

The Minimum Inhibitory Concentrations exerted by the N-salicylidene-2-substituted aniline against various strains of phytopathogenic

Mohamed Yazid Belghit, Zohir Nedjar, Abdelhamid Moussi, Djamel Barkat

Abstract— *Fusarium* genus includes several plant pathogenic fungi that may attack a large number of plants, causing diseases called fusarioses. In our strategy of the fight against this kind of fungus *in vitro*, we tested the acidity and the basicity of the culture medium (PDA) on mycelial growth of this genus of fungus, followed by the determination of the minimal inhibitory concentration of synthetic compounds (Schiff bases) such as N-salicylidene-2-methoxyaniline and N-salicylidene-2-nitroaniline against three species plant pathogenic: *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium verticillioides*, using the agar dilution method at different concentrations of these two Schiff base derivatives and Tachigaren 30% SL as a standard fungicide. We found that the low acidity of the culture medium revealed better mycelial growth. The results show that the inhibition of mycelial growth seems to be proportional to the concentration of these compounds, however a better activity was revealed for the compound N-salicylidene-2-methoxyaniline with Minimum Inhibitory Concentration (MIC) =10 mg / ml against of the *F. culmorum* and *F. graminearum* strains. The two compounds tested showed a diversity of activity for all the fungal strains. This may be due to the presence of the various substituted groups (methoxy and nitro) on the structure of these compounds.

Index Terms— *Fusarium*, culture medium, Schiff bases, Azomethines, fungicide, Potato Dextrose Agar, activity antifungal.

I. INTRODUCTION

Azomethines are the compounds which contain the group $-C=N-$. These compounds are also known as imines, but most often they are known as Schiff bases [1]. In addition, many workers reported a wide range of biological activities of azomethines (Antibacterial, antifungal, anti-inflammatory and antiviral) [2]-[4]. Furthermore, the biological properties of the Schiff bases can be attributed to different groups present in their structures, namely: hydroxyl (OH), methoxy, halogens as well as certain hetero-atoms, such as nitrogen, oxygen and sulfur [5]. The great interest given to these compounds is due mainly to their easy preparation process, their obtaining in good yields and their very varied physicochemical properties [6]-[7], however. The researchers

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always seek to optimize and modify these molecules to arrive to numerous applications [8]-[10].

In economic terms, filamentous fungi belong to the genus *Fusarium*, which includes many plant pathogenic species that can induce disease (fusariosis) in many plants, such as cereals (maize, wheat, barley, and oats), vegetables, ornamentals and many fruit trees. The majority of *Fusarium* species are capable of producing mycotoxins. These are the most important in agriculture and their types are Trichthecenes, Zearalenone and Fumonisin [11] which are produced by the species *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium verticillioides* respectively.

In this work, we studied the mycelial growth of these strains especially on the acidic and basic culture mediums, however the best culture medium was selected to carry out the tests of the effect of the two Schiff base derivatives against three phytopathogenic species (*Fusarium culmorum*, *Fusarium graminearum* and *Fusarium verticillioides*) to determine *in vitro* the minimum inhibitory concentrations of these derivatives.

II. MATERIAL AND METHODS

A. Materials: chemistry and biological

The compounds of azomethine (Fig. 1) were synthesized in our laboratory of molecular chemistry and environment, Department of industrial chemistry, Faculty of Science and Technology, Mohammed Khider-BISKRA University-Algeria [12]-[13].

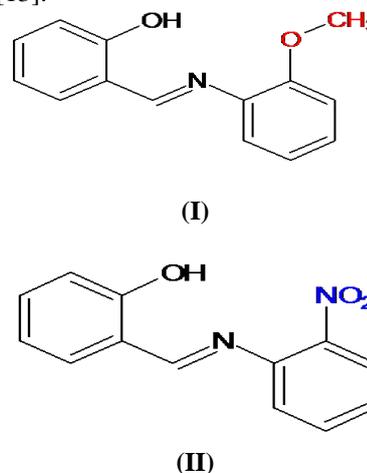


Fig 1: Chemical structures of the N-salicylidene-2-methoxyaniline (I) and N-salicylidene-2-nitroaniline (II)

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Commercial fungicide used: Tachigaren 30% SL (hymexazol) was used as a commercial fungicide.

