First step development of an effective biofungicide against *Lasiodiplodia spp: In vitro* screening of *Trichoderma spp.* for biocontrol potential

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Introduction | Aim of the study | Materials and methods | Results | Discussion | Conclusion

Climate change impacts agricultural production systems, especially in the Mediterranean Basin, a hotspot of global warming (IPCC, 2007).

Pests and diseases are likely to move, following climate change, affecting areas previously immune, and thus less prepared, biologically and institutionally, to manage and control them.

Among these diseases, dieback is caused by a member of the Botryosphaeriaceae family, with economical importance as it can cause significant crop losses.

Lasiodiplodia spp is a pathogen with worldwide distribution especially in Mediterranean countries and occurs in grapevine and others crops (Berraf-Tabbal *et al*., 2020).

Biological control agents (BCAs) offer an alternative to the chemical control of fungal phytopathogens as they can kill or limit the growth of pathogens without harming the host (De la Cruz-Quiroz *et al.,* 2018).

Trichoderma, is a useful, filamentous fungi which have attracted the attention because of it's different activities against various plant pathogens (Fig.1).

IPCC 2007. Intergovernmental panel on climate change staff. Fourth. assessment report–climate change 2007: Migation, vulnerabiitly and adaptation. . Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change (IPCC). Cambridge University Press, Cambridge

Berraf-Tebbal A, Mahamedi AE, Aigoun-Mouhous W, Špetík M, Čechová J, Pokluda R. 2020. *Lasiodiplodia mitidjana* sp. nov. and other Botryosphaeriaceae species causing branch canker and dieback of *Citrus sinensis* in Algeria. PLoS ONE 15(5): e0232448.

De la Cruz-Quiroz R., Roussos S.,Rodríguez-Herrera R., Hernandez-Castillo D., Aguilar C.N. 2018. Growth inhibition of *Colletotrichum gloeosporioides* and *Phytophthora capsici* by native Mexican *Trichoderma* strains. Karbala International Journal of Modern Science 1-7. https://doi.org/10.1016/j.kijoms.2018.03.002

Figure 1. *Lasiodiplodia* and *Trichoderma* T1BB competition for space and nutrient on PDA agar plate after 6 days incubation.

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The present work was then designed to characterize and screen *Trichoderma* isolates with biocontrol ability for a biofungicide development prospect.

Nine (9) *Trichoderma* isolates were isolated from rhizosphere and characterized by studying their growth rate and sporulation index (Fig. 2).

Antifungal activity of *Trichoderma* isolates was evaluated *in vitro* in Petri plate by measuring inhibition percentage of the plant pathogen.

The potent *Trichoderma* isolates showing the maximum inhibition on growth of *Lasiodiploidia spp.* was selected for further studies (Table 1).

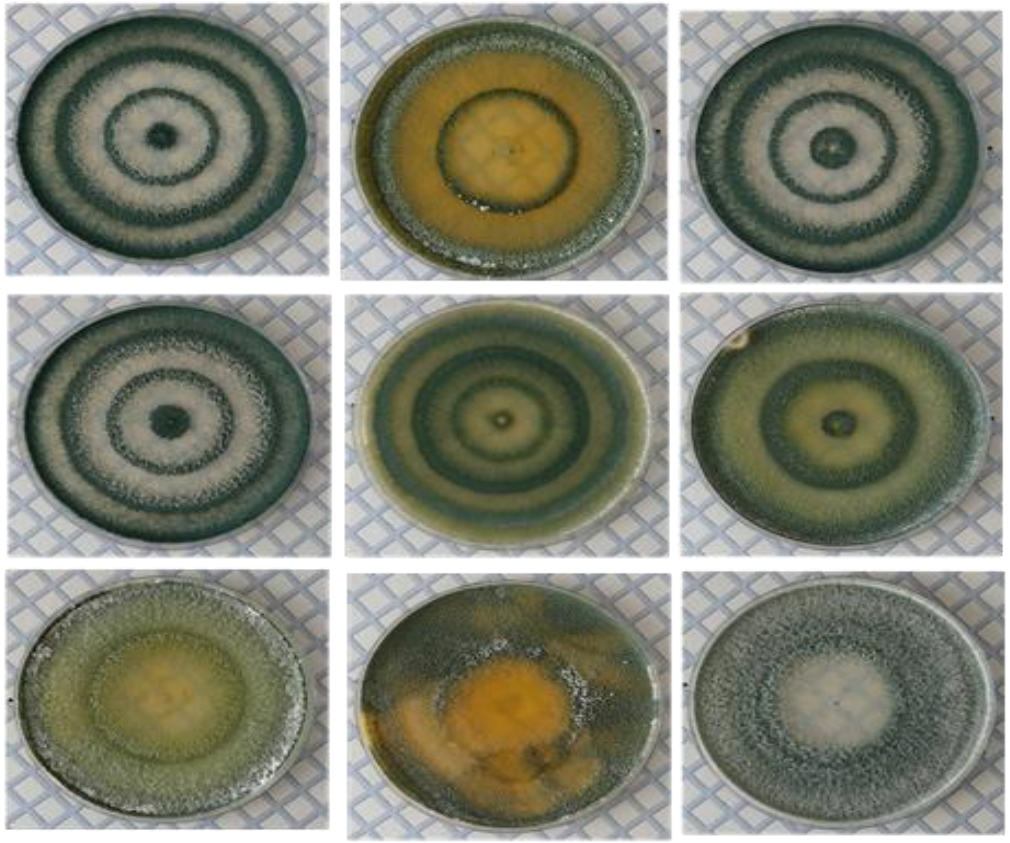

Figure 2. Macroscopic features of the nine isolates of *Trichoderma* used in the study.

