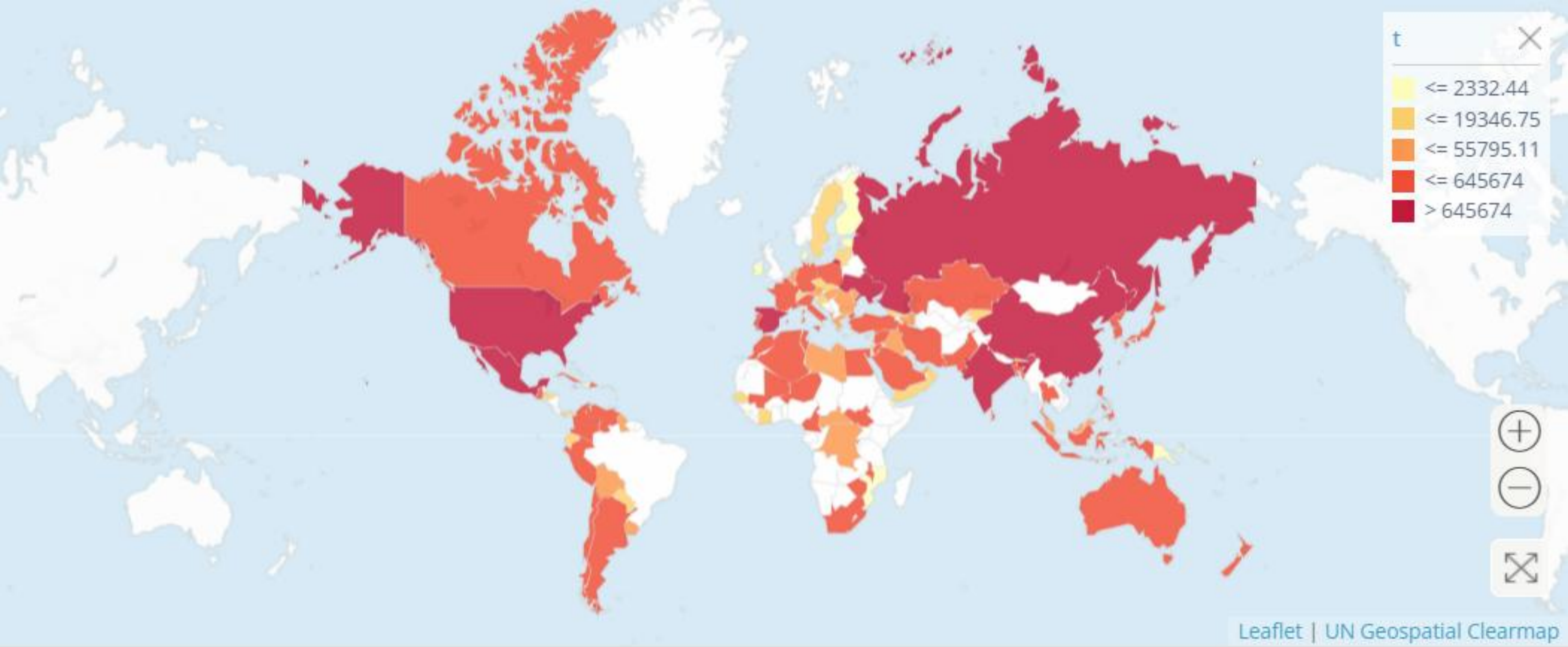
Identification, detection and management of seedborne squash pathogens

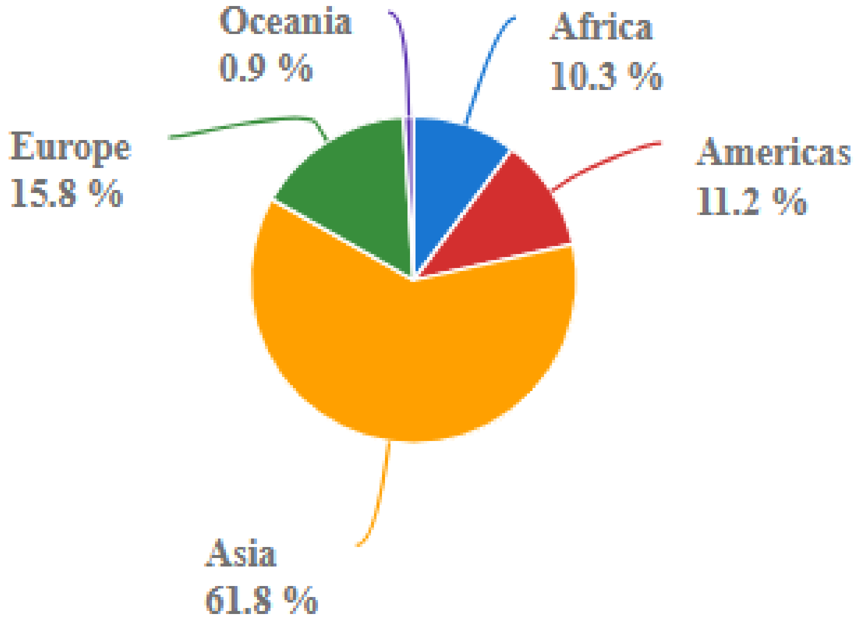
<u>MOUMNI MARWA</u>[1], MUROLO SERGIO[1], LANDI LANDI[1], ALLAGUI MOHAMED[2], ROMANAZZI GIANFRANCO[1]

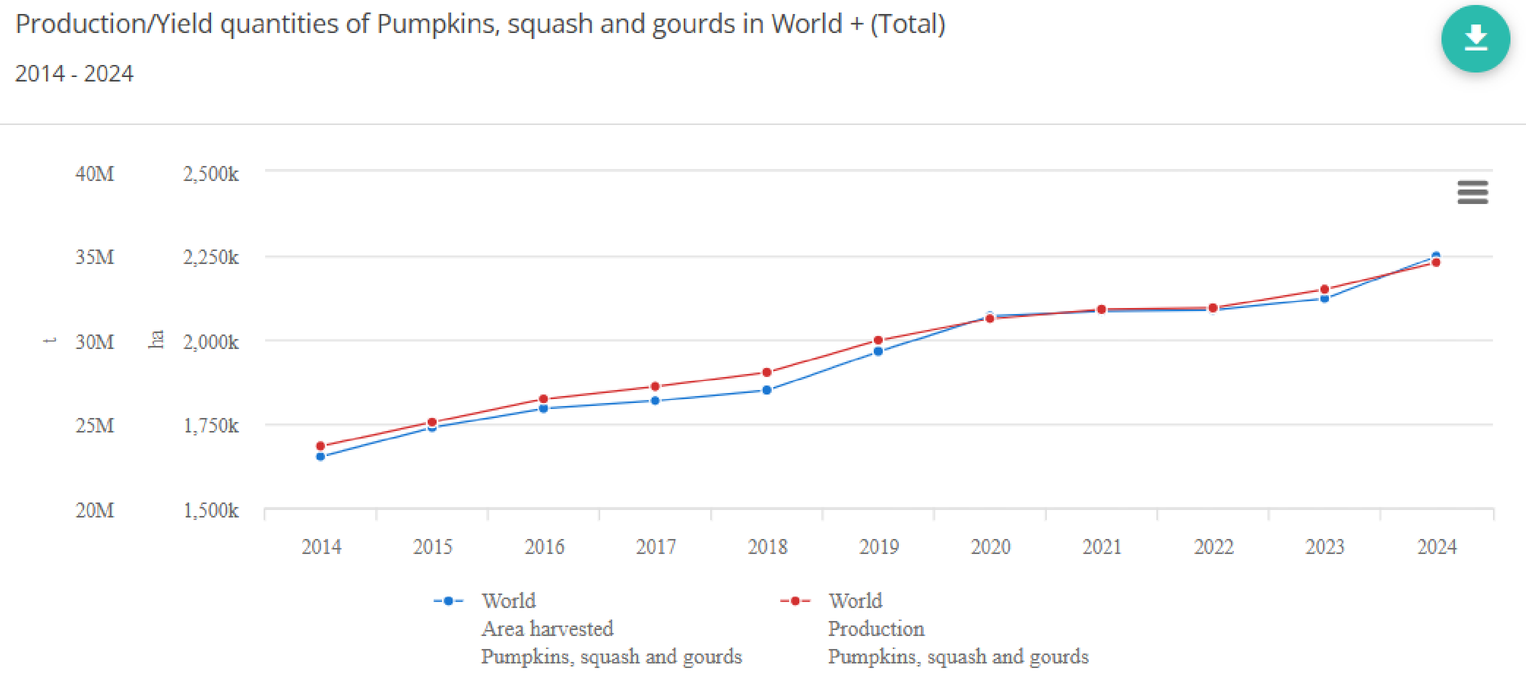
[1] Marche Polytechnic University, Ancona, ITALY;
[2] Institut National de la Recherche Agronomique de Tunisie (INRAT),Tunis, TUNISIA

III COST FOODWASTOP MEETING – ZADAR, CROATIA,
5 FEBRUARY 2026



Production quantities of Pumpkins, squash and gourds by country 2014-2024





Cucurbita spp.

