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Scientific Research

Performance of Natural Antagonists and Commercial Microbiocides towards *in Vitro* Suppression of Flower Bed Soil-Borne *Fusarium oxysporum*

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ABSTRACT

***Fusarium oxysporum* is the causal agent for wilt diseases of many major ornamental and horticultural crops. In this study, we plated a local cut flower grower's soil, with a persistent history of *Fusarium* wilt of scented stock, *Matthiola incana* but not the lettuce rotational crop. This yielded culture plates with characteristic pink to carmine red fungi, together with a mixed bacterial population, a percentage of which was visibly antagonistic to the Fusarium. Using molecular analyses via Polymerase Chain Reaction (PCR) assays, we identified that *Fusarium oxysporum*, *Fusarium culmorum*, *Fusarium equiseti* and *Fusarium venenatum* were prevalent in the soil. The co-habiting bacterial colonies that exhibited strong antagonistic activity (zone of clearance) towards the soil fungi corresponded to *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa* species. Our results arising from an *in vitro* study involving Kirby-Bauer disc-diffusion agar assays, coupled with bio-imaging software techniques demonstrated that the three native soil bacteria were effective inhibitors of all *Fusarium* species tested, while *Bacillus subtilis* exhibited the highest antagonism towards the *Fusarium oxysporum*. Bioassay tests of micro-biocides Prestop (*Gliocadium catenulatum*), Serenade Max (*Bacillus subtilis* QST713) and commercial seaweed extract, AlgiVyt suppressed *in vitro* growth of *Fusarium oxysporum* infecting the scented stock flower to a greater extent, whilst fresh aqueous extracts of garlic (*Allium sativum*) and meadowsweet (*Filipendula ulmaria*) flowers were ineffective towards soil pathogen suppression. This scoping study offers cut flower growers additional options of tapping into populations of antagonistic bacteria found in soil persistently infected with the opportunistic soil phytopathogen *Fusarium oxysporum*, affecting cut flower crops, such as *M. incana*.**

KEYWORDS

***Fusarium oxysporum*; Cut Flower Wilt Disease; Natural Antagonistic Bacteria; *Bacillus subtilis*; Natural and Commercial Plant Extracts; Microbiocides**

1. Introduction

Commercial floriculture worldwide is characterized by high investment and stringent quality demands which often imply high pesticide usage. Of the many soil pathogens, in cut flower business, *Fusarium*, *Pythium and Rhizoctania* are the three major soil-borne pathogens that cause basal stem and root rot, root and crown rot and root and stem rot respectively [1]. The most frequent soil pathogen encountered by the floriculturists in Britain, Ireland and Europe-wide is *Fusarium oxysporum* which has led to serious crop and economic losses in poly tunnels, nurseries and green house crop production systems [2]. Limitations of pesticide usage, regulations, restricted availability and options render them unpopular in terms

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of their environmental, human and soil health impact [3] and cost implications to the greenhouse industry. Chemical fungicides are also increasingly proving either inadequate or ineffective to cope with the severity of *Fusarium* wilt disease losses frequently encountered by many flower growers. This hiatus has led the greenhouse industry and the allied researchers to seek sustainable biological alternatives [4] such as microbial and (medicinal) plant resources as either soil drenches or root dips amongst other conservative crop rotational strategies against *Fusarium* wilt of ornamental crops.

