Effects of Endophytic Non-Pathogenic *Fusarium oxysporum* V5w2 and Mulching on Field-grown Tissue Culture Banana Plants and Root Infection by *Radopholus similis*

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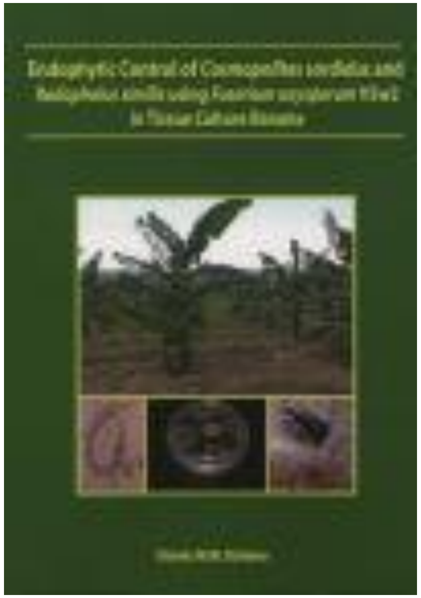
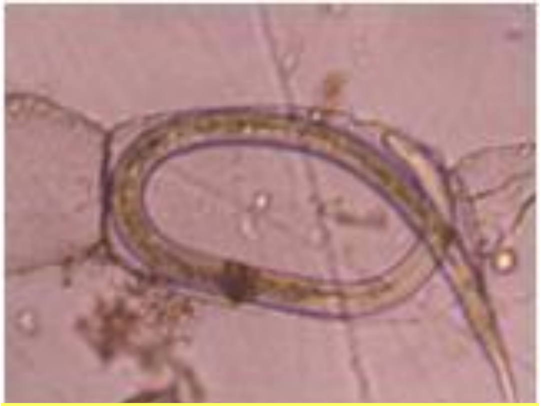


Product of Collaborative Research

- International Institute of Tropical Agriculture (IITA-Uganda)
- Wageningen University and Research Centre, The Netherlands

1. Background

- The root burrowing nematode *Radopholus similis* has been a serious pest of banana in East Africa.
- Crop losses as high as 50% through toppling have been reported.
- Control had focused towards clean planting materials produced by Tissue Culture (TC).
- Conversely, TC also eliminates endophytic protective microbes, producing plants that are more vulnerable to nematode attack [1].



Radopholus similis

Toppled banana plant

- Focus shifted towards re-introduction of protective endophytic microbes into TC banana, as a Biological Control component of IPM [1].

- *Fusarium oxysporum* V5w2, a fungus, was mentioned to have Biological Control potential against *R. similis* and other nematodes [2].

- Mechanisms of action included induced resistance, antibiosis, and direct parasitism [1].

***F. oxysporum* V5w2 (middle)**

TC banana plant

- In the year 2006, *F. oxysporum* was considered ready for adoption by banana farmers in East Africa [2].

- This was an Award Winning Science: *"The material, intentionally infected with a beneficial fungus, offers protection against pests and diseases and has been delivered to farmers via an innovative public-private partnership* [3]*"*

- However, this process still required verification on performance as a biological control agent, in relation to biotic and abiotic factors, under different agro-ecosystems.

Endophyte-enhanced banana tissue culture plant produced in the nursery of Tissue Culture company [2].

2. The Experiment

2.1. Goal

To provide data for the approval of transfer of *F. oxysporum* V5w2-treated banana plants to farmers.

2.2. Objective

To elucidate the combined effect of *F. oxysporum* V5w2 and mulch against *R. similis* in tissue culture banana plants under field conditions.

2.3. Hypothesis

Combined application of resistance-inducing *F. oxysporum* V5w2 and mulch suppresses *R. similis* and promotes growth and yields of tissue culture banana plants under field conditions.

3. Materials and Methods

- On-station at the International Institute of Tropical Agriculture (IITA), Sendusu Field Station, Uganda.
- Between October 2006 and February 2009.
- Three factor (2 × 2 × 2) RCBD
- *Fusarium oxysporum* V5w2 **(+/-)**; *Radopholus similis* **(+/-)**; Mulch **(+/-)**
- 8 treatments, 3 replicates (blocks), n=20 plants
- One crop cycle (i.e. the inoculated mother plant only).No repeat in space or time.