Fungal material

Three fungal strains of the genus *Fusarium* such as *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium verticillioides* were collected from the laboratory of Plant Pathology and Molecular Biology, the Higher National Agronomic School (El Harrach-Algiers, Algeria).

B. Methods

Preparation of culture medium; the PDA (Potato Dextrose Agar) used as a culture medium for the mycelial growth of the strains to be tested was prepared as follows:

-200g Potato +20g Dextrose + 15 g Agar + 1000ml of distilled water

-The Sterilization in a autoclave at 120° C for 30 min

-PDA Standard (pH of is equal 5.8)

Effect of pH; this test is to determine the optimum pH of the culture medium for a better growth of the strains. A range of pH (4, 5, 7.5 and 9) is obtained by the addition of 0,1N HCl or 0,1N NaOH in standard culture medium (pH = 5.8). The Petri plates were seeded of from a young and pure culture of seven days. The incubation was performed in an incubator at 28° C for 7 days. The average of two perpendicular diameters of mycelial growth was measured daily for 7 days of incubation.

Determination of the minimum inhibitory concentrations (in vitro); to test in vitro the effect of the compounds on the inhibition of mycelial growth of the three species (*F.culmorum*, *F.graminearum* and *F. verticillioides*) using the agar dilution method [14]. This method consists in diluting the two Schiff base derivatives in 20 ml / ml of the PDA culture medium in a melting state, and this for each concentration of these compounds (C₁= 1mg /ml; C₂= 2mg /ml; C₃= 4mg /ml; C₄= 8mg /ml; C₅= 10mg /ml; C₆=11mg /ml; C₇=12mg /ml).

After solidification of the mixture, the seeding was carried out by 5 mm diameter culture disks placed on the center of each Petri dish. Then we determined the minimum inhibitory concentration (MIC).This is the lowest concentration of the Schiff base inhibiting any growth of the mycelium visible to the naked eye after 7 days of incubation at 28° C.

The tests were carried out in the solvent DMSO is the most used and the most suitable. We carried out the same experimental protocol for commercial fungicide (Standard).

III. RESULTS AND DISCUSSION

A. The influence of ph of culture medium on mycelial growth

The pH of the medium affects the diametrical growth of the three species. The optimum of mycelial growth (Figs 2 and 3) is 86.06 mm to 76.97mm, 80.65mm to 70.84 and 65.27 to 75.47mm for strains of *F. culmorum*, *F. graminearum* and *F. verticillioides* respectively. Is observed at pH 5 to 7.5