In cut-flower production, new antifungal biocides of bacterial (*Pseudomonas*, *Bacillus*) and fungal (e.g. *Trichoderma*, *Gliocadium*) genus have been recently registered in Europe, but have yet to be adopted on a large scale by floricultural farmers in Ireland as effective antagonists against soil-borne pathogens. Literature evidence highlights the recent trend towards finding natural biological remedial solutions for soil fungal phytopathogens that may exist in their own complex habitat [5]. Data are also gathering momentum that either fresh or spent compost dressings and in tandem with antagonistic *Gliocadium* spp., and mycoparasitic *Clonostachys rosea* fungi themselves could act as disease suppressants [6]. Prompted in part by our recent research with natural plant resources in search of complimentary therapy antibiotics for medical pathogens [7-10], we extended our scouting expedition for biological control agents relevant to specific plant diseases such as *Fusarium* wilt. Options involving manipulation of specific antagonists/antibiotics isolated from *Fusarium* suppressive soils for disease management are a widely practised classical methodology for soil-borne fungal infection containment. However, in the light of a paucity of suppressive soil sites locally, the isolation, and evaluations of efficacy of naturally evolved antagonists of soil-borne fungal pathogens in local floriculture soil infested with *Fusarium* spp. are practicable and prudent.

We present an *in vitro* scoping study examining antagonistic performance of culture suspensions of native antagonistic bacteria isolated from horticultural soils with chronic *Fusarium* infection, where stock (*Matthiola incana*) crops are alternated with lettuce (*Lactuca sativa*) as rotational cropping history. Together with the natural biota, commercial microbiocides and aqueous extracts of garlic and meadowsweet flowers were evaluated for their efficacy against soil-borne *Fusarium* species, also isolated from the same soil.

2. Experimental

2.1. Isolation of Antagonistic Bacteria and Fungi from Flower Bed Soil

Naturally occurring bacteria and wild type *Fusarium* species were all isolated (in this study) from the soil of a poly tunnel house of a local flower grower (Greenisland Flowers, Co. Armagh, Northern Ireland, BT62 1XB). Soil bacterial isolates were grown in LB broth and the fungi on potato dextrose (PD) media. The putative colonies of bacteria (on LB agar plate) and fungi (on potato dextrose agar) were single colony purified. The bacteria that grew alongside the fungal colonies that exhibited antagonistic zone were specifically picked out. The adjacent fungal colony to the bacterial inhibitory zone was initially assessed for their presumptive *Fusarium* genera wild type culture characteristics such as colour and hyphal features (white-mild pink hypal extensions, intense carmine red pigmentation). A *Fusarium oxysporum* (16602) culture was obtained from LGC Standards, Teddington, Middlesex, UK.

2.2. Molecular Identification of Antagonistic Bacteria and Fungi in the Flower Soil Bed

The identity of the putative antagonistic bacteria adjacent to individual *Fusarium* morpho-types was further confirmed by means of Polymerase Chain reaction (PCR) assays. For *Fusarium* genera wild type identification, a loop full of fungal hyphae from the culture plate was carefully transferred to 50 µl of sterile nucleic acid free water in an Eppendorf, vortexed vigorously, and an aliquot subjected to rapid DNA extraction and amplification procedures described by the manufacturer (RedExtract, Sigma) using 18S rDNA universal ITS regions: ITS1 (TCC GTA GTT GAA CCT GCG G) as forward and ITS 4 (TCC TCC GCT TAT TGA TAT GC) as reverse primers [11]. For bacterial molecular identification, colonies were purified by single colony changes on LB agar. DNA was obtained using a sterile wire loop, and a single pure colony was suspended in 50 µl in an Eppendorf and held in a boiling water bath (95˚C, 10 min) and immediately cooled in an ice bath (4˚C, until use) and the bacterial DNA obtained in the above manner was added to the PCR reaction buffer containing universal bacterial 16S rDNA forward and reverse primers [12], 27F (5'-AGA GTT TGA TC[A/C] TGG CTC AG-3') and 1492R (5'-G[C/T]T ACC TTG TTA CGA CTT-3'). The primers were added to the reaction mixture containing bacterial DNA (~15ng) in a total volume of 50µl of MasterMix (Invitrogen). In both cases, the PCR reaction cycles were set at 94˚C, 3 min (one hold), 94˚C, 30s, 53˚C, 30s, 72˚C, 1 min (35 cycles), followed by a final extension step of 10 min, 72˚C. Resulting sequences were confirmed from chromatogram analysis and confirmed sequences were compared with those stored in the GenBank using the BLASTn alignment software (http://www.blast.genome.ad.jp/).