4.0. Results

4.1. Effects of *Radopholus similis* treatment on field-grown tissue culture banana plants

Stage	Parameter	Control	*R. similis*	Effect (%)	Response	Interpretation
Preharvest	Plant height	**56.0 a**	**50.2 b**	**-11.6**	Negative	Plant growth suppression
(After 125 days)	Number of leaves	6.3	6.1	-3.3	*ns**	.
	Leaf length	**60.2 a**	**54.5 b**	**-10.5**	Negative	Plant growth suppression
	Leaf width	**29.4 a**	**26.9 b**	**-9.3**	Negative	Plant growth suppression
	R. similis	**33 b**	**1333 a**	**97.5**	Positive	Successful infection
	Helicotylenchus	58	93	37.6	*ns*	.
	Meloidogyne	1558	340	-358.2	*ns*	.
	Dead roots	**1.3 b**	**4.7 a**	**72.3**	Positive	Root damage
	Necrosis	**0.9 b**	**1.4 a**	**35.7**	Positive	Root damage
	Feeder roots	2.3	2.4	4.2	*ns*	.
Harvest	Time	514	522	1.6	*ns*	.
(After 175 days)	Plant height	271	239	-11.8	*ns*	.
	Girth	**48.6 a**	**42.6 b**	**-12.3**	Negative	Plant growth suppression
	Diameter	21.5	19.3	-10.2	*ns*	.
	Leaves	3.7	3.7	0.0	*ns*	.
	Hands	**6.5 a**	**5.6 b**	**-13.8**	Negative	Plant growth and yield suppression
	Fingers	96.5	75.4	-21.9	*ns*	.
	Bunch weight	11	8.6	-21.8	*ns*	.
	**Not statistically significant*					

Conclusion 1: *Radopholus similis* damages roots leading to the suppression of growth and yields of field-grown tissue culture banana plants.

4.2. Effects of *Fusarium oxysporum* V5w2 treatment on field-grown tissue culture banana plants

Stage	Parameter	Control	*F. oxysporum* V5w2	Effect (%)	Response	Deductions
Preharvest	Plant height	**56.6 a**	**50.7 b**	**-10.4**	Negative	Plant growth suppression
(After 125 days)	Number of leaves	**6.4 a**	**5.9 b**	**-7.8**	Negative	Plant growth suppression
	Leaf length	**60.2 a**	**54.6 b**	**-9.3**	Negative	Plant growth suppression
	Leaf width	**29.5 a**	**26.8 b**	**-9.2**	Negative	Plant growth suppression
	R. similis	558	863	54.7	*ns**	.
	Helicotylenchus	30	118	293.3	*ns*	.
	Meloidogyne	383	1418	270.2	*ns*	.
	Dead roots	2.9	2.9	0.0	*ns*	.
	Necrosis	5.4	9.2	70.4	*ns*	.
	Feeder roots	2.4	2.3	-4.2	*ns*	.
Harvest	Time	**546 a**	**490 b**	**-10.3**	Negative	Plant growth suppression
(After 175 days)	Plant height	257	259	0.8	*ns*	.
	Girth	46.4	45.9	-1.1	*ns*	.
	Diameter	20.7	20.5	-1.0	*ns*	.
	Leaves	3.7	3.6	-2.7	*ns*	.
	Hands	6.2	6.1	-1.6	*ns*	.
	Fingers	87.9	87.7	-0.2	*ns*	.
	Bunch weight	9.9	10.3	4.0	*ns*	.
	**Not statistically significant*					

