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In vitro Antagonistic Effect of *Trichoderma koningii* Audemas and *Trichoderma longibrachiatum* Rifai on Mango Malformation Disease in Southern Senegal

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***Abstract* – The antagonistic action of Trichoderma koningii Audemas (M35) and Trichoderma longibrachiatum Rifai (M37) was studied in vitro against 13 strains of Fusarium sp. isolated from symptoms of mango malformation disease in southern Senegal. The antagonists and pathogens were cultured on Petri dishes containing PDA medium in direct and remote confrontation for 6 days at 28°C. The 2 Trichoderma species showed inhibitory activity on mycelial growth of the 13 Fusarium strains tested at varying rates according to the strain and confrontation method as well. Inhibition percentages in the presence of *Trichoderma koningii* Audemas (M35) ranged from 13.13 to 57.67 in the direct confrontation and from 3.33 to 33.33 in the remote confrontation tests. In the presence of *Trichoderma longibrachiatum* Rifai (M37), the inhibition percentages went from 09.80 to 37.03% in the direct confrontation. An antagonistic capacity against Fusarium strains was also observed in remote confrontations where the inhibition percentages ranged from 13.33 to 33.33%. Competition and mycoparasitism were the main modes of action shown to inhibit mycelial growth in the 13 strains tested. These results show the potential for using Trichoderma species as a means of controlling mango malformation disease.**

***Keywords* – Antagonist, Fusarium, Mango Malformation Disease, Senegal, Trichoderma.**

I. Introduction

The mango tree (*Mangifera indica* L.) belonging to the anacardiaceae family is considered to be one of the most important fruit crops in tropical regions. The Mango sector has been promising in recent years and a source of economic growth in many countries. World mango production is estimated to reach beyond 43 million tons [1]. Several countries in the West Africa sub-region, including Senegal, are currently spearheading their export activities [2].

In Senegal, mango production has long existed in traditional forms, especially in the south and centre of the country. The country's soil and climate conditions offer great potential for the expansion of mango production [3]. In addition, the downward trend of the main cash crops such as groundnuts and millet has led farmers to seek alternative income-generating activities. This situation has boosted a dynamic of promotion and development of fruit arboriculture and in particular the mango sector. As an example, crop production practices in the south and centre of the country were modernized in traditional orchards and new plantations were created for export. The improvement of the mango sector and the application of appropriate technologies throughout the value chain offer labor and employment opportunities, especially for women and rural youth. The mango sector has become the most dynamic in fruit exports [4].

As a consequence, the mango tonnages have increased to reach 60% of the country's fruit production, over an estimated 150,000 tons per year [5].

Despite the significant growth of the mango sector and the economic interest shown by the population, the yields remain low in Senegal compared to those of other African countries. These low yields could be due to factors like the many diseases that attack the mango tree, including mango malformation disease [6-9]. This disease, also known as mango fusarium head blight, is probably considered to be the most devastating disease in mango trees [10, 9]. This observation is to put in relation with its widespread and destructive nature, and also because of its confusing etiology since its discovery, despite several years of research. Economic losses have been estimated in several countries at between 10 to 90% [9]. The disease manifests itself as a vegetative and floral malformation that directly reduces yield because the affected panicles become sterile [11, 12]. Several abiotic and biotic constraints are associated with mango malformation disease. After several years of continuous research, fungi of the genus Fusarium spp. have been most often reported as the main cause of this disease [9, 13]. A diversity of fusarium species has been identified all over the world. The disease was first recorded in West Africa in southern Senegal in 2009 in Tandouk, Bignona department [8]. It was subsequently also reported in Mboro in the Thies region in 2012 [8]. The disease has been progressing steadily in the South since its discovery. If appropriate control measures, generated by research, are not undertaken, the impact of this disease could result in the complete cessation of mango production activity in the southern regions of the country. In addition, the presence of an infestation outbreak in mango orchards in the south that supply large cities such as Dakar with fruit may expose the Niayes area (next to Dakar), which accounts for 80% of exported volumes, and other West African countries to contaminations. Following several decades of research, no effective control methods have yet been reported [9, 13]. In order to explore control methods for mango malformation in Senegal, the antagonistic activity of *Trichoderma koningii* Audemas (M35) and *Trichoderma longibrachiatum* Rifai (M37) was assessed against 13 strains of *Fusarium sp*.

