pseudomonas fluorescens*; C = Combination of *Bacillus subtilis* and *Pseudomonas fluorescens;* Var = Varieties

Effect of Different Concentrations of *Pseudomonas fluorescens* and *Bacillus subtilis* on Root Length and Yield Parameters of Cowpea Varieties Infested with *Fusarium oxysporum* using different application methods

Combined microbial treatments at higher concentrations (C1 and C2) produced superior results across all measured parameters. C1 (10^9 CFU/ml) recorded the longest root length (10.52 cm), highest number of flowers (3.85), pods (2.70), and yield (8.43 g), closely followed by C2 (10^8 CFU/ml). These findings confirm a strong synergistic effect between *Pseudomonas fluorescens* and *Bacillus subtilis* at optimal concentrations in enhancing plant performance and suppressing *Fusarium oxysporum*.

High-concentration single inoculants (B1, B2, P1, P2) also showed relatively good results, with comparable root lengths (9.44–10.00 cm) and yields (6.98–7.58 g), though not significantly different from one another. In contrast, low-concentration treatments (P4, B4, P3) recorded the poorest performance, particularly P4, which showed the shortest root length (7.52 cm), fewest flowers (0.81), lowest pod number (0.41), and minimal yield (1.38 g). This underscores the concentration-dependent effectiveness of microbial inoculants.

Application method had a significant impact on all parameters, with soil amendment consistently yielding the best results—root length (12.55 cm), number of flowers (4.43), pods

(3.20), and yield (9.37 g). Foliar spray was moderately effective, while the untreated control showed the poorest performance. These findings highlight the superior efficacy of soil-based delivery in enhancing plant growth and disease resilience.

Among cowpea varieties, Kanannado Brown exhibited the best overall performance, with the longest roots (9.90 cm) and highest yield (7.81 g), followed by IT89KD-288. Aloka Local had the lowest yield (3.24 g), although there were no significant varietal differences in flower and pod counts. The better performance of Kanannado may be linked to its stronger inherent resistance or better responsiveness to microbial treatments.

Significant interaction effects were found between treatment and application method for number of flowers, pods, and yield, indicating that the success of microbial treatments depended on how they were applied. Additionally, application method and variety interactions were significant for root length and yield, suggesting that different cowpea varieties responded differently to application techniques. However, treatment and variety interactions were not significant, indicating a generally uniform response to treatments across varieties.

Table 6: Effect of Different Concentrations of *Pseudomonas fluorescens* and *Bacillus subtilis* on Root Length(cm) and Yield Parameters of Cowpea Varieties Infested with *Fusarium oxysporum* using Different Application Methods.

Factors	R/LENGTH (cm)	N/FLOWERS	N/PODS	YIELD(g)
Treatments (CFU/ml)				
B1 (10^9)	10.000^{ab}	3.444^{abc}	2.333^{a}	7.489^{ab}
B2 (10^8)	9.704^{abc}	3.407^{bc}	2.222^{a}	7.575^{ab}
B3 (10^7)	8.815^{cde}	2.370^{d}	1.259^{bc}	4.952^{cd}
B4 (10^6)	8.000^{ef}	1.815^{e}	0.852^{c}	3.222^{d}
C1 (10^9)	10.519^{a}	3.852^{ab}	2.704^{a}	8.432^{a}
C2 (10^8)	10.370^{ab}	3.926^{a}	2.481^{a}	8.340^{a}
C3 (10^7)	9.333^{bcd}	3.000^{c}	2.296^{a}	5.929^{bc}
C4 (10^6)	8.852^{cde}	2.222^{de}	2.074^{ab}	4.608^{cd}
P1 (10^9)	9.444^{bcd}	3.519^{ab}	2.185^{ab}	7.074^{ab}
P2 (10^8)	9.481^{a-d}	3.593^{ab}	2.148^{ab}	6.984^{ab}
P3 (10^7)	8.593^{de}	2.037^{de}	1.111^{c}	3.931^{d}
P4 (10^6)	7.519^{f}	0.815^{f}	0.407^{c}	1.375^{e}
Application Methods				
Control	4.528^{c}	0.796^{c}	0.463^{c}	1.574^{c}
Foliar spray	10.583^{b}	3.278^{b}	1.852^{b}	6.533^{b}
Soil amend	12.546^{a}	4.426^{a}	3.204^{a}	9.371^{a}
Varieties				
Aloka Local	8.361^{b}	2.870^{a}	1.583^{b}	3.242^{c}
IT89KD-288	9.398^{ab}	3.037^{a}	1.833^{ab}	6.428^{b}
Kanannado Brown	9.898^{a}	2.593^{a}	2.102^{a}	7.807^{a}
Interactions				
Trt x app methd	NS	<.001	0.024	<.001
Trt x Var	NS	NS	NS	NS
App methd x Var	<.001	NS	NS	<.001

Means along column with different superscripts for each factor are significantly different at P<0.05 using Fitcher's LSD; NS = Not significant; WAT = Weeks after treatment; B = *Bacillus subtilis*; P = *Pseudomonas fluorescens*; C = Combination of *Bacillus subtilis* and *Pseudomonas fluorescens;* Var = Varieties; Trt = Treatments

DISCUSSION

The agricultural productivity, essential for global food security, confronts ongoing challenges from diverse pathogens that can detrimentally impact crop health and yield (FAO, 2019). Among these challenges is Fusarium oxysporum, a notorious soil-borne pathogen recognized for causing wilt diseases in a broad spectrum of plant species, with cowpea (Vigna unguiculata) particularly vulnerable (Michielse and Rep, 2009; Jha *et al.,* 2020; Sampaio *et al*., 2020). Fusarium wilt poses a substantial threat to cowpea production, resulting in significant yield losses and economic hardships for farmers (Bhar *et al*., 2021).