Viruses
- CMV
- ZYMV
- SqMV

Bacteria
- *Pseudomonas syringae*
- *Xanthomonas cucurbitae*
- *Acidovorax avenae*

Fungi
- *Stagonosporopsis cucurbitacearum*
- *Fusarium* spp.
- *Alternaria* spp.
- *Paramyrothecium roridum*
- *Albifimbria verrucaria*

Main diseases cucurbits → **Seeds**

Seeds represent an efficient vehicle for the dispersion of seedborne pathogens

Indian Phytopathology (2024) 77:257–269
https://doi.org/10.1007/s42360-024-00723-3

REVIEW ARTICLE

Seed-borne fungi: challenges in seed health testing for biosecurity and agricultural sustainability

Jameel Akhtar[1] · Pardeep Kumar[1]

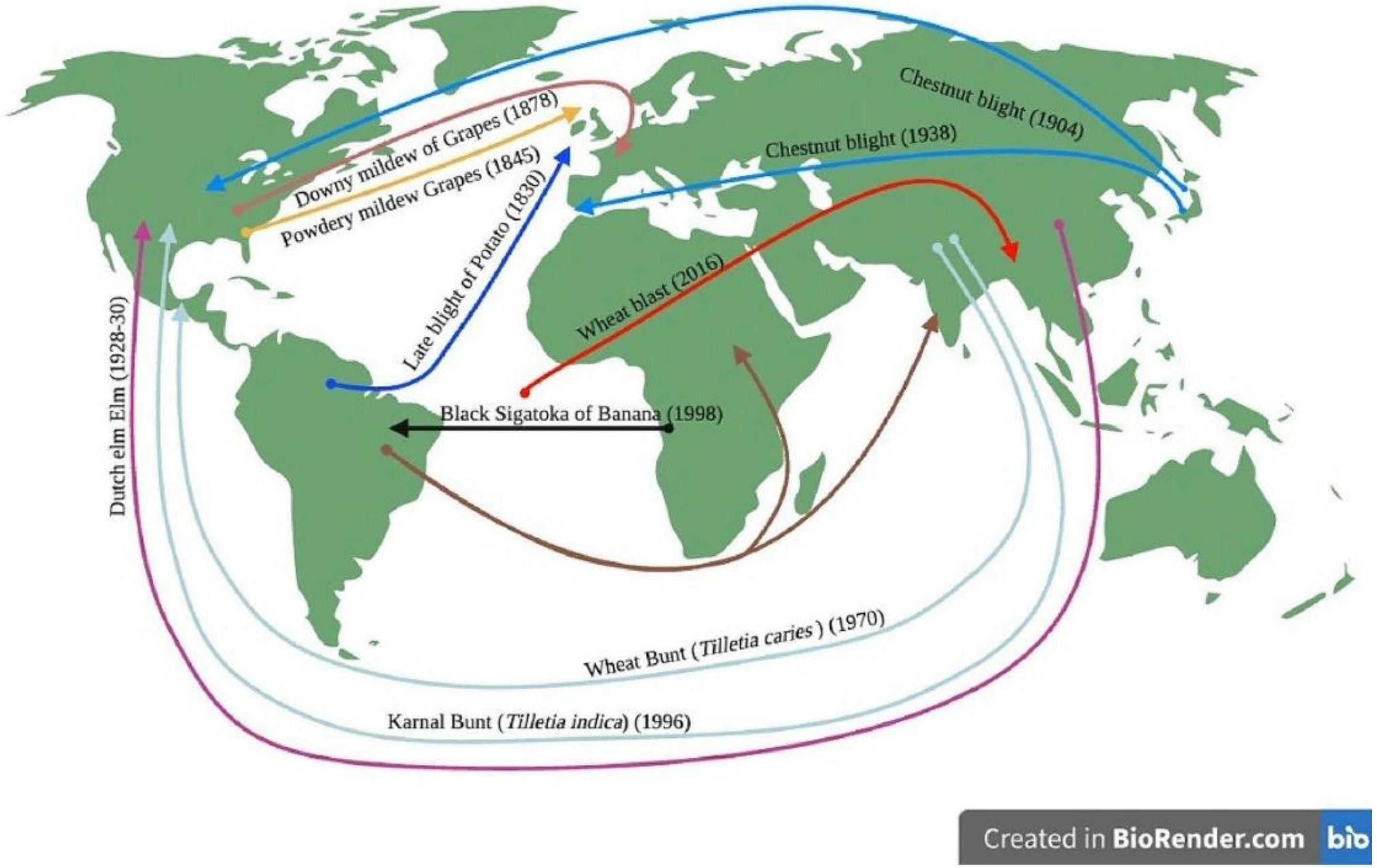
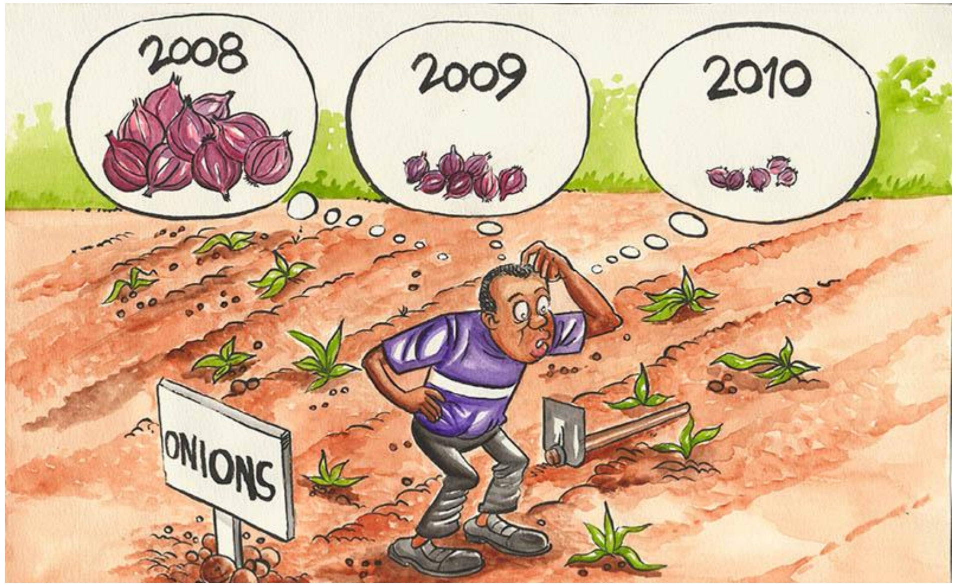


Fig. 1 Global invasion of seed-borne plant pathogenic fungi

Table 1 Global invasion of some seed-borne plant pathogenic fungi

Pathogen	Disease	Crop	Introduced		Year
			From	To	
Phytophthora infestans	Late blight	Potato	Peru	Ireland	1830
Uncinula nicator	Pow-dery mildew	Grapes	USA	England	1845
Plasmopara viticola	Downy mildew	Grapes	USA	France	1878
Ophiostoma ulmi	Dutch elm	Elm	Asia	USA	1928-30
Hemileia vastatrix	Rust	Coffee	Brazil	Africa, Asia	1970
Tilletia caries	Bunt	Wheat	India	Mexico	1970
T. indica	Karnal bunt	Wheat	India	USA	1996
Magnaporthe gressea pathoype *Triticum*	Blast	Wheat	Brazil	Bangladesh	2016
Crypho-nectria parasitica	Chest-nut blight	Chestnut	Asia	USA	1904
C. parasitica	Chest-nut blight	Chestnut	Asia	Italy	1938
Pseudocerco-spora fijiensis	Black Sigatoka	Banana	Africa	Brazil	1998

Source Akhtar et al. (2021)



PhytoFrontiers™ | 2023 • 3:71-74 https://doi.org/10.1094/PHYTOFR-03-22-0029-FI

PhytoFrontiers™
An Open Access Journal from The American Phytopathological Society
APS

Perspective

Seed Health Testing: Doing Things Right

Gerrit A. Hiddink[1] | Roland Willmann[2] | Joyce H. C. Woudenberg[3] | Rose Souza-Richards[3,†] |

Abstract

Since seeds can be a route for pathogen introduction, they are routinely inspected and tested to prevent pest outbreaks and introduction into new territories. The need for high throughput, short lead times, and cost reduction has played an important role in the development and application of techniques in seed health testing. Examples are molecular and serological techniques, such as ELISA and PCR assays, which are commonly called indirect tests. After signal detection in ELISA or PCR assay a seed lot is a suspect lot that requires further investigation for a final conclusion about the health status of the seed lot, since these tests do not provide any information about pathogen viability or pathogenicity. The seed industry uses them as a prescreen to identify healthy seed lots and in combination with classical methods, commonly called direct tests, to confirm viability of the target pathogen and demonstrate its pathogenicity. However, outside industry, indirect tests are increasingly used to make a final decision on the health status of a seed lot. This has led to a growing number of seed lots being rejected when the risk of introducing a pathogen to importing countries may have been negligible. We propose that investments continue to be made in the development of high-throughput prescreening detection methods like HTS and PCR assays, but together with direct tests that enable accurate assessment of the risks involved when target pathogens are detected using indirect tests. Close collaboration between molecular scientists and classical phytopathologists is essential.