II. Materials and Methods

Fungal Pathogens:

The 13 Fusarium strains studied were isolated from floral and vegetative malformations of mango trees. Sampling was carried out on roads in southern Senegal every 50 ± 5km over 30 mango trees at a rate of 10 m chosen at random in 3 directions per locality (Figure 1).

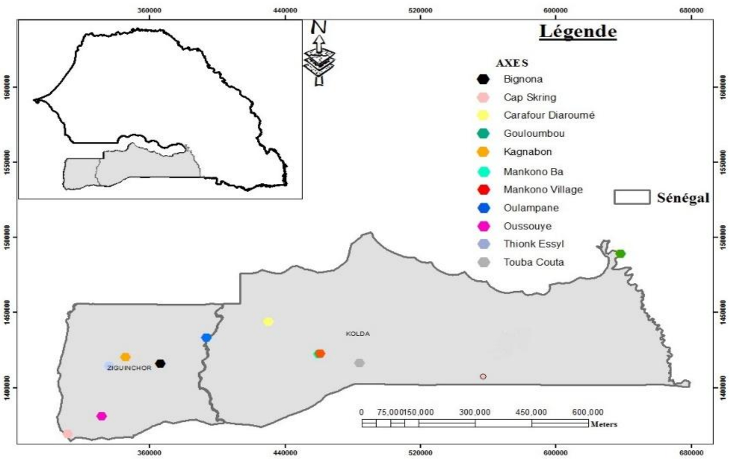

Fig. 1. Location of sampling sites on roads in southern Senegal.

Antagonistic Agents:

Trichoderma koningii Audemans (M35) and *Trichoderma longibrachiatum* Rifai (M37) (Figure 2) were obtained from the National Centre for Scientific and Technical Research in Rabat, Morocco and were grown on a Potato Dextrose Agar (PDA) medium.

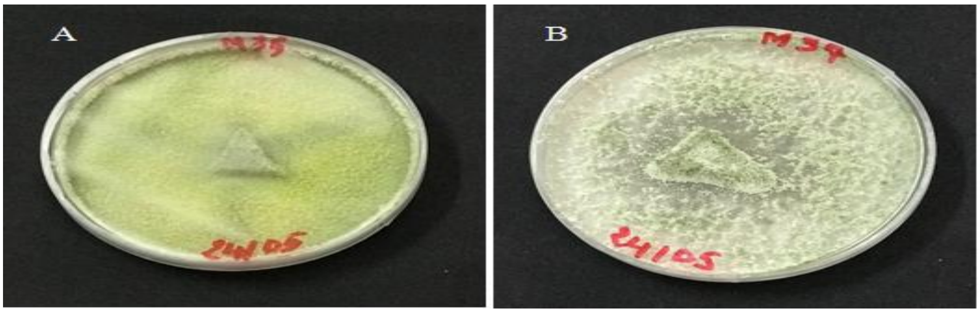

Fig. 2. *Trichoderma koningii Audemas (A)* and *Trichoderma longibrachiatum Rifai (B)* in culture on PDA medium.

A. *Direct Confrontation Test*

For the evaluation of Trichoderma antagonistic activity on the 13 Fusarium strains, the technique of Benhamou and Chet, 1993 was used [14]. For this purpose a 5mm diameter of agar disc colonized by Fusarium sp. mycelium was placed at one end on the Petri dish containing PDA. After 2 days, another Trichoderma mycelium carrot was inoculated on the other end of the same petri dish at a distance of about 5 cm figure 3 [15]. The controls consist only of the pathogen and antagonist grown separately on 2 Petri dishes containing PDA. Daily measurements of the colony diameter were made until the two fungi meet of stop growing towards each other and form an inhibition zone between colonies. The percentage of mycelial growth inhibition were calculated using the formula below [16].

$$PICR = [(R1 - R2) / R1] \times 100$$

PICR : Percentage of inhibition of radial growth.

R1 : Diameter of the control.

R2 : Diameter tested.