The plant growth-promoting activity of PGPR bacteria has been reviewed in detail by Santoyo *et al.,* (2016) and (Xia *et al.,* 2015; Santoyo *et al.,* 2016). *Bacillus* and *Pseudomonas* species are widely known as invaluable resources for plant growth promotion and the suppression of disease symptoms (Sundaramoorthy & Balabaskar, 2013; Chaves-López *et al.,* 2015). Over the last few decades, several studies have reported on the beneficial aspects of Bacillus spp. as biocontrol and biofertilizer agents; e.g., *Bacillus licheniformis, Bacillus subtilis, Bacillus cereus, Bacillus pumilus, and Bacillus amyloliquefaciens* (Pane & Zaccardelli, 2015; Han *et al.,* 2016).

The plant growth promotion and other beneficial aspects of *Bacillus* strains can be attributed to their ability to enhance the production of phytohormones such as auxin (IAA), and gibberellic acid (Gamalero & Glick, 2011).

The present research was conducted to evaluate the biocontrol efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* on *fusarium* wilt and yield performances of cowpea varieties (Alocal local, IT89KD-288 and Kanannado Brown) using three application methods (Control, Foliar spray and Soil Amendment).

The results demonstrate that the combined application of *Pseudomonas fluorescens* and *Bacillus subtilis*, especially at higher concentrations (e.g., C1: 10^9 CFU/ml), significantly enhanced various growth parameters of cowpea varieties infested with *Fusarium oxysporum*, including plant height, number of leaves, chlorophyll content (SPAD values), and root length. Soil amendment consistently emerged as the most effective application method across all parameters, underscoring the role of root-zone colonization in nutrient uptake and sustained plant-microbe interaction. These findings align with earlier studies by Akhtar *et al.,* (2010), Samavat *et al.,* (2011) and El-Mohamedy *et al.,* (2015), who emphasized the benefits of co-inoculation in promoting plant growth, nodulation, leaf development, and disease suppression over single-strain treatments.

The superior performance of the combination treatments is attributed to synergistic mechanisms such as auxin and siderophore production, phosphorus solubilization, systemic resistance induction, and enhanced antioxidant activity (Leveau & Lindow, 2005; Van Loon & Glick, 2004; El-Sharkawy *et al.,* 2015; Wong *et al.,* 2021; Sivasakthi *et al.,* 2014). The dose-dependent improvements observed reinforce the importance of microbial density and compatibility, as also reported by Zhu *et al.,* (2020) and Siddiqui *et al.,* (2001). While varietal differences were evident, particularly in root length (with Kanannado Brown showing the longest roots), the positive impact of microbial inoculants was largely consistent across cowpea genotypes. These findings validate the co-inoculation of compatible PGPR strains like *B. subtilis* and *P. fluorescens* as a robust bioformulation strategy to boost plant vigor, enhance

photosynthetic efficiency, and improve root architecture in *Fusarium*-challenged environments.

The combined application of *Pseudomonas fluorescens* and *Bacillus subtilis*, especially at higher concentrations (e.g., C1: 10^9 CFU/ml), significantly enhanced the reproductive parameters of cowpea varieties infested with *Fusarium oxysporum*, including the number of flowers, number of pods, and overall yield. Soil amendment consistently outperformed foliar spray and control across all yield components, producing the highest flower count (4.43), pod number (3.20), and yield (9.37g), highlighting the crucial role of application method in maximizing the effectiveness of PGPR treatments. The superior performance of the C1 and C2 treatments reflects the synergistic action of the two strains, likely driven by multiple plant growth-promoting mechanisms such as the synthesis of auxins, cytokinins, gibberellins, siderophores, antibiotics, phosphorus mobilization, and systemic resistance induction (Sivasakthi *et al.,* 2014).

These results support earlier findings by Akhtar *et al.,* (2010) and Akhtar & Azam (2014), who demonstrated that mixed inoculation enhanced pod formation and flower development under *Fusarium* stress in legumes. The significant interactions observed between treatment and application method, as well as between variety and method, emphasize the importance of optimizing both microbial formulation and delivery strategy.

The higher yields recorded in Kanannado Brown (7.81 g) suggest a potential genotype-specific compatibility with microbial inoculants and/or stronger resilience to *Fusarium* infection. Additionally, the dose-dependent trend observed where higher microbial concentrations consistently produced superior reproductive outcomes and lower concentrations (e.g., P4) resulted in minimal yield (1.38 g) confirms the importance of microbial density in bio formulation efficacy. These findings align with Estevez de Jensen *et al.,* (2002), who reported increased yield through Bacillus-Rhizobium co-inoculation under *Fusarium* stress, and further reinforce the value of deploying compatible, high-density PGPR combinations via soil amendment for improving reproductive output and productivity in disease-prone agro-ecosystems.

CONCLUSION

Application of higher concentrations of *P. fluorescens* and *B. subtilis* significantly improved cowpea Plant height, number of leaves, chlorophyll content, root length, number of flowers, number of pods, and overall yield in the presence of *Fusarium oxysporum*, indicating that concentration is a critical factor in the efficacy of these biocontrol agents. Soil amendment proved to be the most effective application method, resulting in significantly enhanced growth and yield parameters compared to foliar spray and untreated control. This suggests that root-zone delivery of microbial inoculants allows for better colonization and interaction with the plant rhizosphere.

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