Keywords: biological relevance, direct test, indirect test, seed movement

Plant Disease • 2020 • 104:1335-1350 • https://doi.org/10.1094/PDIS-04-19-0741-RE

e-Xtra*

Morphological and Molecular Identification of Seedborne Fungi in Squash (*Cucurbita maxima, Cucurbita moschata*)

Marwa Moumni,[1,2,3] Mohamed Bechir Allagui,[3] Valeria Mancini,[1] Sergio Murolo,[1] Neji Tarchoun,[4] and Gianfranco Romanazzi[1,†]

[1] Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, 60131 Ancona, Italy
[2] National Agricultural Institute of Tunisia, 1082 Tunis, Tunisia
[3] Laboratory of Plant Protection, National Institute for Agronomic Research of Tunisia, University of Carthage, 2080 Ariana, Tunisia
[4] Laboratory of Vegetable Crops, High Agronomic Institute of Chott Mariem, Sousse, Tunisia

Smptomatic fruits

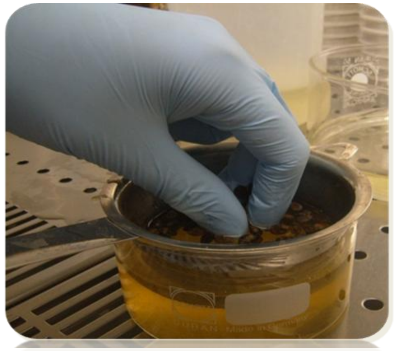
Asymptomatic fruits

4 regions; 6 Farmers; 29 Fruits; 3 cv. Aspen, Naples Long, Butternut

5 regions; 20 Farmers; 37 Fruits; 3 cv. Bjaoui, Galaoui, Batati

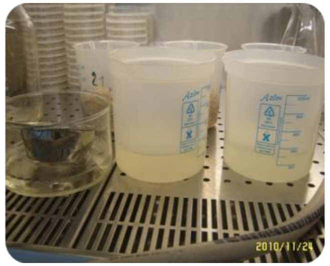
Morphological identification

Blotter test



200 Seeds/Lot

1



2

After 14 days of incubation

LAS software (version 3.8; Leica DFC 295),

3

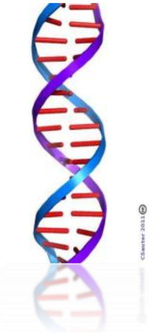
Pycnidia, Perithecia, Conidia

50 unitis

Molecular identification

DNA extraction from mycelia

93 isolates



PCR amplification using specific primer

Design of specific primers

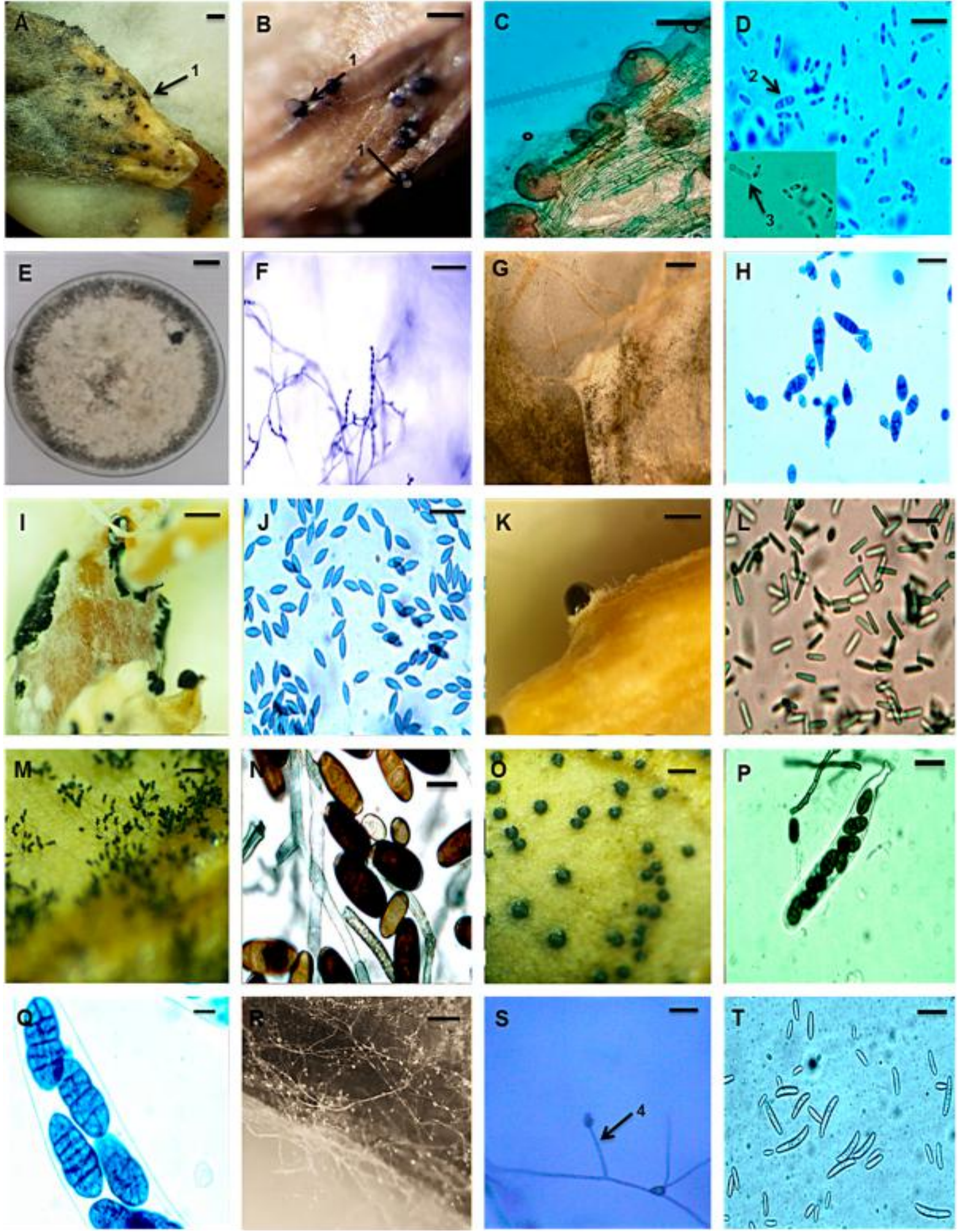
Genes	Primer
ITS region	ITS1/ITS4
TUB	EF1-728F/EF1-986R
CALM	CAL228F/737R
EF 1α	Bt2a/Bt2b
HIS	CYLH3F/CYLH3R

14 fungal species were detected from the squash seeds

- *Alternaria alternata*
- *Stagonosporopsis cucurbitacearum*
- *Fusarium fujikuroi*
- *Fusarium solani*
- ***Albifimbria verrucaria****
- ***Stemphylium vesicarium****
- ***Pramyrothecium roridum****
- *Fusarium oxysporum*
- *Curvularia spicifera*

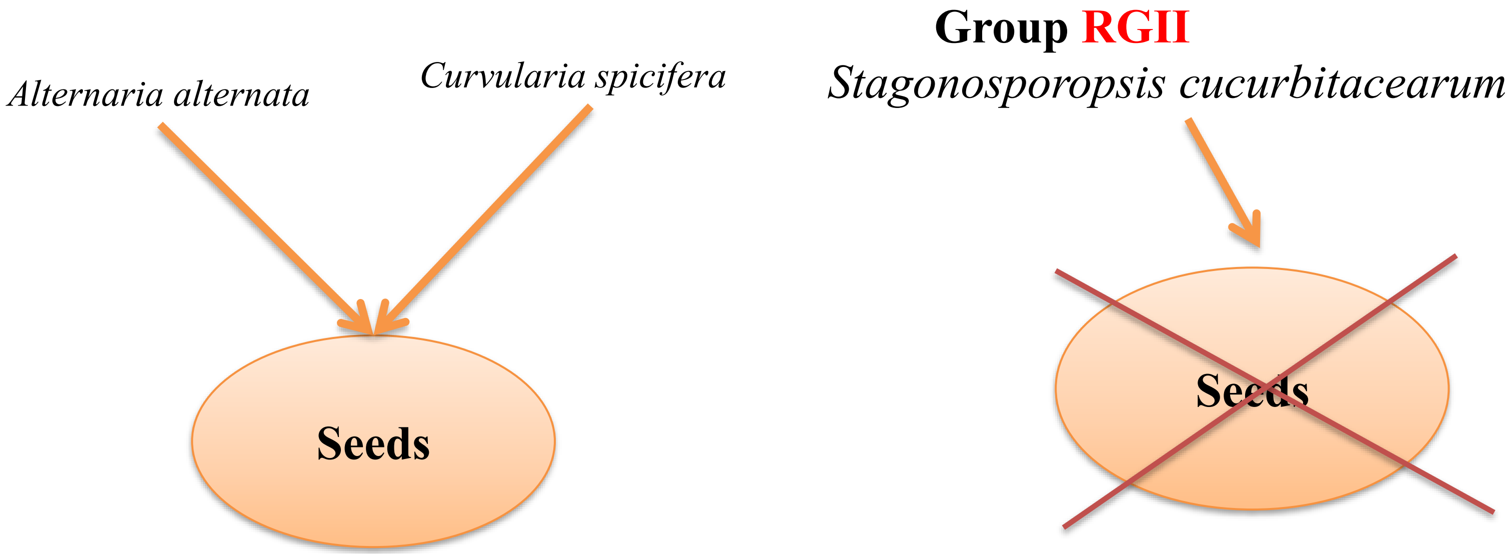
Specific primers designed

Target species	Primer name[a]	Sequence (5' to 3')
Albifimbria	Myroverr F1	5’-TGTGAACCTTACCATATTGTTGC-3’
verrucaria	Myroverr R1	5’-CGTTCCAACTGCGAGGTTGT-3’
Paramyrothecium roridum	Myroror F1	5’-CCCTTTGTGAACCTTACCTAT-3’
	Myroror R1	5’-AGCTCCAATGCGAGTTGTG-3’
Stemphylium vesicarium	Pleo F	5’-TACACAATATGAAAGCGGGTTG-3’
	PleoR	5’-AAGGCTGATTCAAAGTGCAAG-3’
	Pleo F1	5’-ATTCACCCATGTCTTTTGCG-3’
	PleoR1	5’-AAATGTGGTCTTGATGGATGC-3’