2.3. Plant Extracts and Commercial Materials

For bioassays [7] in this study, herbaceous specimen *Fi-*

lipendula spp (meadowsweet) was freshly collected from a local forest (with the kind permission of Ulster Folk and Transport Museum, Northern Ireland); Garlic (*Allium sativum*) was purchased from a retailer (Sainsburys, UK); Prestop (*Gliocadium catenulatum*), and Serenade Max (*Bacillus subtilis* QST713) were gifted for research purposes (Greenisland Flowers, Co. Armagh, Northern Ireland, BT62 1XB); and a seaweed commercial extract Algavyt Zn/Mn, supplied by Agriges (www.agriges.com) was obtained from our agrochemicals stocks held at SAFSD, Agri-Food Biosciences Institute, Newforge Lane Belfast, BT9 5 PX, Northern Ireland, UK). A fresh stock solution of 0.2% w/v of natural extracts (garlic and meadowsweet) was made just before use. The wettable powder (WP) formulations of commercial microbiocides (Serenade Max and Prestop) were freshly prepared and when required, they were re-suspended in sterile water to yield a final concentration of 1.9×10^8 colony forming units (cfu) ml^{-1} respectively. A working stock of 1.2×10^8 cfu's ml^{-1} of freshly growing log phase culture suspensions of native bacterial isolates were obtained from this study and the above preparations were used for bioassays.

2.4. Antagonism Assays Using Antibiotic (Kirby-Bauer) Disc Assays

From stock *Fusarium* species cultures 6mm diameter plugs were excised and transferred individually to the Cartesian co-ordinate centre of four directional segments marked previously using a fine-tip marker pen of fresh plates of PDA, incubated for 3 days at ambient temperature to facilitate natural contours of hyphal growth to advance. The culture plates were examined using a binocular microscope and the outline of the perimeter of the hyphae was carefully traced by marker pen. At a distance of 10 mm to the "east and west" of the culture, a small mark was made with the marker pen. Three sterile discs (Mast Group Ltd, Merseyside, UK) were saturated with a total of 60 µls of an assay component and the discs were stacked and placed at the 10 mm distance mark. At intervals of 3 days the extent of the hyphal growth (mm) was marked. The area covered by each culture was measured and recorded using the bio-imaging technique [13] to assess inhibition/growth promoting properties of novel agents on moulds, using the Autochemisystem UVP Bioimaging system (UVP Products, Cambridge, UK), supported by LabWorks software package. Compared to recording clearance zones by bacterial lawn, the irregular contours of fungal growth is problematic in antifungal assays. Initially the instrument was set on white light and to an exposure ratio of 490:500, with a constant focus of 47% calibration. Using the "Area Density" tool, (in pixels) the entire "area of individual plate" was measured first, recorded, and then using freehand "draw" tool followed on by the "area of irregular contours" of fungal growth co-integrated within the Cartesian coordinates estimated to reflect inhibition effects (clearance zones) as seen like an undulated coastline.

2.5. Statistical Analyses

The arbitrary ratio, *fungal growth*, was calculated for each treatment as the ratio of total surface area occupied by fungal growth/total surface area of the plate. Statistical analyses were performed employing the student t-test and values >0.05 (5%) was considered not significant.