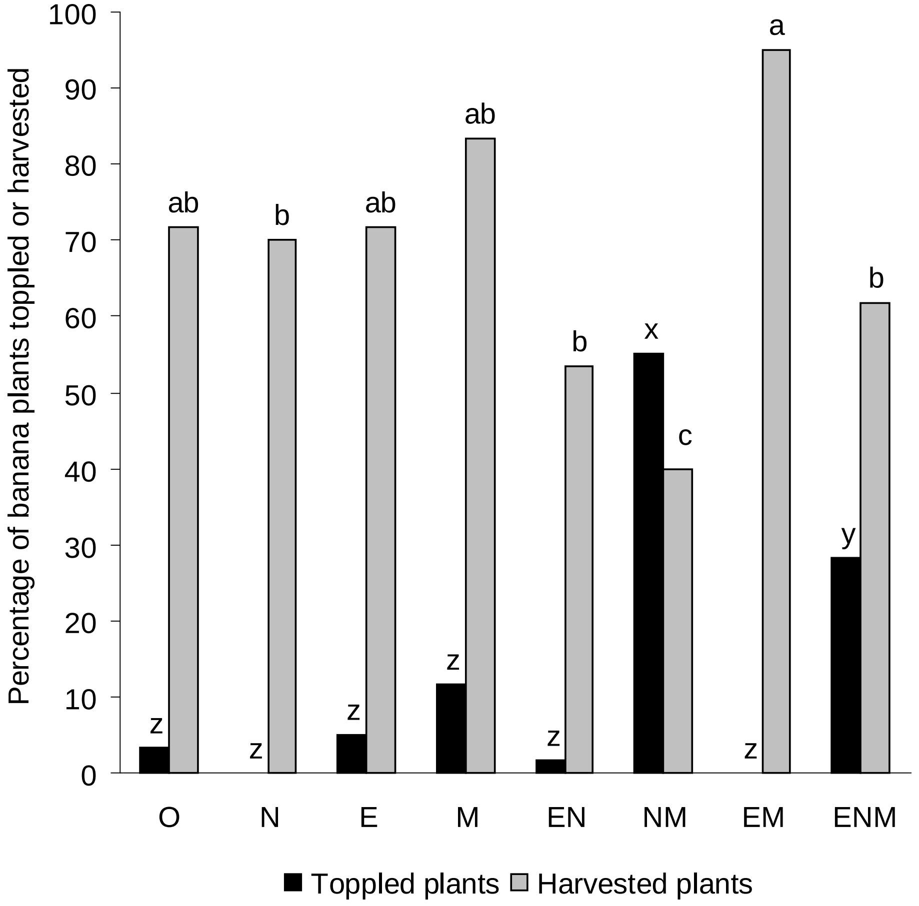
Conclusion 2: *Fusarium oxysporum* V5w2 suppresses the growth of field-grown tissue culture banana plants.

4.3. Effects of mulch application on field-grown tissue culture banana plants

Effects of mulch application on field-grown tissue culture banana plants						
Stage	**Parameter**	**Control**	**Mulch**	**Effect (%)**	**Response**	**Deductions**
Preharvest	Plant height	**47.8 b**	**58.4 a**	**22.2**	Positive	*Plant growth improvement*
(After 125 days)	Number of leaves	**5.9 b**	**6.4 a**	**8.5**	Positive	*Plant growth improvement*
	Leaf length	**52.9 b**	**61.9 a**	**17.0**	Positive	*Plant growth improvement*
	Leaf width	**26.1 b**	**30.2 a**	**15.7**	Positive	*Plant growth improvement*
	R. similis	983	440	-55.2	*ns**	.
	Helicotylenchus	110	40	-63.6	*ns*	.
	Meloidogyne	278	1583	469.4	*ns*	.
	Dead roots	3.9	2	-48.7	*ns*	.
	Necrosis	8.3	6.1	-26.5	*ns*	.
	Feeder roots	2.3	2.4	4.3	*ns*	.
Harvest	Time	514	519	1.0	*ns*	.
(After 175 days)	Plant height	**230 b**	**284 a**	**23.5**	Positive	*Plant growth improvement*
	Girth	**40.5 b**	**51.5 a**	**27.2**	Positive	*Plant growth improvement*
	Diameter	**18.6 b**	**22.5 a**	**21.0**	Positive	*Plant growth improvement*
	Leaves	**3.1 b**	**4.2 a**	**35.5**	Positive	*Plant growth improvement*
	Hands	**5.5 b**	**6.8 a**	**23.6**	Positive	*Plant growth and yield improvement*
	Fingers	**72.5 b**	**102.4 a**	**41.2**	Positive	*Plant growth and yield improvement*
	Bunch weight	**7.7 b**	**12.4 a**	**61.0**	Positive	*Plant growth and yield improvement*
	**Not statistically significant*					

Conclusion 3: Mulch improves growth and yields of field-grown tissue culture banana plants.