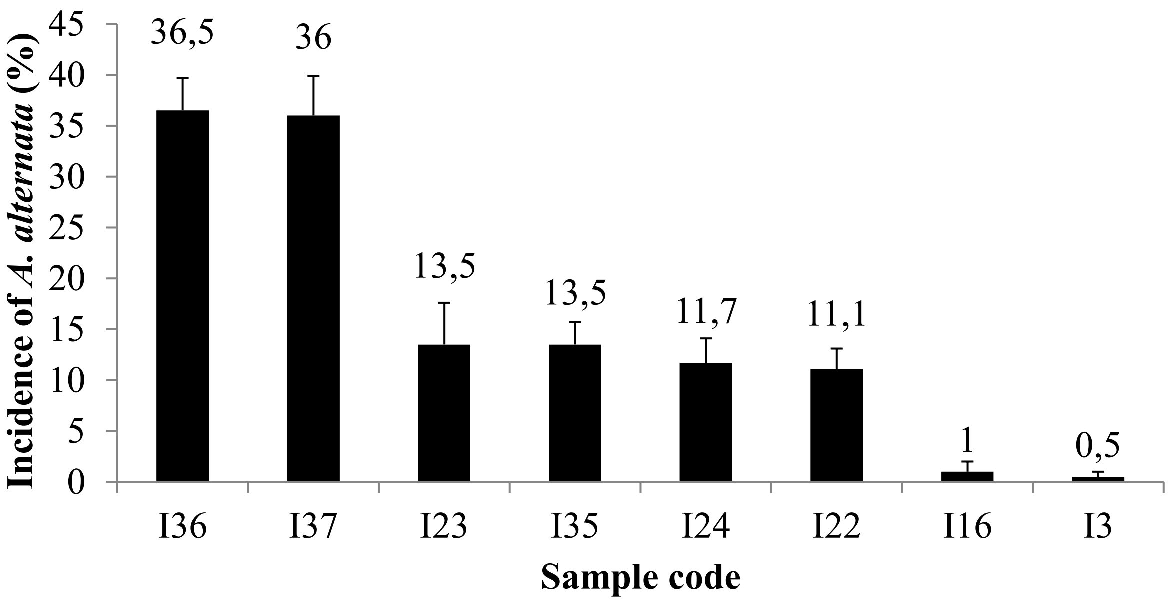
ITALY

20 symptomatic fruits



9 asymptomatic fruits

Incidence of *A. alternata* (%)



TUNISIA

Group RGII

A. alternata *S. cucurbitacearum* *C. spicifera*

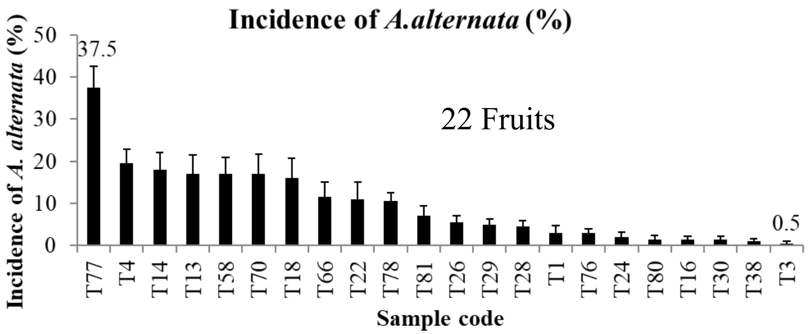
11 Symptomatic fruits

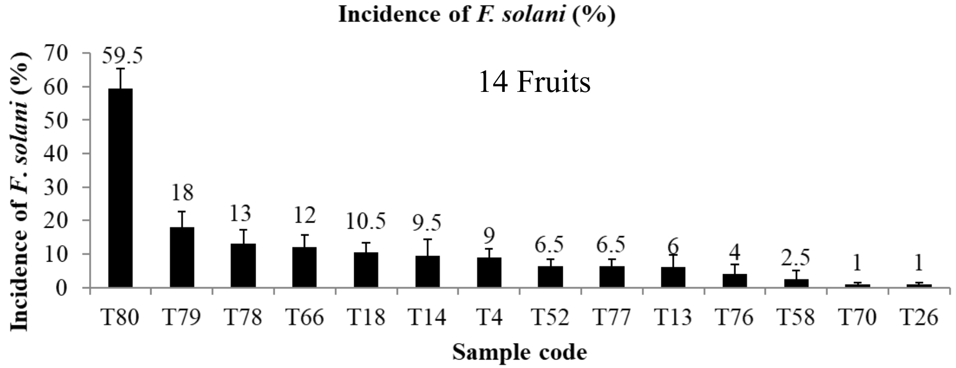
Indirect invasion

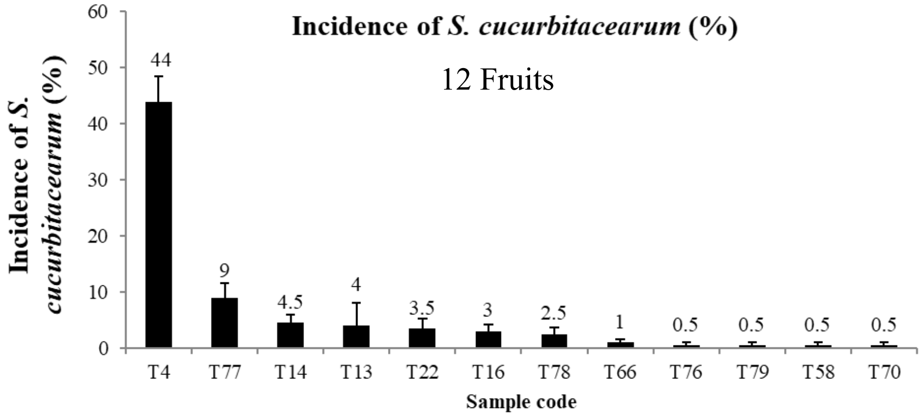
seeds

seeds can be infected indirectly through the fruit

26 Asymptomatic Fruits







Present in the first time in Tunisia and Italy on *Cucurbita*

frontiers in Microbiology
ORIGINAL RESEARCH
published: 11 January 2022
doi: 10.3389/fmicb.2021.764447

Detection and Quantification of *Stagonosporopsis cucurbitacearum* in Seeds of *Cucurbita maxima* Using Droplet Digital Polymerase Chain Reaction

Sergio Murolo[1], Marwa Moumni[1,2], Valeria Mancini[1], Mohamed Bechir Allagui[2], Lucia Landi[1] and Gianfranco Romanazzi[1*]

[1] Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Ancona, Italy, [2] Laboratory of Plant Protection, National Institute for Agronomic Research of Tunisia, University of Carthage, Ariana, Tunisia

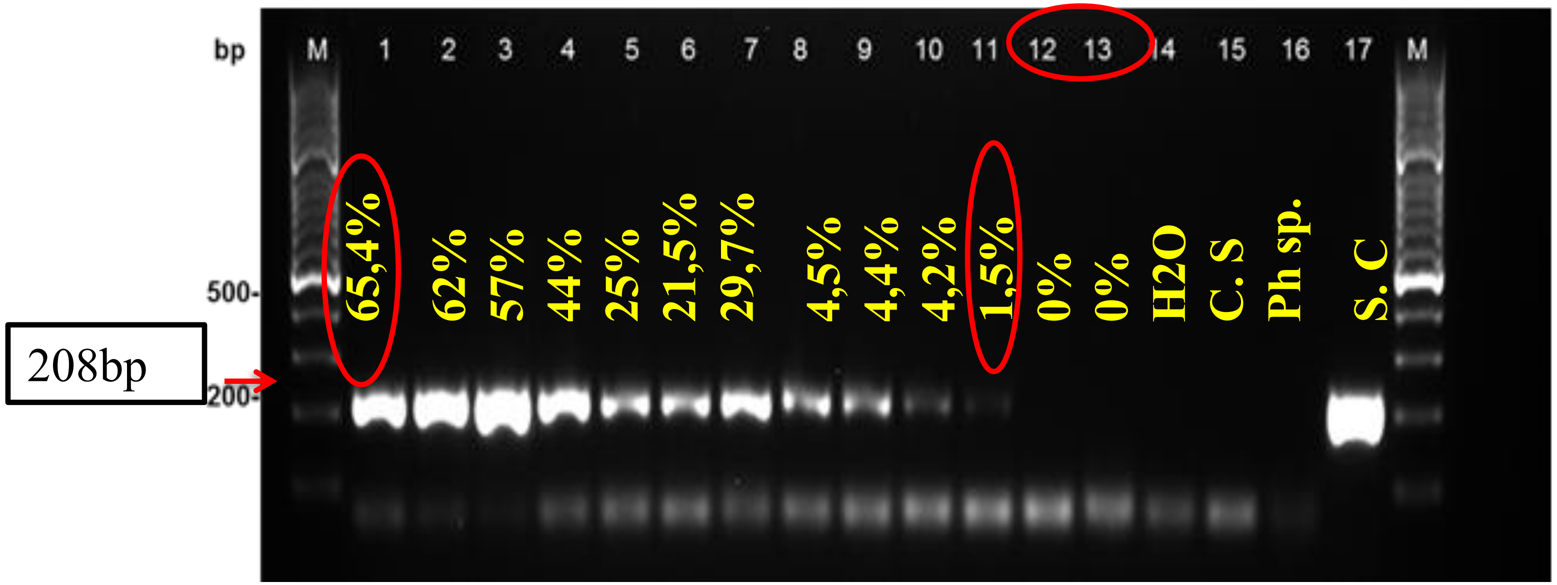
18 Asymptomatic fruits

Incidence of *Stagonosporopsis cucurbitacearum* on seeds

1.5% to 65.4%

Specific primers designed for dectection ***S. cucurbitacearum***

DBF1 (5’-TCGAATGGCTCAGAGAAGGT-3’)
DBR1 (5’-AAGTCCACGTCAGACCCATC-3’)