3. Results and Discussion

3.1. Isolation and Identification of Fusarium Species and Antagonistic Native Bacteria

The soil samples were obtained from a local flower grower's farm (Greenisland Flowers, Co Armagh). The farm has a series of polytunnels for mainly floriculture and the soil was collected from notorious tunnel house no. 31 which has a history of *Fusarium* infestation (**Figures 1(A)** and **(B)**) of the cut flower scented stocks (*Matthiola incana*). With the above in mind, the soil sampling was carried out closest to the plug plant roots and when the scented stock plug transplants were 3 - 4 weeks old. The culture plate technique yielded three bacterial colonies that were adjacent to the fungal colonies in the same plate and exhibited antagonism towards the mycota. The bacteria were carefully isolated, purified on several rounds of plating and individual pure colonies were identified using molecular analyses involving PCR of the DNA and gene sequencing followed by bioinformatic query (Blastn) for its taxonomic tree similarity and identity of the bacterial genomes held in the databases (GenBank). The results indicated that there were three specific bacteria viz., *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa* exhibiting antagonism in the same PDA plate carrying presumptive *Fusarium* spp. For purposes of this scoping study, PCR analyses of the DNA extracted from culturable *Fusarium* spp., followed by bioinformatic queries of fungal genome databases was adequate. Molecular analyses results indicated that *Fusarium oxysporum*, *F. venenatum F. culmorum* and *F. equiseti* were the dominant *Fusarium* populations prevalent in the tunnel house no.31 soils; Such findings corroborate molecular investigations [14] to discriminate *Fusarium* spp., that are common soil inhabiting phytopathogens of small grain wheat and barley crops. Results based on traditional microbiology (colour and hyphal features in the culture plate—white-mild pink hypal extensions, intense carmen red pigmentation) revealed that the *Fusarium oxysporum* comprised at least six morphological sub-types. It was beyond the scope of this study to dissect molecular taxonomy of *Fusarium* spp, corresponding antagonistic bacteria for their range, diversity,

molecular population dynamics (metagenomics) and phylogeny at this stage. High-throughput next generation sequencing and metagenomic analyses in real-time [e.g. 15,16] in tandem with our on-going direct soil DNA extractions (unpublished) employing RedExtract (Sigma-Aldrich), Maxwell 16 DNA extractor kits (Promega) protocols would ascertain a holistic understanding of microbial community distributions in the flower bed soil.

3.2. *In Vitro* Antagonism of Native Soil Bacteria towards *Fusarium* Species

The 3 soil bacteria isolated were effective inhibitors of all *Fusarium* species tested. The bio-imaging software generated Cartesian calculus of area of the zone of clearance (inhibition), and the variations thereupon due to irregular fungal growth contours and (**Figure 1(F)**) converted the digital pixel values into percentages (ca. ~3700 of the area equalled one percent). This method gives a more real-effect estimate of the *in vitro* inhibition performance, *i.e.* mean inhibitory potential (*mip*%) of the antibiotic disc assays than manual analogue bidirectional values obtained for proximal and distal points of the growth of the test fungi against the antagonistic challenger in the Petri plate bioassays. For better clarity and reference, the wild type *Fusarium* spp (CF68A, CF71A, CF106A and FS2E) and native bacteria (M17 and Beech) were given identity tags (shown in parantheses) corresponding to the precise location it was originally isolated within the soil beds. The *in vitro* growth of *Fusarium oxysporum* wild types isolated in this study (**Figure 2**) from cut flower growers soils was suppressed strongly by *Bacillus subtilis*-M17 (*mip* ca. 41.6%); *Bacillus subtilis*-Beech (*mip* ca. 45.1%) and both native *B. subtilis* isolates displayed antagonism (*mip* ca. 41.5%) towards the type strain *Fusarium oxysporum* (16602). The other two non-pathogenic bacilli, *Bacillus amyloliquifaciens* (*mip* ca. 45.5%) and *Paenibacillus polymyxa* (*mip* ca. 61.4%) was slightly less effective than others. Using similar strategy of *in vitro* scoping via antibiotic disc assays, we recently reported an example of phytopathogen infested soil serving as a prospective reservoir for isolating disease suppressants; we isolated two forest soil inhabiting *Bacillus* spp and *Clitocybe nebularis*, a forest fairy ring macrofungi as potent biological agents exhibiting strong suppression against the devastating soil oomycetes dieback phytopathogen *Phytophthora ramorum* prevalent in the local forests with a history of dieback outbreaks [17].