4.4. Combined effects of *R. similis*, *F. oxysporum* V5w2 and mulch on field-grown tissue culture banana plants



Radopholus similis (N) causes toppling of banana plants when mulch (M) is applied resulting in fewer harvested plants.

Ref: N vs. NM

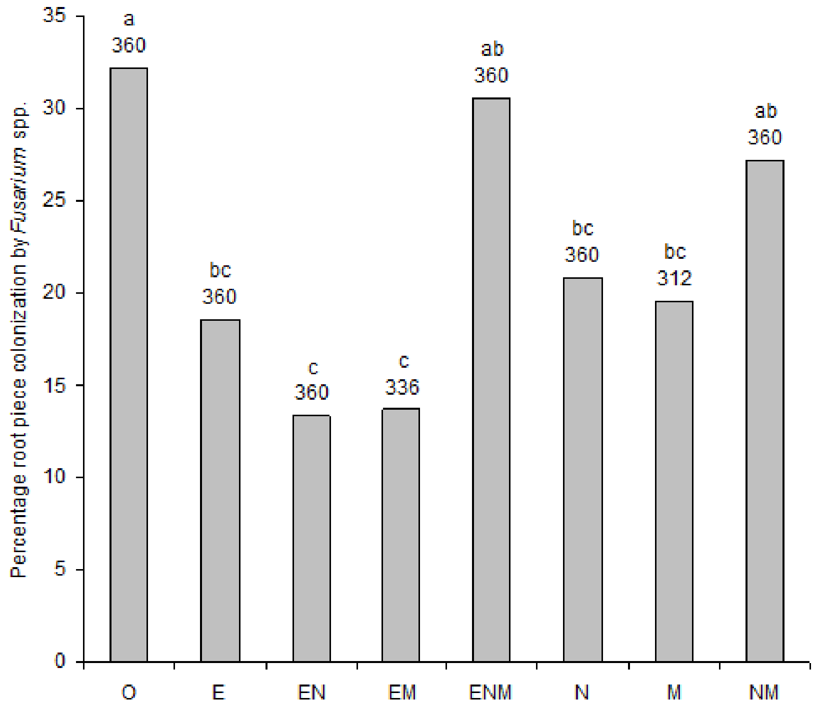
Fusarium oxysporum V5w2 (E) reduces the rate of toppling in nematode-infected and mulched banana plants.

Ref: NM vs. ENM

Conclusion 4: Large size of mulched banana plants makes them topple more when their roots are infected by *R. similis*; but the application of *F. oxysporum* V5w2 reduces their size and hence the chances of toppling.

4.5. Detection of *F. oxysporum* V5w2 in banana roots

This is considered crucial in associating the observed effects of *F. oxysporum* V5w2 on banana plants.