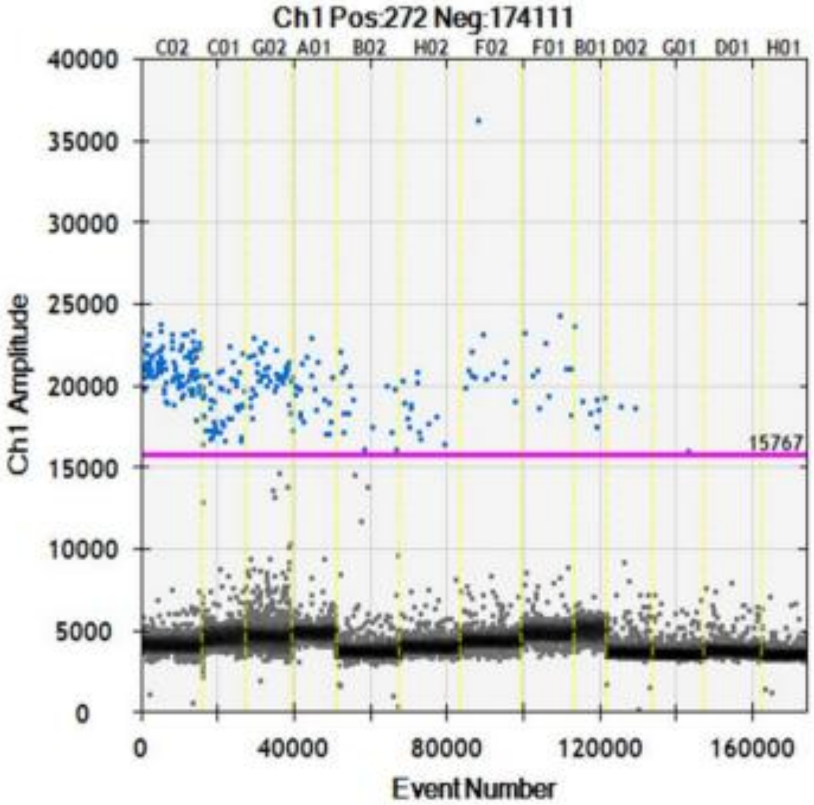
Quantitative real-time PCR

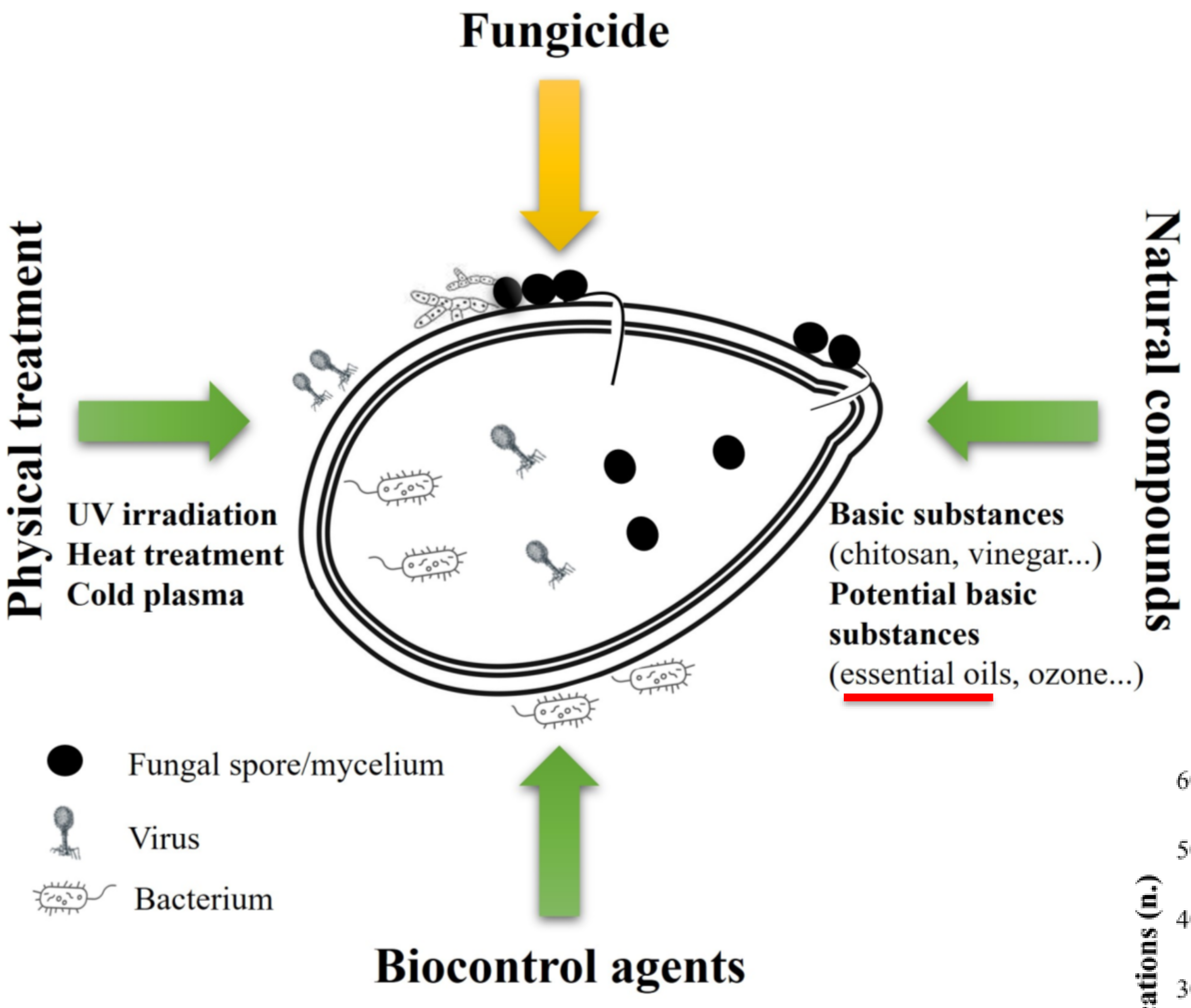
Sample code	Blotter method (%)	Molecular detection Conventional PCR[b]	ddPCR	
			Positive events	Absolute quantification (copies/μl)
T86	25.0 ± 2.3	+	22	2.2
T93	6.1 ± 2.4	+	6	0.8
T83	49.0 ± 4.1	++	48	5
T87				0.89
T101				0
T8				0.99
T85				0.95
T90				0.2
T96				0.93
T4				0.35
T95	15.0 ± 2.6	+	13	0.97
IHS[a]	0.0 ± 0.0	–	0	0
WC[a]	–	–	0	0

[a]IHS, healthy seed control; [b]WC, water control.
–, no amplification; +/–, very weak amplification, +, weak amplification; ++, moderate amplification; +++, strong amplification.

Blotter and ddPCR tests showed a high degree of correlation ($R^2 = 0.986$, $p \leq 0.01$).

Droplet Digital Polymerase Chain Reaction





Food Security
https://doi.org/10.1007/s12571-023-01384-2

REVIEW

Recent innovative seed treatment methods in the management of seedborne pathogens

Marwa Moumni[1] · Guro Brodal[2] · Gianfranco Romanazzi[1]

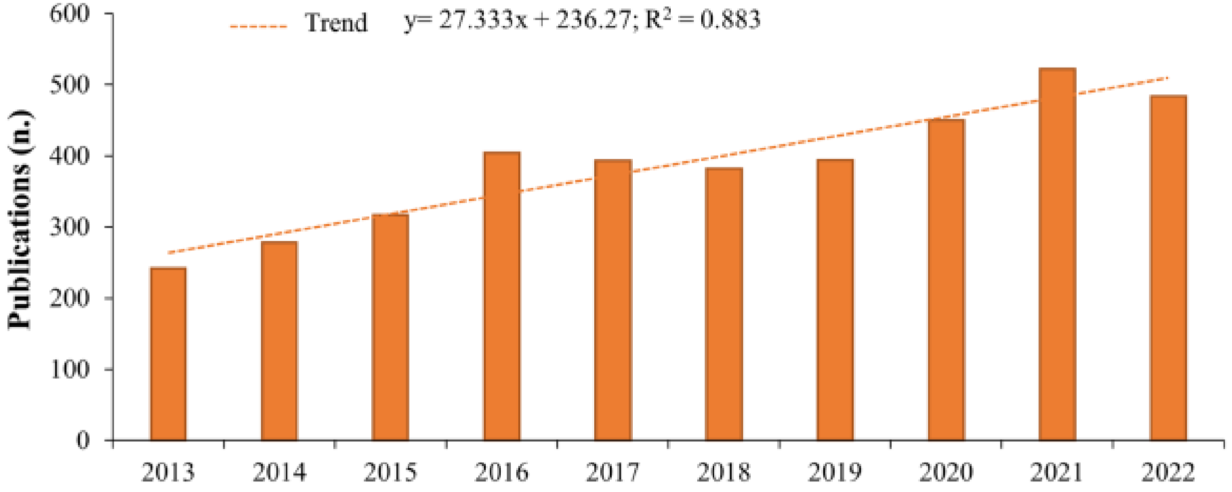


Fig. 1 Number of publications available on Scopus through searches with keywords 'Seed treatment' in 'Article title, Abstract, and Keywords' published over the last 10 years (Source: Scopus, accessed on 25 July 2023; https://www.scopus.com)