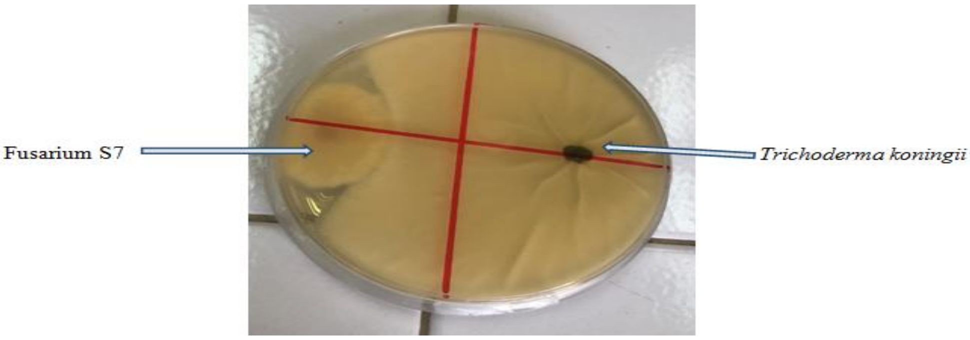

Fig. 3. Growth of a Fusarium strain (S7) and *Trichoderma koningii* in a Petri dish containing PDA medium 6 days after transplantation.

B. *Indirect Confrontation Test*

For the detection of volatile substances. The method of Deniss and Webster, 1971b was used [17]. It consists in transplanting a 5mm diameter agar disc of Trichoderma sp. (M35 or M37) and a *Fusarium sp*. carrot of the same diameter and age (6 days) in two separate petri dishes at the same time. Subsequently the lids were removed and an assembly is carried out by superimposing the two petri dishes so that the Trichoderma box is at the bottom and the Fusarium box at the top. The assembly of the two boxes was reinforced with layers of parafilm to avoid any loss of volatile substances. The control was done by superimposing the two boxes, the top one containing a Fusarium carrot, while the bottom one contains only the PDA medium. The experiment was repeated 3 times for each isolate. The boxes were incubated at 28°C. The average diameter of the treated colonies was recorded 6 days later. Mycelial growth inhibition was estimated as a percentage compared to controls using the previous formula.

C. *Data Analysis*

The data were analyzed with the statistical software R 3.5.1. A two-factor analysis of variance was performed using the aov function of the agricolae package. The Student Newmann and Keuls Multiple Comparison Test (SNK) was done using the SNK test function of the agricolae package.

III. Results

Direct Confrontation Test

The effectiveness of the 2 antagonists: *Trichoderma koningii* Audemans (M35) and *Trichoderma longibrachiatum* Rifai (M37) varied from one strain to the other (Figure 5). Figure 4 indicates that confrontation with M35 resulted in a reduction in mycelial growth of all pathogens tested after 6 days of incubation. Indeed, the percentages of inhibitions of strains 17, 7, 9, 8 and 3 were between 12.12% and 25%. For the other strains, their inhibition rates ranged from 31.70 to 57.14. Strain 6 was the most sensitive with an inhibition of 57.14%.

In the presence of M37, the lowest inhibition percentages ranged from 3.12% to 17.14% for strains S2, S5, S3, S11 and S26 (Figure 4). The inhibition percentages of the other strains ranged from 20 to 37.34. Strain 8 was the most sensitive with an inhibition percentage of 37.34.

Definitely, there is a clear antagonistic activity of the two Trichoderma species on the 13 Fusarium strains tested. The species *Trichoderma koningii* Audemans (M35) showed a higher significant antagonistic effect than *Trichoderma longibrachiatum* Rifai (M37) for the 13 Fusarium strains. Only strains S53 and S6 showed a non-significant difference.

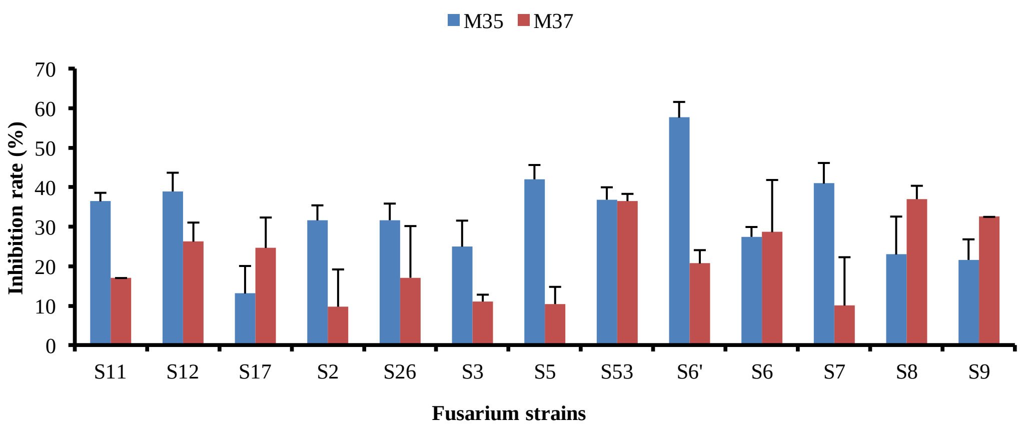

Fig. 4. Percentages of mycelial growth inhibition during direct confrontation between Fusarium and Trichoderma strains (M35 et M37).