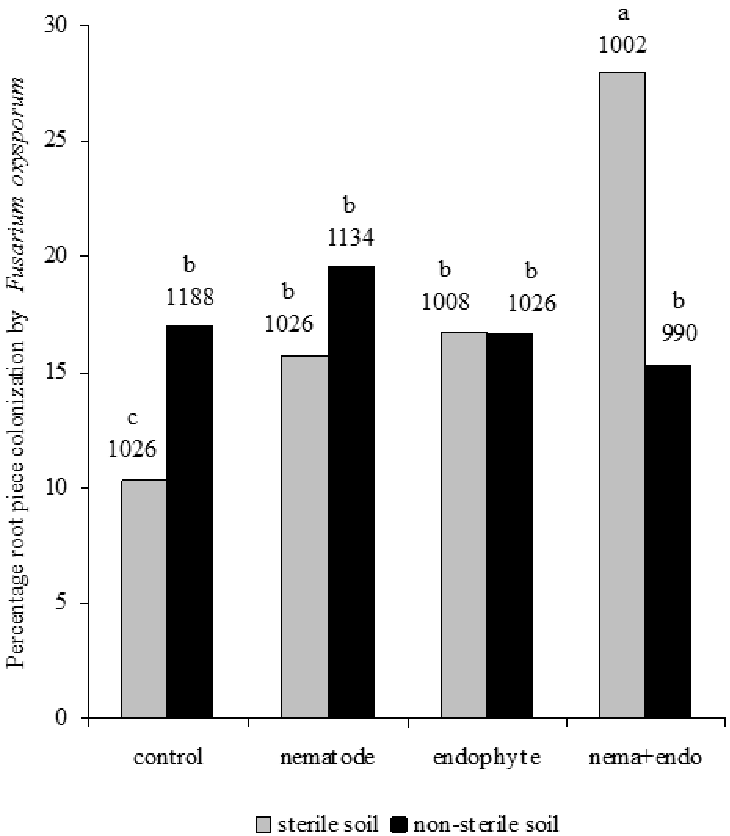


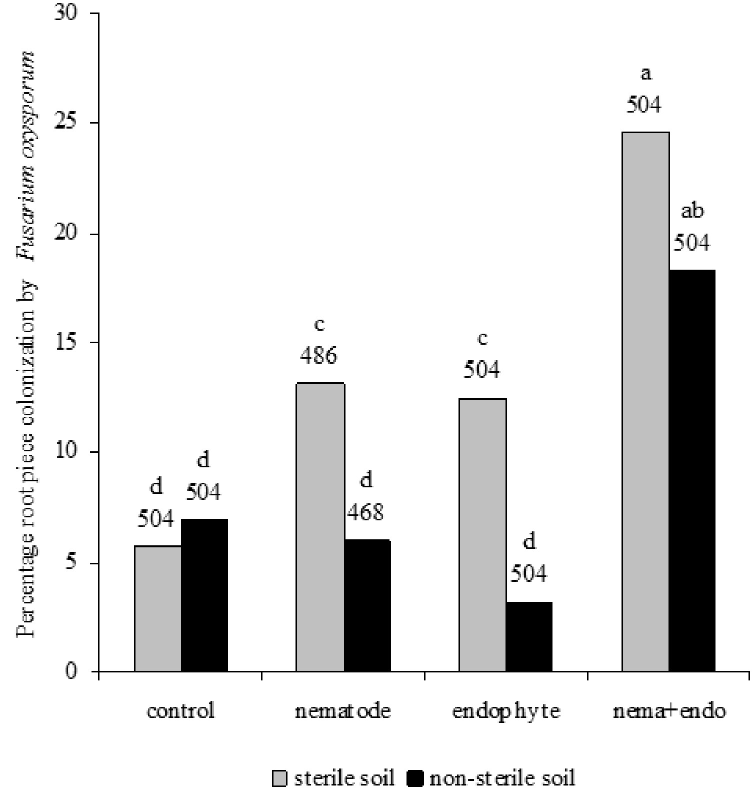
***Fusarium* spp. and other endophytes from banana roots grown under non-sterile soil conditions**

Conclusion 5: Trends in data on percentage root piece colonization were inconsistent and hence unreliable, because wild-type *F. oxysporum* V5w2 was utilized, and there was heavy contamination with *Fusarium* and non-*Fusarium* endophytes.