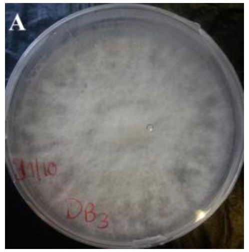

antibiotics

MDPI

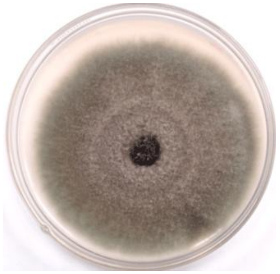
Article

Antifungal Activity and Chemical Composition of Seven Essential Oils to Control the Main Seedborne Fungi of Cucurbits

Marwa Moumni [1,2], Gianfranco Romanazzi [2,*], Basma Najar [3], Luisa Pistelli [3], Hajer Ben Amara [1], Kaies Mezrioui [1,2], Olfa Karous [4], Ikbal Chaieb [5] and Mohamed Bechir Allagui [1]



S. cucurbitacearum

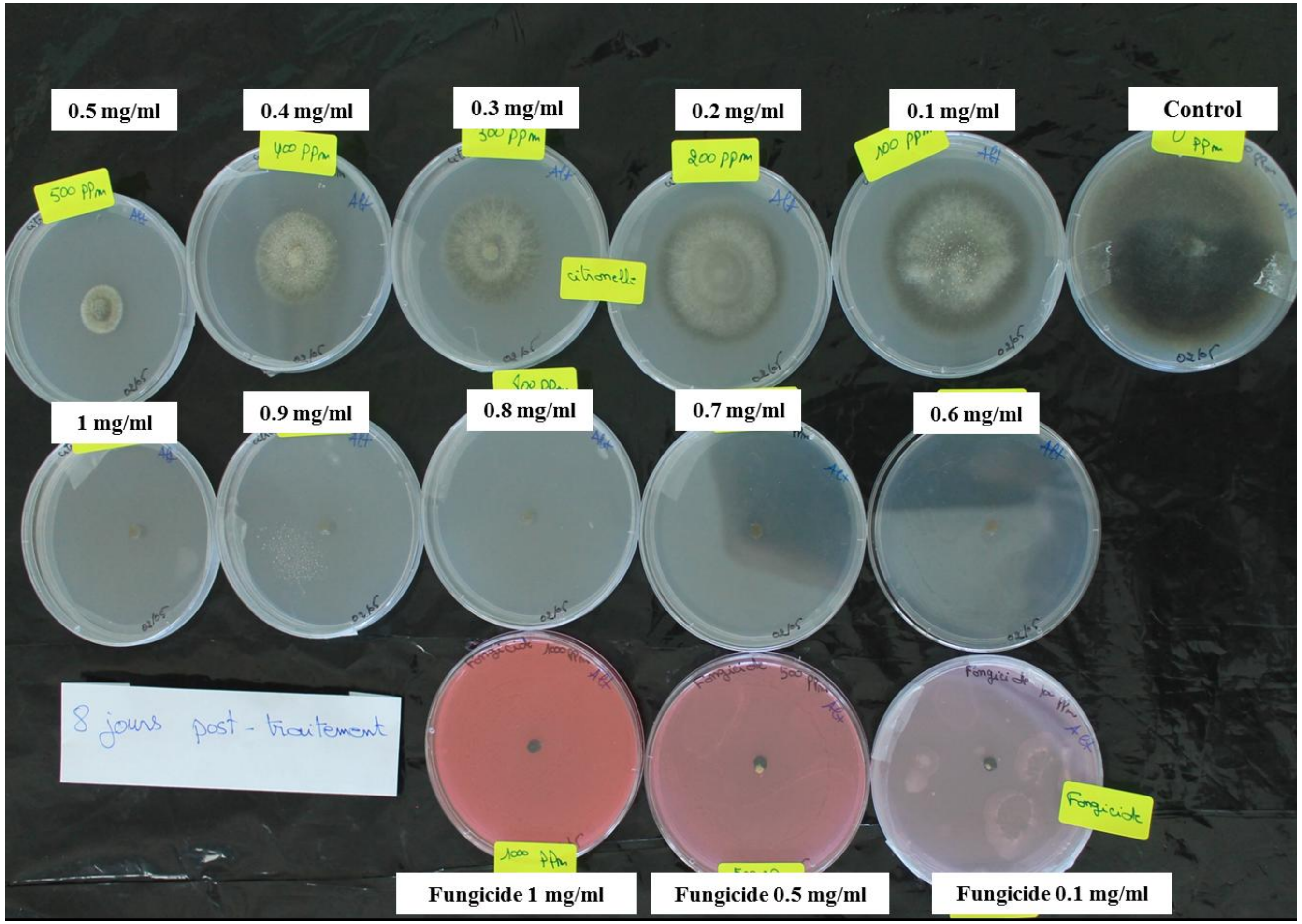


A.alternata

PDA + 7 EOs

0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9 and 1 mg/mL

Mycelial growth inhibition of ***Alternaria alternata*** by lemongrass EO

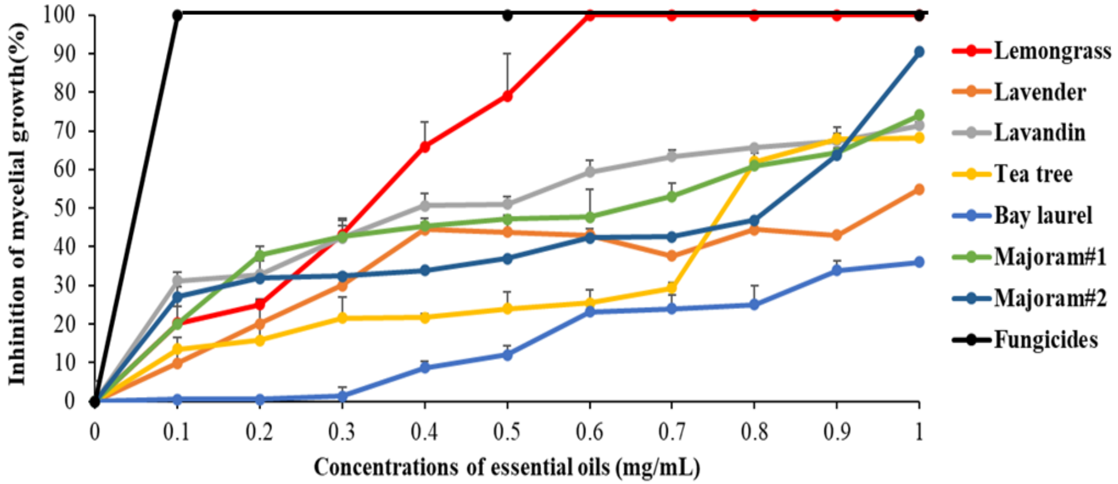


antibiotics MDPI

Article

Antifungal Activity and Chemical Composition of Seven Essential Oils to Control the Main Seedborne Fungi of Cucurbits

Marwa Moumni [1,2], Gianfranco Romanazzi [2,*], Basma Najar [3], Luisa Pistelli [3], Hajer Ben Amara [1], Kaies Mezrioui [1,2], Olfa Karous [4], Ikbal Chaieb [5] and Mohamed Bechir Allagui [1]



Mycelial growth inhibition of *Stagonosporopsis cucurbitacearum* by the seven essential oils