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Trichoderma sporulation index

Sporulation index was evaluated in Erlenmeyer Flask with PDA as culture media (Fig. 3) at 29°C during 5 days.

Spore suspension was prepared and inoculated inside the culture medium.

Five days after incubation, 100 mL of distilled water containing tween 80 (0,01%) were added onto the flasks and the spores scraped using an electronic stirring.

Figure 3. *Trichoderma* cultures onto Erlenmeyer Flask.

Dual confrontation

The dual confrontation method was used to screen the bioagents as described by Dennis and Webster (1971). Results was expressed in inhibition percentage.

The Bell scale (Bell et al., 1982) ranging from 1-5 was used to determined the degree of antagonism of each bioagent in confrontation with *Lasiodiploidia spp.* with rate 1 being the most antagonist fungi.

Dennis, C. and Webster, J. 1971. Antagonistic properties of species groups of *Trichoderma*. Transactions of British Mycological Society 57: 25-39.
Bell, D.K., Wells, H.D. and Markham, C.R. 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology, 72: 379-382.

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Table 1. *In vitro* mycelial growth rate and sporulation index of *Trichoderma* isolates.

Trichoderma isolate	MGR	SI
Trichoderma 1BB	0.8	10.1
Trichoderma 2BB	1.1	8.7
Trichoderma 3BB	0.7	4.4
Trichoderma 4BB	0.8	3.4
Trichoderma 5BB	0.7	3.0
Trichoderma 6BB	1.1	8.7
Trichoderma 7BB	1.2	6.4
Trichoderma 8BB	1.2	10.1
Trichoderma 9BB	1.2	9.9

MGR: mycelial growth rate ($mm.h^{-1}$); **SI**: sporulation index ($x10^9$spores/g CS).

Table 2. *Lasiodiplodia spp* inhibition obtained after 3 , 6 and 9 days during dual confrontation in Petri plates.

Trichoderma isolate	Growth inhibition (%)			Bell Scale
	3 Days	6 Days	9 Days	
Trichoderma T1BB	44.4 ± 3.5	55.6 ± 2.6	75.4 ± 3.2	1.0
Trichoderma T2BB	31.8 ± 4.0	42.3 ± 3.0	51.2 ± 5.0	2.0
Trichoderma T3BB	25.3 ± 6.0	35.3 ± 3.1	53.1 ± 2.4	2.0
Trichoderma T4BB	25.9 ± 2.8	35.8 ± 4.0	49.3 ± 1.5	1.7
Trichoderma T5BB	21.5 ± 4.4	21.5 ± 2.2	21.5 ± 2.0	3.7
Trichoderma T6BB	30.6 ± 2.5	40.1 ± 2.8	50.8 ± 2.5	2.3
Trichoderma T7BB	44.4 ± 3.1	52.8 ± 4.0	64.1 ± 2.4	1
Trichoderma T8BB	35.8 ± 2.0	47.5 ± 3.1	60.0 ± 3.1	1.3
Trichoderma T9BB	34.9 ± 4.5	44.9 ± 6.2	58.1 ± 6.6	2.3

Results (Table 1 and 2) showed that, *Trichoderma* 7BB, *Trichoderma* 8BB and *Trichoderma* 9BB exhibited the maximum mycelial growth rate of 1.2 $mm.h^{-1}$. While the higher solubilization index ($10.1x10^9$spores/g CS) was achieved by *Trichoderma* 1BB and *Trichoderma* 9BB. Nine days after incubation, the greatest inhibition (75.4%) was developed by *Trichoderma* T1BB followed by *Trichoderma* T7BB (64.1 %). This fungi also shown higher antagonism potential with a complete colonization of the medium culture.

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Figure 4. Selected isolates after 3, 6 and 9 days incubation during dual culture test. The first line shows the competition between *Lasiodiploidia* (left) and *Trichoderma* T1BB (right) while the second line shows the competition between *Lasiodiploidia* (left) and *Trichoderma* T7BB (right).

- The results obtained (Fig. 4) could be explained by *Trichoderma's* ability to compete for space and nutrients in ecological niche as it is known as a fast-growing fungus.
- *Trichoderma* is also known to act against to plant pathogens by using various mechanisms such as the production of cell wall hydrolytic enzymes, antifungal metabolites, antibiotics, mycoparasitism (De la Cruz-Quiroz et al., 2018).
- These results suggest that the evaluated fungi are fast growing microorganisms with the ability to compete for space in ecological niches.

De la Cruz-Quiroz R., Roussos S.,Rodríguez-Herrera R., Hernandez-Castillo D., Aguilar C.N. 2018. Growth inhibition of *Colletotrichum gloeosporioides* and *Phytophthora capsici* by native Mexican *Trichoderma* strains.

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Thank you for your kind attention