4.6. Data from own supportive experiments

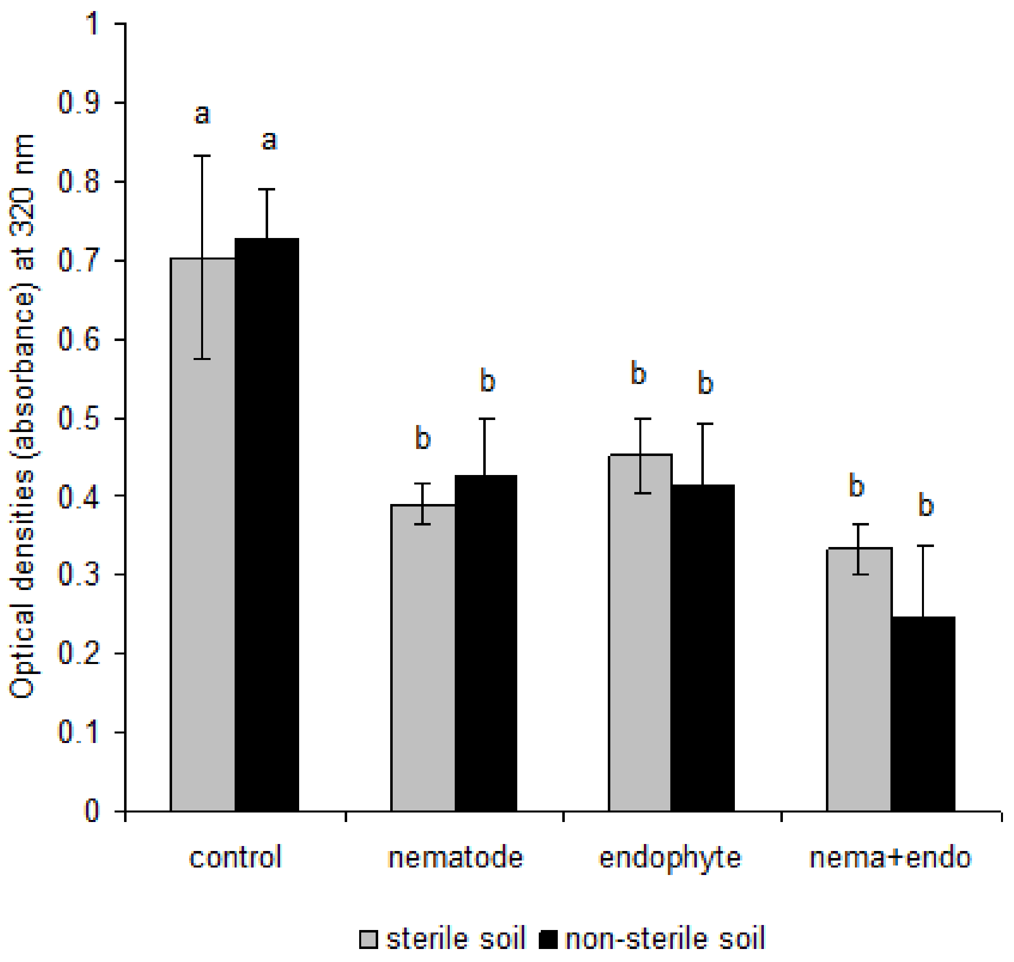
4.6.1. Percentage root piece colonization by *F. oxysporum* V5w2 in controlled screenhouse experiments with potted plants.





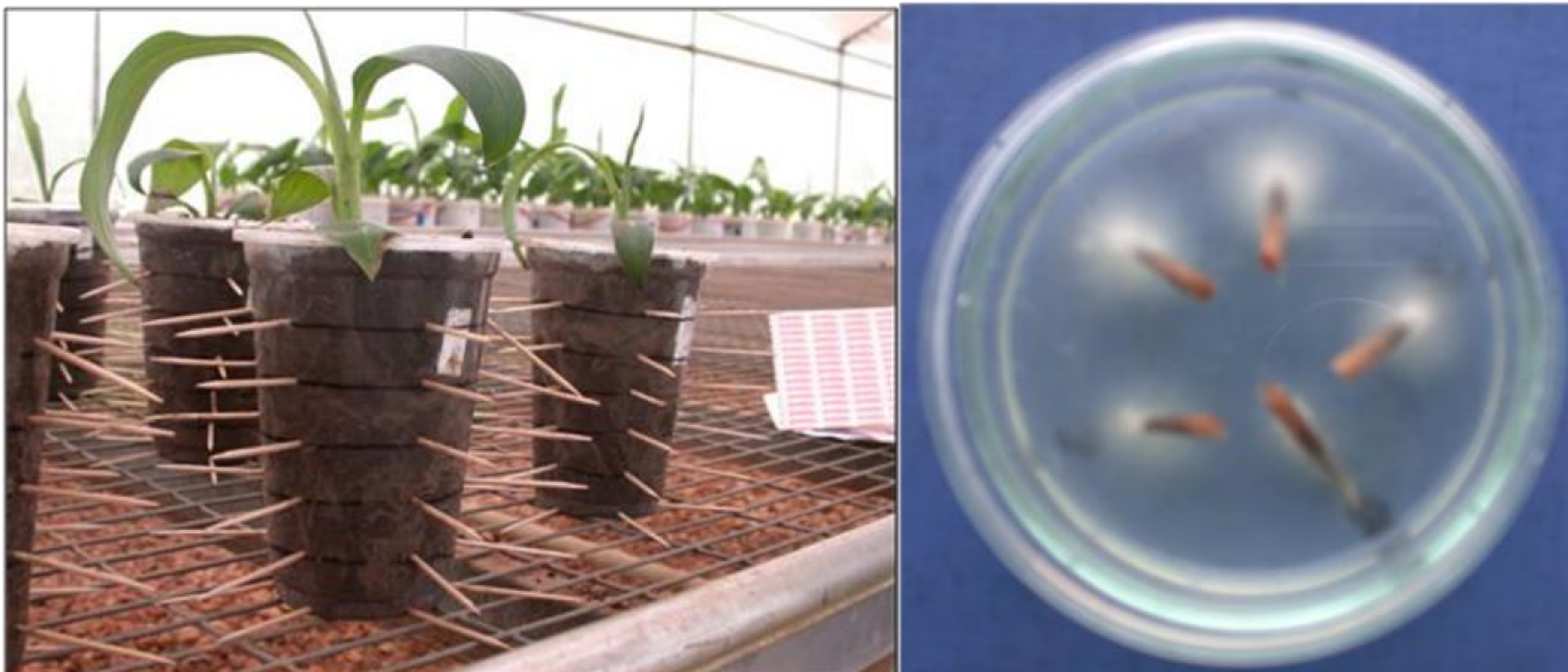
Conclusion 6: The level of *Fusarium* spp. colonization increases when *F. oxysporum* V5w2 is inoculated, especially when *R. similis* is present, but the visual method does not clearly differentiate the inoculated endophyte, especially in non-sterile soil.