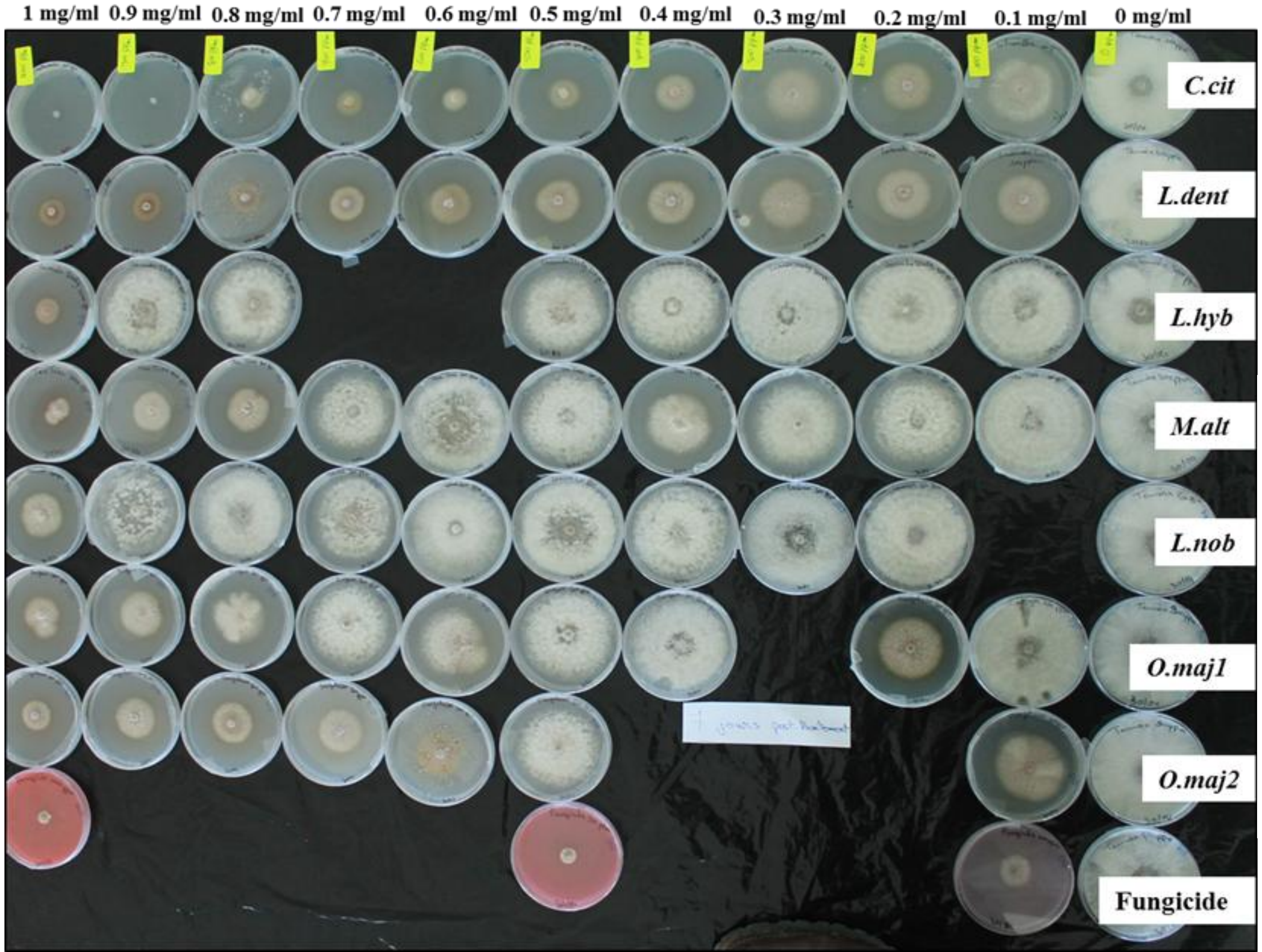
antibiotics

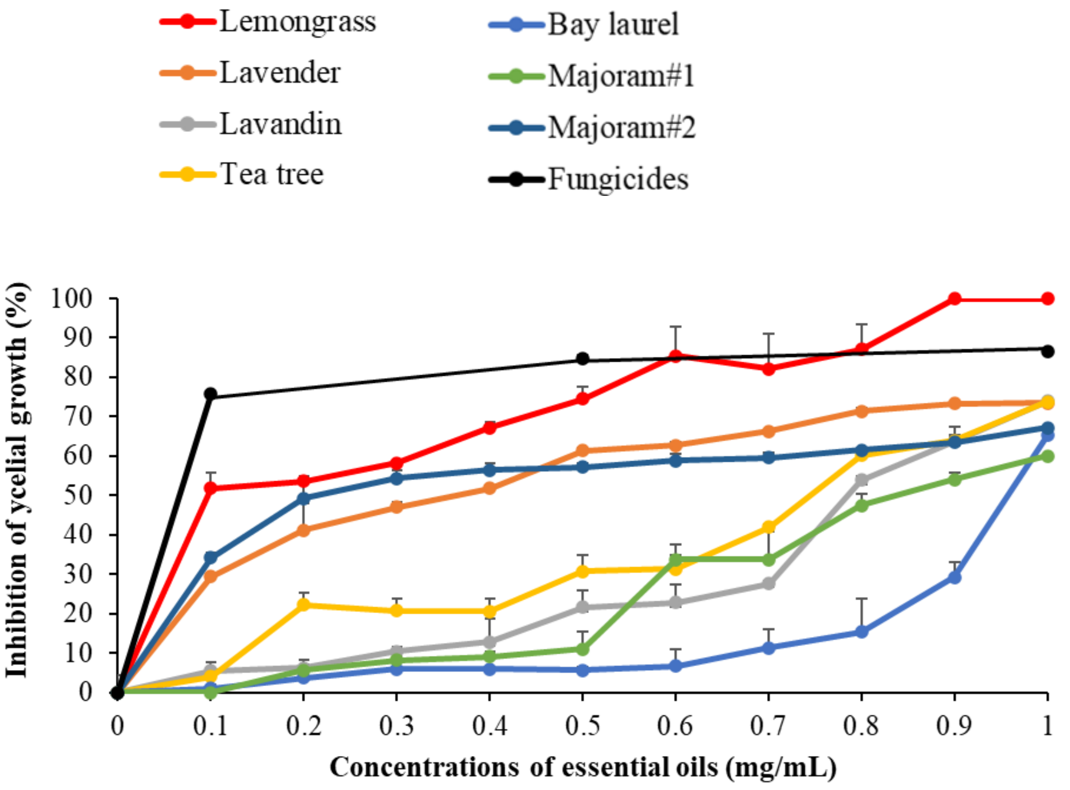
MDPI

Article

Antifungal Activity and Chemical Composition of Seven Essential Oils to Control the Main Seedborne Fungi of Cucurbits

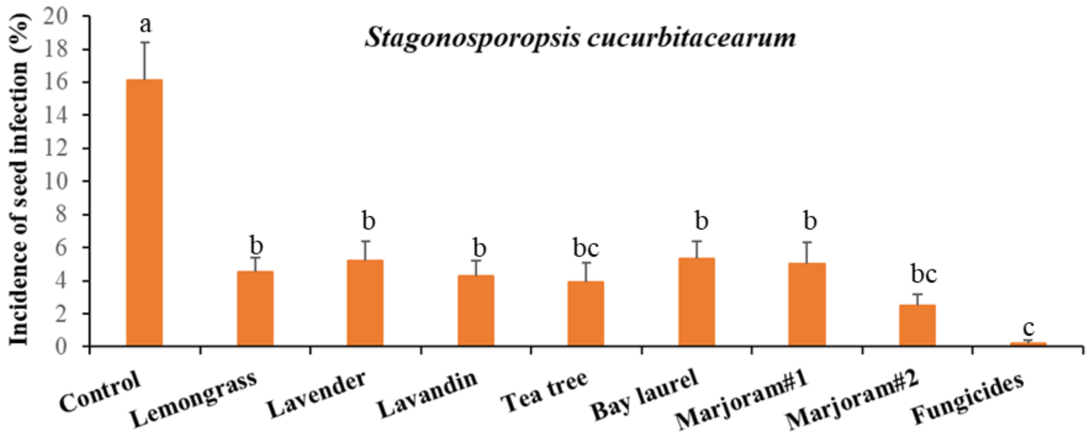
Marwa Moumni [1,2], Gianfranco Romanazzi [2,*], Basma Najar [3], Luisa Pistelli [3], Hajer Ben Amara [1], Kaies Mezrioui [1,2], Olfa Karous [4], Ikbal Chaieb [5] and Mohamed Bechir Allagui [1]