3.3. Antagonism and Synergism of Native Bacteria and Fungi in Soil Microcosms

Our data from the co-culture plate carrying native bacteria and fungi when overviewed in the light of the intensive farming practices of flower growers and their traditional crop (lettuce) rotation that reign in new races of *Fusarium* spp., [18,19] further heightens the need to have a closer look at the natural antibiosis between soil fungal pathogens and native bacteria in soils with a persistent history of *Fusarium* wilt disease. Interestingly, we observed that whilst *M. incana* plants were seriously affected by *Fusarium oxysporum* infection (**Figure 1**), the leafy vegetable plants had no visible symptoms of *Fusarium* wilt (data not shown) due to *Fusarium oxysporum* f.sp. *lactucae*, the latter of which was not detected by culture plate and sensitive PCR assays; this may be due to fungal-bacterial consortia promoting lettuce growth [20]. To this end, it is important to also bear in mind that bacterial ectosymbionts and endosymbionts of *Fusarium*

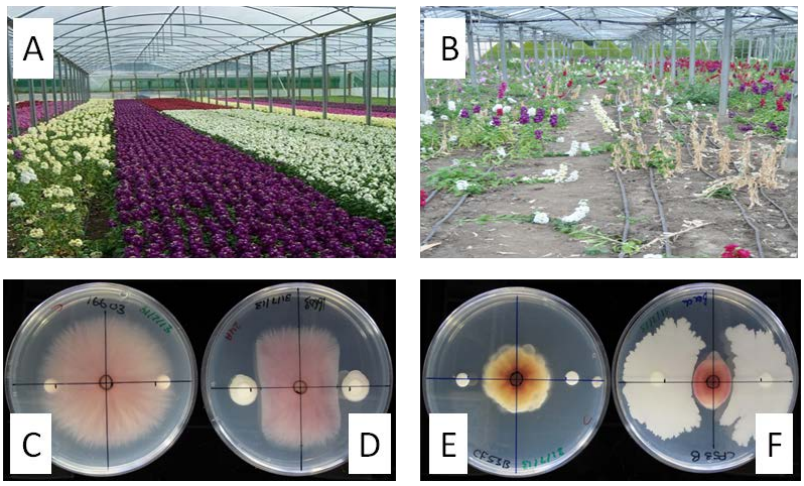

Figure 1. Biological control of *Fusarium* spp by commercial and natural microbiocides. Polytunnel greenhouse grown healthy scented cut flower stock in *Fusarium* suppressed soil (A) and symptomatic *Fusarium* wilt disease infested soil (B). Kirby-Bauer Petri plate assays (C)-(F) demonstrating antifungal activity against *Fusarium oxysporum*: untreated control (C), Prestop and Serenade Max (D), AlgiVyt Mn/Zn (E), *Bacillus subtilis* (F).