4.6.2. Optical density of banana root extracts



Conclusion 7: Optical density of banana root extracts reduces with the inoculation of plants with *F. oxysporum* V5w2 and *R. similis.*

4.6.3. Rhizosphere-toothpick method for detecting the banana endophyte *Fusarium oxysporum* V5w2



TC banana plants inoculated with *F. oxysporum* V5w2 grown in sterile soil with wooden tooth-pick probes *(left)*; and *Fusarium* growth from toothpicks in PDA *(right)*

Conclusion 8: *Fusarium oxysporum* V5w2 colonizes banana rhizosphere when inoculated by the root-dip method, an aspect that needed to have been considered in the interpretation of results from previous studies.

4.6.4. Wilt-like symptoms in banana plants co-inoculated with *F. oxysporum* V5w2 and *R. similis* under P-deficient conditions

Tissue Culture banana plants inoculated with *F. oxysporum* V5w2 and *R. similis* grown with sterile beach sand supplied with P-deficient solution *(right two)*, and controls without the two organisms *(left two)*.

Conclusion 9: *F. oxysporum* V5w2 and *R. similis* interact to cause wilt-like symptoms in banana plants, because their independent applications did not result in these symptoms.

5.0. Critical issues concerning this study [4,5,6,7]

5.1. Biological control potential

(a) Current understanding

Fusarium oxysporum V5w2 is a known biological control agent of *Radopholus similis*, and hence results of this study are unreliable because there was no nematode suppression.

(b) Debatable facts

- The present work was done between 2006 and 2010. There was no evidence in previous researches published between 1995 and 2010 to support biological control potential of *F. oxysporum* V5w2 in the field.

- The only field trial on *F. oxysporum* V5w2 provided data on endophyte colonization and persistence in banana roots, without data on nematode control [8].

- Studies providing some information on biological control potential of *F. oxysporum* V5w2 have been published after the year 2009 [9,10, 11, 12], when the outcomes of the current study were already known.

5.2. Non-pathogenicity of *Fusarium oxysporum* V5w2

(a)Current understanding

F. oxysporum V5w2 has been well studied and found to be non-pathogenic, and hence results of this study are unreliable because there were wilt-like symptoms and plant growth suppression.

(b) Debatable facts

- Symptomless status of the original source plant is not a strong basis for claiming non-pathogenicity of *F. oxysporum* V5w2 [1,2,13].

- Previous researches did not provide data on plant growth and yields that would have partly addressed non-pathogenicity and plant growth promotion [8,14,15].