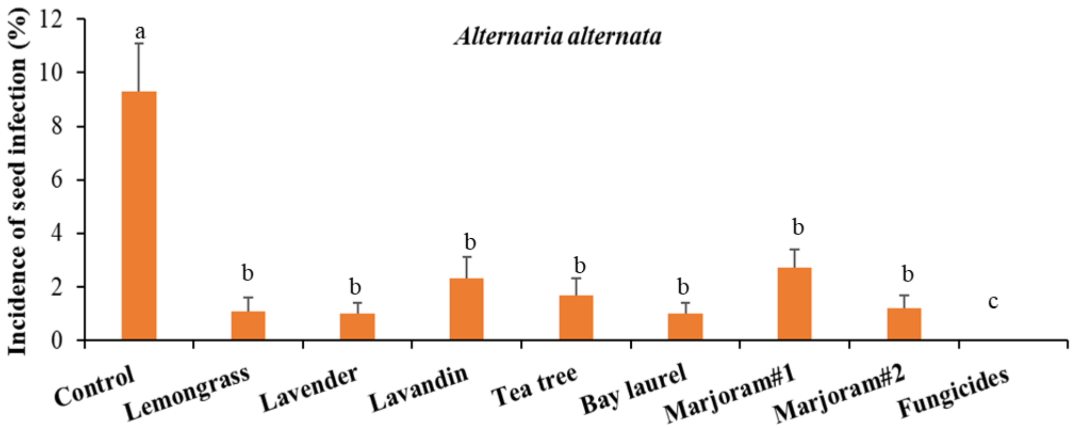


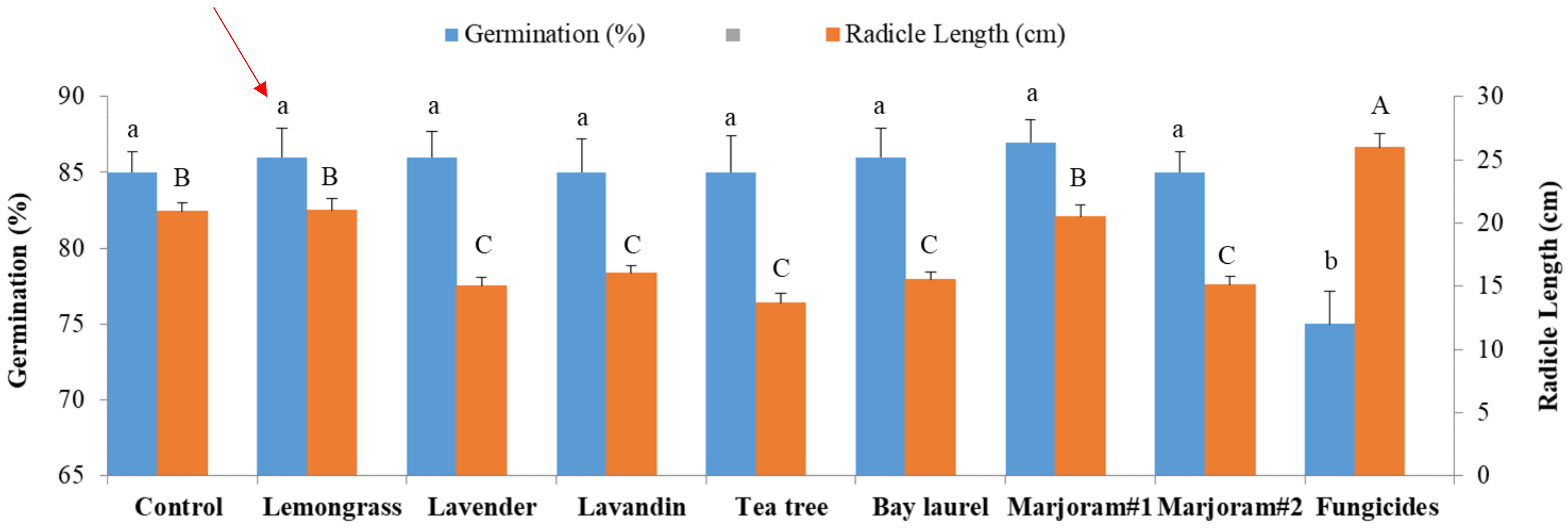


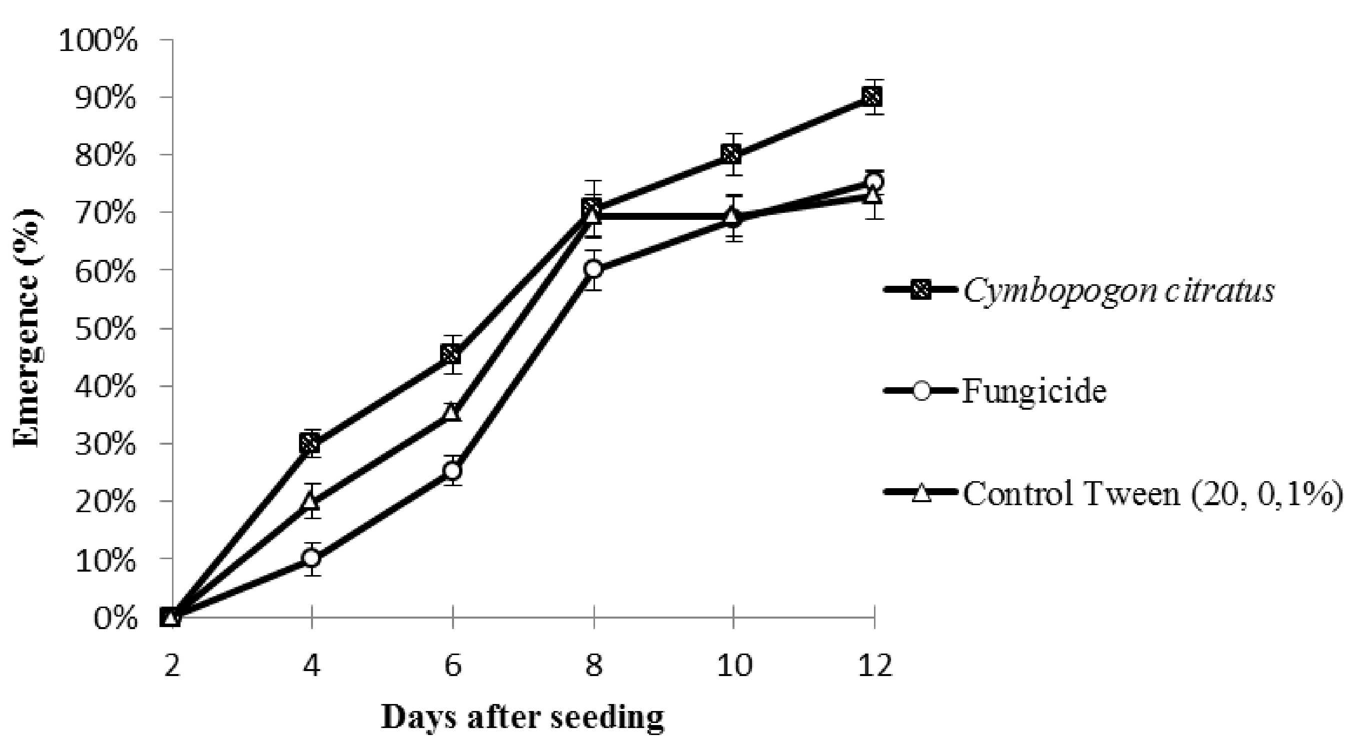

molecules
MDPI
Article
Evaluation of Seven Essential Oils as Seed Treatments against Seedborne Fungal Pathogens of *Cucurbita maxima*
Marwa Moumni [1,2], Mohamed Bechir Allagui [2], Kaies Mezrioui [1,2], Hajer Ben Amara [2] and Gianfranco Romanazzi [1,*]
400 Naturally infected seeds/Treatment
1
0.5 mg/mL concentration
Blotter test
2
3
After 14 days of incubation
Germination rate of seeds and length of the radicle

Effects of seven essential oils at 0.5 mg/mL on incidence of seed infections









Treatment	Plantlets infection (%)	Disease incidence (%)			Plantlets lengths (cm)
		Leaves	stems	roots	
C. citratus	30.0±4.4 b	18.7±3.3 a	28.3±4.5 a	18.1±4.3 a	30.5±1.1 b
Fungicide	16.8±1.9 a	9.7±1.6 a	17.3±1.9 a	17±2.1 a	30.2± 1.2 b
Control	49.9±2.9 c	30.3±5.2 b	43±4.4 b	43.1±3.2 b	23.3±1.2 a

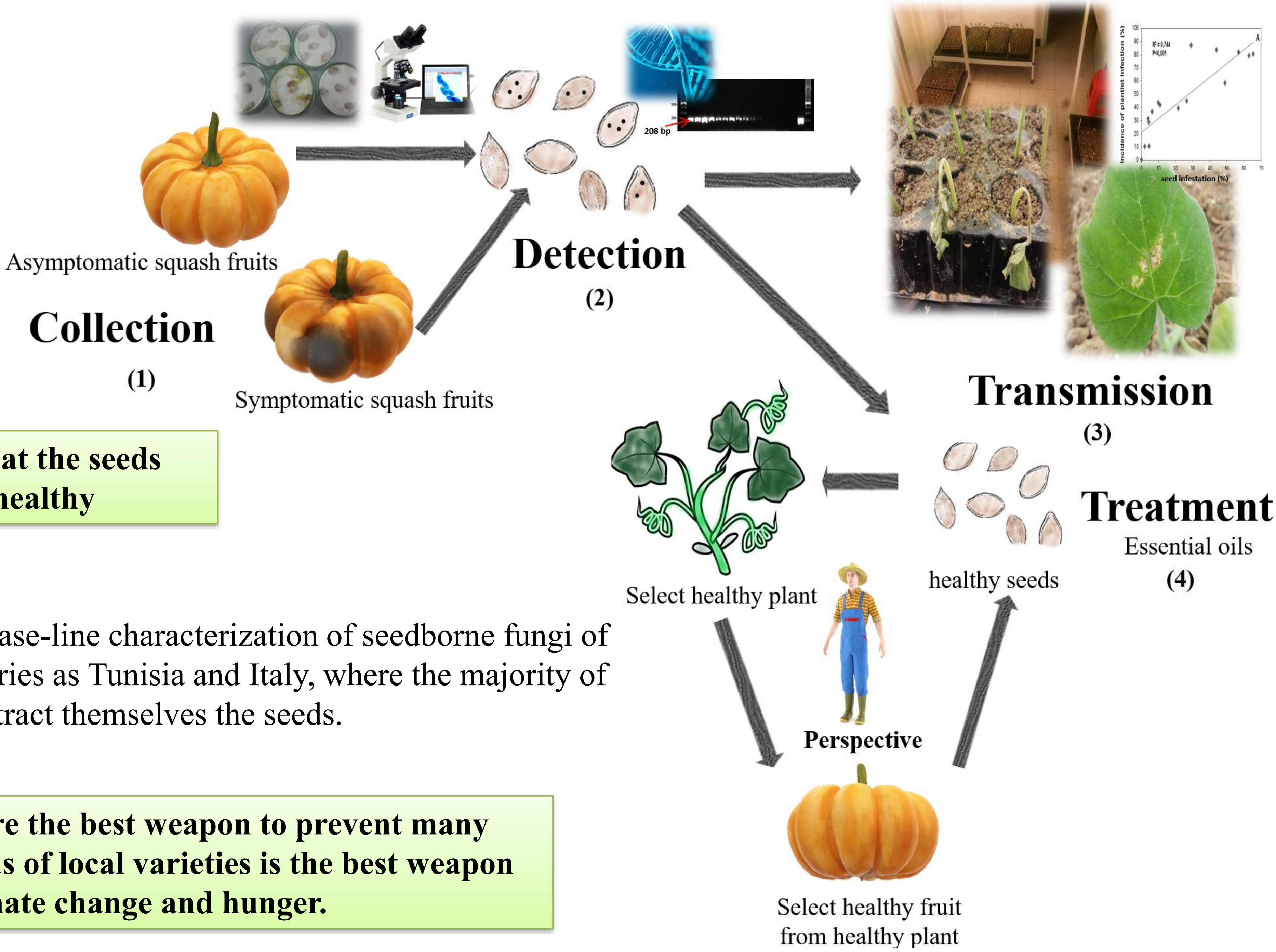
Conclusions

The cultural practices

↓

Routine visual inspection of fruit

Not enough to guarantee that the seeds inside these fruits are healthy



This study represents the first base-line characterization of seedborne fungi of squash in Mediterranean Countries as Tunisia and Italy, where the majority of growers extract themselves the seeds.

Uses of healthy seeds are the best weapon to prevent many diseases, and sowing seeds of local varieties is the best weapon against climate change and hunger.

UNIVERSITÀ POLITECNICA DELLE MARCHE

THANK YOU FOR YOUR ATTENTION

Prof. Gianfranco ROMANAZZI
Prof. Mohamed Bechir ALLAGUI
Prof. Sergio MUROLO
Prof. Lucia LANDI
Dr. Valeria MANCINI
Dr. Kaies MEZRIOUI
Dr. Hajer BEN AMARA

For further info: m.moumni@staff.univpm.it