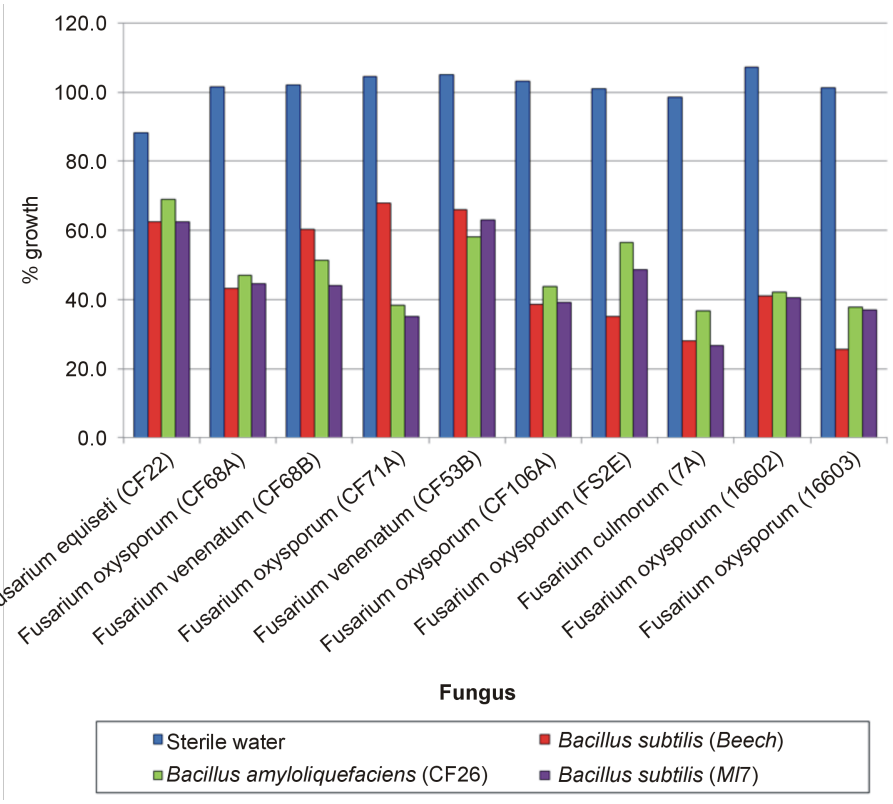

Figure 2. Inhibitory effect (%) on growth of Fusarium cultures by antagonistic bacteria.

oxysporum hyphae are commonly encountered in the rhizosphere and mycosphere zones of intense pathogen-host plant relationships [21,22], and the complex mechanisms of antibiosis, signalling, exudates, metabolite exchange, chemotaxis, microbial cross-talking (quorum sensing), symbiotic, host pathogenicity/virulence [23] determination.

3.4. Antifungal Efficacy of Commercial Microbiocides versus Native Bacteria

Commercial microbiocides (Serenade Max, Prestop), herbaceous aqueous extracts of garlic and meadowsweet flowers and a biostimulant seaweed extract commercial product (Algavyt Mn/Zn) and culture suspension aliquots of native bacilli *Bacillus subtilis* wild type isolates obtained from this study) were examined (**Figures 1(C)-(F)**) for their *in vitro* efficacy of inhibition of *Fusarium* spp prevalent in the soil beds of flower crops. Results indicated (**Table 1**) that in general the commercial microbiocide formulations supplied at 0.2% (ca. 1.9×10^8 cfu ml^{-1}) to the antibiosis assay discs showed a greater mean inhibitory potential (~30.8%) towards *in vitro* growth suppression of all *Fusarium* spp., isolated from the flower bed soil. Serenade Max (*Bacillus subtilis* strain QST 173) and Prestop (*Gliocadium catenulatum*) displayed impressive growth reductions (*mip* ca. ~17% ± 2.0%) and (*mip* ~ca. 27% ± 2.0%) of *Fusarium oxysporum* strain CF106A (isolated from the flower soil bed). The inhibitory potential of these two microbiocides were almost similar to those of the growth inhibition (*mip* ca ~16% + 2.0%) evinced when *Fusarium oxysporum* type strain (16602) was challenged. The culture suspension aliquots of the two antagonistic bacilli isolated from the *Fusarium* infested soil were also effective inhibitors causing (*mip* ~ca. 46% ± 2.0%) *in vitro* growth reduction of all *Fusarium* spp., tested; interestingly, the above broad spectrum antifungal effects of native soil bacilli was in relative terms only approximately ~15% less potent than the commercial biocides. The native antagonistic bacilli isolated in this study also exhibited about half the specific inhibitory potential (~ca 38% ± 2.0%) of that of Serenade Max in terms of *in vitro* growth reduction of the phytopathogenic *Fusarium oxysporum* CF106A strain isolated in this study. Given that the native antagonistic bacteria in this study emerged from a chronic *Fusarium* wilt disease prevalent soil, our data suggests that they possess resilient antifungal combative competitiveness and warrants investigations to ascertain their persistence.