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Microscopic observations made at the contact areas between Trichoderma mycelia and Fusarium strains showed changes marked by mycelial lysis, cytoplasm vacuolation and cord transformation of the pathogen mycelial filaments (Figure 5).

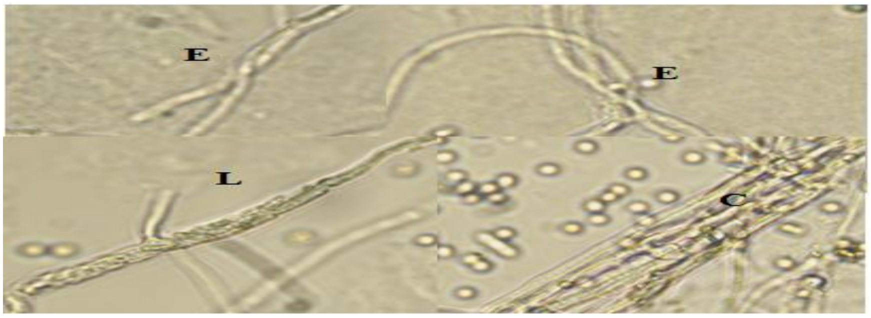

Fig. 5. Mycelial modifications of fusarium strains (E: Winding, C: cord transformation and L: lysis) in the presence of the antagonists (*T. koningii* and *T. longibrachiatum*).

Indirect Confrontation Test

Results obtained after 6 days of incubation showed that the mycelial growths of the controls are greater than those obtained in the presence of *Trichoderma koningii* Audemas M35 or *Trichoderma longibrachiatum* Rifai M37. The percentages of inhibitions varied from one strain to the other with the presence of M35 or M37 (Figure 6 and figure 7).

With the presence of M37, the highest inhibition percentages are observed with strains S17 (33.33%), S53 (31.32%), S11 (30.23%) and S12 (28.07%), and the lowest percentages with strains: S2 (11.11%), S26 (12.22%), S7 (13.09) and S9 (13.33). The other strains displayed an intermediate response.

With the presence of M35, the highest inhibition percentages were obtained with strains S17 (33.33%) and S3 (25.40%) and the lowest with strains S7 (3.33), S8, S9 (4%), S2 (6%), S5 (7.52%), S11 (8.6%), S26, S12 (11%) and S6, S6' (14%).

Fusarium strains that show a significantly different response between the two Trichoderma species are S11, S2, S5, S53, S7, S8 and S9. The other strains (S12, S17, S26, S26, S3, S6, S6, S6') showed responses that were not significantly different for the two Trichoderma species.

The remote confrontation shows that M37 has a higher antagonistic power than M35.

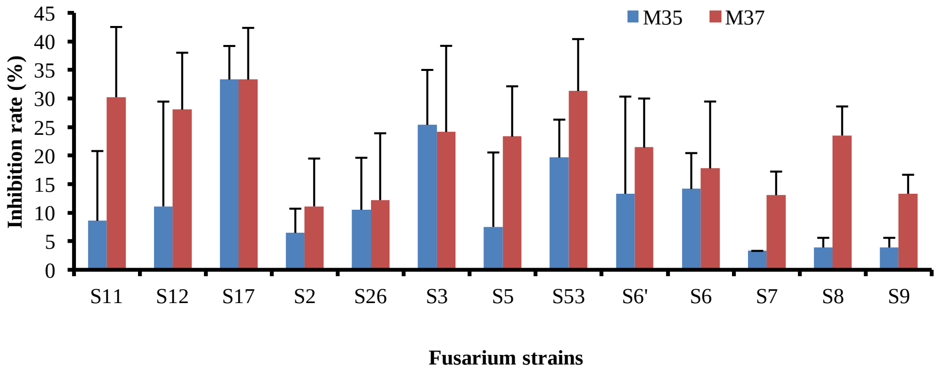

Fig. 6. Percentages of mycelial growth inhibition during indirect confrontation between Fusarium and Trichoderma strains (M35 et M37).