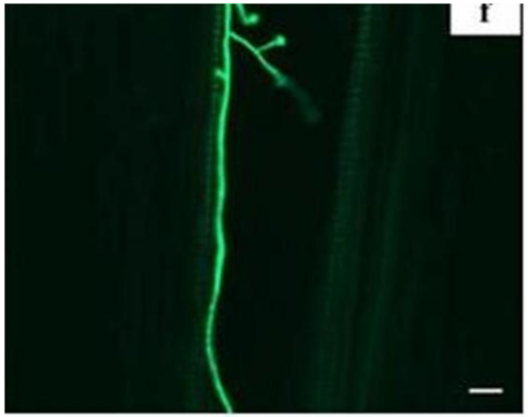
- Some studies only provided data on endophyte colonization of banana roots [8,14,15,16].

- Studies providing some information on non-pathogenicity of *F. oxysporum* V5w2 have been published from the year 2009 [9,11,12], when outcomes of the current study were already known.

5.3. Proof for successful inoculation of *Fusarium oxysporum* V5w2

(a)Current understanding

Banana roots have to be wounded to facilitate entry of non-pathogenic *F. oxysporum* V5w2 and kill *R. similis*; and hence results of this study are unreliable because inoculation was done with intact roots.



"Where the tips of roots were broken prior to dipping in an endophyte spore suspension, mycelia could be seen growing parallel to the inner walls of the xylem vessels [16]

(b) Debatable facts

- *Radopholus similis* inflicted wounds that allowed the entry of *F. oxysporum* into banana roots, leading to high percentage root piece colonization by *Fusarium* spp.

- "Infection of banana plants by pathogenic *F. oxysporum* is always through injured roots, especially via deep wounds that expose the xylem, and there is no evidence of the fungus having an ability to attack living cells of the main root [17]".

5.4. Mechanism of action by *Fusarium oxysporum* V5w2

(a)Current understanding

Mechanism of action by *F. oxysporum* V5w2 against *R. similis* is induced resistance, exclusively endophytically; which has been confirmed by use of molecular tools to detect activated enzymes and their associated reactions[10,18,19,20,21].

(b) Debatable facts

- Banana plants react to *F. oxysporum* V5w2 to limit its endophytic colonization[8,18], and hence this is not a friendly interaction as has been portrayed.

- The wilt-like symptoms and plant growth suppression in banana plants inoculated with *F. oxysporum* V5w2, are evidences of unfriendly interactions that usually involve undesirable microbes[22,23,24,25].

- Confirmation that *F. oxysporum* V5w2 colonizes the rhizosphere, where it takes up nitrogen, may imply that the enzymatic reactions associated with induced resistance are also artifacts of nitrogen uptake by the fungus in the rhizosphere[26,27].

5.5. Emerging researches on *Fusarium oxysporum* V5w2

(a)Current understanding

- *"Releasing endophyte-enhanced TC plants to farmers has created an efficient and novel plant protection option and constitutes a much sought-after alternative to pesticide use in commercial production. At present, F. oxysporum strain V5w2 is being commercially registered in Kenya*[20,21]*".*
- *Fusarium oxysporum* V5w2 among other strains has been found to suppress populations of *Pratylenchus goodeyi* and *Helicotylenchus multicinctus* in banana roots by between 45 to 60% [11,12].
- Banana yield improvement ranging between 20-35% have been realized with the application of *F. oxysporum* V5w2 and other strains of the endophyte[11,12].

(b) Debatable facts

- These recent studies did not investigate *Radopholus similis* and mulch, so their applicability to the present work may be minimal.

- Retrospective application of the results to the decision of transferring *F. oxysporum* V5w2-inoculated TC banana back in 2006 remains debatable[20]. "*Because the concept of using nonpathogenic fungi that colonize the endorhiza for biological control is still relatively new, information on this subject in the form of published literature is scarce, in the form of Ph.D. theses or in unpublished form* [28]".

6. Conclusion

▪The benefits of mulching in banana production were evident, while the enhancing effect of inoculating tissue culture plants with *F. oxysporum* V5w2 could not be verified.

▪Data in the current study do not support the transfer of *F. oxysporum* V5w2-treated banana plants to farmers, because the plants suffer from reduced performance.

7. Way forward

▪Engage global stakeholders in developing policies to guide the use of Biological Control Agents, which currently face regulatory challenges[29].

Acknowledgements

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The current authorship is in compliance with the nature of the findings, interests of researchers, and the collaboration of institutions.

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