The subdued antifungal *in vitro* performance of the native isolates compared to the commercial microbial product counterparts may be due to a number of reasons, including the lower viable bacterial load ($cfuml^{-1}$) in the assay disc, the nature, range and strength of the antibiotic

Table 1. Efficacy of commercial antagonistic agents challenged against flower soil bed prevalent *Fusarium* species.

	F equiseti CF22	*F venenatum* CF53B	*F culmorum* 7A	*F oxysporum* A1	*F oxysporum* 16602	Mean (%) Inhibition of *Fusarium* sp
Formulations						
Serenade Max[1]	50.0	45.7	25.0	17.8	16.4	30.8
Prestop[2]	37.9	44.3	46.3	27.7	24.5	31.1
AlgaVyt Mn/Zn[3]	134.5	48.6	40.0	64.3	59.1	69.3
Native bacteria						
Bacillus subtilis (Beech) WT[4]	62.5	66.1	27.9	38.5	40.9	47.1
Bacillus subtilis (M17) WT[4]	62.4	62.9	26.7	39.1	40.4	46.3
Plant extracts						
Allium sativum[5]	92.2	83.7	94.2	81.4	90.3	88.2
Filipendula[6]	100	96.3	86.0	95.9	93.2	94.2
Control*	100	100	100	100	100	0
S. E. ±	2.8	2.3	2.9	2.7	2.4	-

Efficacy values shown correspond to percentage growth of the fungal colony on the Petri plate following the challenge by the antagonistic agent held on the disc. [1]*Bacillus subtilis* strain QST173; [2]*Gliocadium catenulatum*; [3]Commercial biostimulant seaweed extract (Agriges); [4]Wild Type native bacilli isolated from flower bed soils in this study; [5]Garlic; [6]Meadowsweet flowers. Serenade Max, Prestop and AlgaVyt Mn/Zn were arbitrarily added at 0.2% of the commercial formulation. In the case of the WP formulations—Serenade Max and Prestop, the bacterial and fungal viable counts respectively and the suspension cultures of our bacilli isolates set at ca. 1.0×10^8 cfuml^{-1}.

constituents in them. The superior performance of biological control of *Fusarium* wilt disease severity in begonia flower plants by Serenade Max and Prestop has been reported before [24] via *in vitro* plate assays and potted plants in greenhouse. Soil-borne *Fusarium* wilt is among the most difficult to control and regarded as most destructive diseases of many major ornamental, horticultural crops. Owing to *Fusarium* species niche role as natural harmless saprophytes enriching soil biogeochemistry, they possess indefinite survival in the soil. However, they may turn pathogenic due to human intervention such as ornamental crop practices and affect the yield and quality of the produce. Introducing either a large population of antagonistic "naturalised" bacteria (e.g. drenching treatments) such as those evaluated in this study or commercial alien microbial formulations (e.g. Serenade Max) are likely to proliferate as dominant and active suppressors of soil *Fusarium* species in the scented flower plant root/ rhizosphere. The inhibition of the soil-borne phytopathogen by the native bacteria or the microbiocide propagules would rely on survival/fitness to combat in the rhizosphere of either Stock flower or alternate lettuce plant roots and the exudates, chemistry, molecular and physiological responses would also influence the overall *in vivo Fusarium* wilt disease control efficacy.