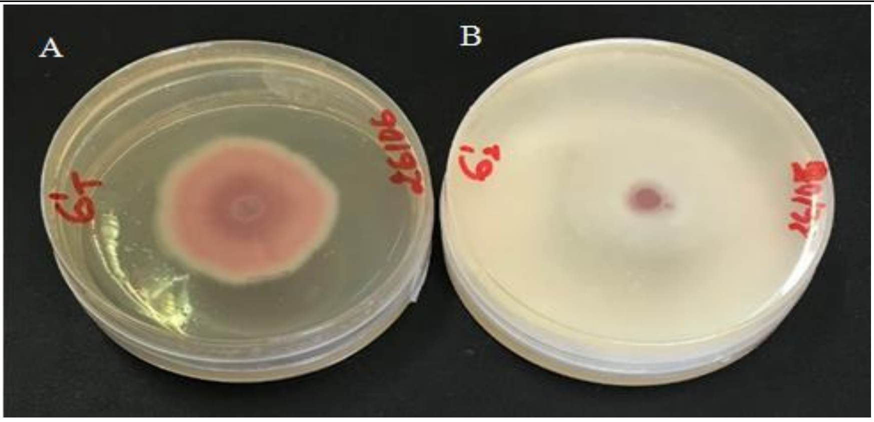

Fig. 7. Growth of S6' strain and *T. koningii* in a Petri dish containing PDA (A) control and (B) during remote confrontation 6 days after transplanting.

IV. Discussion

Mango malformation disease is a major constraint to mango production throughout the world in general and in Senegal in particular. Its control still cannot rely of a recognized effective method, opening the way for research on that matter. The *in vitro* biological control tests carried out revealed that the 2 strains of Trichoderma (*Trichderma koningii* Audemas M35 and *Trichoderma longibrachiatum* Rifai M37) have an inhibitory effect on the mycelial growth of all Fusarium strains at varying degrees. Authors such as Acevas *et al*., 2001; Kumar *et al*., 2012; Arenas *et al.*, 2018 have demonstrated the inhibitory activity of Trichoderma species on mycelial growth in some Fusarium species including *Fusarium oxysporum*, *Fusarium solani* and *Fusarium subglutinans* [18], [19], [20]. These results are in line with those of Kumar *et al*., 2012, which showed that Trichoderma species have a strong inhibition power on Fusarium species. The antagonism obtained in the direct confrontation was stronger than in the remote confrontation. This could be explained by the fact that some Trichoderma species, including *T. koningii* and *T. longibrachiatum*, use several modes of action depending on the nature of the pathogen to exercise their antagonistic power [20]. Despite the absence of direct contact between the Fusarium strains tested and the 2 Trichoderma strains (M35 and M37), the latter were able to exert inhibitory activity on the development of Fusarium colonies. It should be noted that M37 showed a higher percentage of inhibition than M35 during the remote comparison on all strains. This could be explained by the ability of Trichoderma species to produce volatile substances that are able to limit and even stop the development of the pathogen. During the direct confrontation, the Trichoderma colonies occupied the largest surface of the Petri dish. Trichoderma strain M35 showed the highest percentage of inhibition. According to Sharma, 2011 this type of interaction indicates competition [21]. Beyond the 6-day incubation period, the 2 Trichoderma species invaded the colonies of the Fusarium strains except Fusarium strain 53. They sporulated on the mycelium of the Fusarium cultures showing a high myco-parasitic power. This kind of invasion was observed by Benhamou and Chet, 1993 and then by Daami-Remadi and El Mahjoub, 2001 when carrying out a direct confrontation on PDA medium between *T. harzianum* and *Fusarium oxysporum* f. sp. radis-lycopersici [14], [22]. The non-invasiveness of Trichoderma strains on the mycelium of Fusarium strain 53 could be explained by antibiosis. In fact, some Trichoderma species have the potential to produce toxic metabolites that have a biocidal effect by inhibiting the growth of various pathogens [23].

Furthermore, a winding of the Trichoderma mycelium on that of Fusarium was also observed on the myceliu-

-m of most Fusarium strains. The ability illustrates the high myco-parasitic power of both Trichoderma species on Fusarium.

V. Conclusion

The results of this study lead to the conclusion that the 2 Trichoderma species (*T. koningii* Audemas M35 and *T. longibrachiatum* Rifai M37) have an antagonistic power against the 13 Fusarium strains tested. This conclusion derives from both direct confrontation and remote activity through volatiles. Beside competition, an additional mode of action in terms of mycoparasitism have been identified. These results encourage *in vivo* tests with Trichoderma strains against Fusarium species associated with mango malformation disease.

Acknowledgement

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