3.5. Antifungal Efficacy of Seaweed Biostimulants and Herbaceous Plant Extracts

We tested commercial seaweed Algavyt Mn/Zn (Agriges) that is usually applied for crop nutrient biostimulant (and dressing supplement for Zinc/Manganese) against its antifungal efficacy (if any) upon soil phytopathogenic *Fusarium* spp., isolated in this study. The phycoproduct AlgiVyt produced an overall *Fusarium* growth suppression (*mip* ~ca 69 ± 2.0) in our *in vitro* bioassays (**Table 1**). To our knowledge, the antifungal potential of this algal product is not known or reported before. However, as seen in visual evaluation (**Figure 1(E)**), the commercial algal cocktail (containing approximately 5% ca. millimolar levels of Zn/Mn supplements) of Algavyt Mn/Zn when assayed (at 0.2%), may have contributed to the fungicidal effects normally associated with commercial Mancozeb metallic fungicides apart from underwhelmed antimicrobial potentials of alga themselves. For instance, some of the components in seaweeds are well documented [25] to act as crop plant biostimulants, fungal elicitors etc among other desirable traits such as suppressants of biotic and abiotic stresses for crop protection and crop production. Seaweeds are yet untapped natural resources in the sea-locked British Isles and remains an attractive proposition to the local farmers for biological means of soil infested fungal pathogen management. In our bioassay, the aqueous garlic (*Allium sativum*) and local forest meadowsweet (*Filipendula* spp) flower extracts respectively were ineffective towards all *Fusarium* challenged in the antibiotic disk assay (**Table 1**). Our results were in contrast to the knowledge upon garlic extracts, in that they are normally regarded as strong candidate for broad spectrum phytopathogen suppression applications, and due its potency attributed to its soluble volatiles [26]. Likewise a number of herbaceous and medicinal plant resources [27-29] have been demonstrated

to have potent antifungal activity, but our antibiotic disc assays showed little or no impact on the *in vitro* inhibition of *Fusarium* spp growth (*mip* ~ca 89 ± 2.0) with either garlic or the medicinal plant extracts.

3.6. Chemical Constituents of Antagonistic *Bacillus* spp.

Our results indicated that the culture suspensions of *B. subtilis* were potent suppressants of *Fusarium* spp. *In vitro* antagonism of *Bacillus* spp. is widely known [30] and linked to high microbial antibiosis, and activity found in soils with high organic matter content such as in horticulture composted beds and in woodlands. The antibiotic production of the *Bacillus subtilis* in natural environments is well documented. *Bacillus* spp., offers additional scope as natural reservoir for biocides; they are a heterogeneous group of Gram-positive, facultative anaerobic, endospore-forming natural soil inhabitant bacteria producing unique enzymes, rare antibiotics against a range of fungal and bacterial plant pathogens [31] and known to produce potent antifungal lipoprotein (leu-7) biosurfactant components [e.g. 32] in the cell-free spent culture medium. In the light of the above, the diffusate from either the native wild type *Bacillus subtilis* or that of commercial strain Serenade Max soaked assay discs in the Kirby-Bauer assay plates can be expected to possess antifungal components that may act against phytopathogens. It is difficult to qualitatively ascertain at present, that our native bacilli isolates produced similar or variant antibiotic components than those reported before. However, the strong *in vitro* antifungal efficacies of native bacilli against *Fusarium oxysporum* growth are encouraging to pursue further greenhouse and field scale evaluations.

4. Conclusion

In conclusion, the *in vitro* antagonistic performance of the soil bacterial co-habitants, viz., root colonising non-pathogenic bacilli tested in this study appear promising and are complemented by antifungal activities of commercial bacterial/fungal microbiocides and seaweed extracts towards soil-borne phytopathogens. In contrast to a traditional search for antibiosis in suppressive soils, our present study on antagonism between pathogenic fungi by suppressant cohabitant native bacteria in a "sick" soil itself served to encourage further considerations for natural combinatorial options (e.g. mixed inocula of native antagonistic bacteria and the commercial microbiocides) for biological solutions for soil pathogen control. In the light of the widespread recurrent incidents of *Fusarium* wilt disease in cut flower plants, the development of a sustainable eco-friendly multiple disease control strategy is urgently needed. This scoping study offers the local flower growers lacking typical wilt suppressive soils in their narrow farmland range available, the potential to tap the habitat's own antagonistic natural resources potential for reducing the exacerbation of soil-borne opportunistic phytopathogen *Fusarium oxysporum*. Future emergent evidence and innovation for ecologically viable plant and soil disease management strategies including development of alternative non-chemical greener products [33] warrant impact assessments in greenhouse and field trials with a range of potential biological control agents for successfully combating the devastating wilt disease and to sustain the floriculture industry.

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