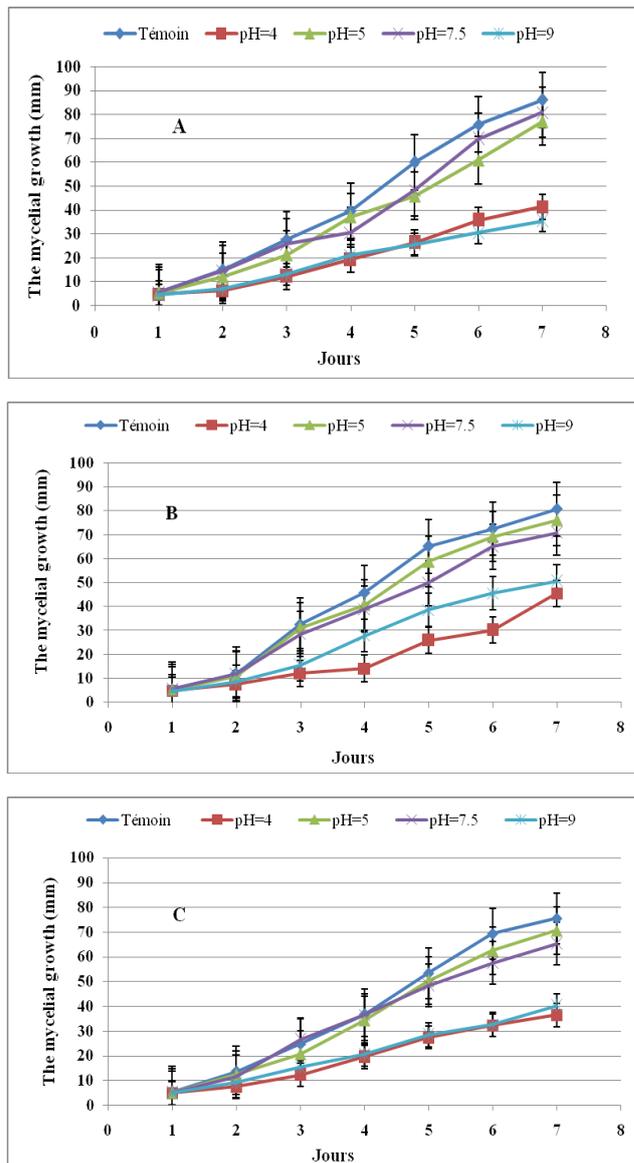


Fig. 2: Kinetics of mycelial growth of the three species to different value of pH of the culture medium (A: *F. culmorum*, B: *F. graminearum* and C: *F. verticillioides*).

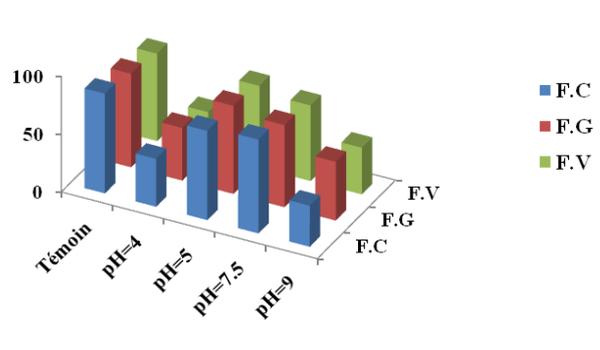


Fig. 3: Effect of pH on the mycelial growth of three species of *Fusarium* after seven days of incubation

The results obtained show that the perfect growth of the mycelium at a pH = 5.8 and strains able to tolerate pH values of 5 and 7.5 while their development back in media with pH of about 4 and 9.

B. Determination of the MIC

The results of the evaluation of the absence of mycelial growth of our compounds were obtained according to the above method. The results of the MIC values of compounds are shown in table I.

All fungal strains were inhibited by the compounds **I** and **II** at different MIC values and are compared with the fungicide .

Different values of MIC obtained allow us to see that the Schiff base (**I**) shows improved activity relative to the base (**II**) on *F.culmorum* and *F.graminearum*.

The figs 4 and 6 show that the highest activity of (**I**) due to the presence of the methoxy group (electron donor) on the aniline with the exception of the nitro group (electron-withdrawing) leads to a low activity is shown in Figs 5 and 7.

Table I: The values of the Minimum Inhibitory Concentrations of the Schiff bases

Compounds	<i>Fusarium culmorum</i>	<i>Fusarium graminearum</i>	<i>Fusarium verticillioides</i>
	Minimum Inhibitory Concentrations (mg/ml)		
(I)	10	10	>12
(II)	12	11	>12
Fungicide	11	08	12

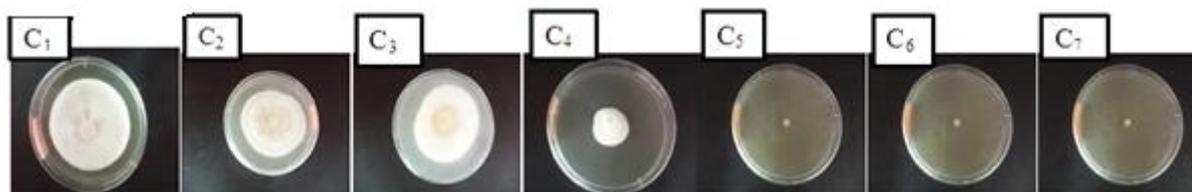


Fig.4: Effect of N-salicylidene-2-methoxyaniline on inhibition of *F.culmorum* growth

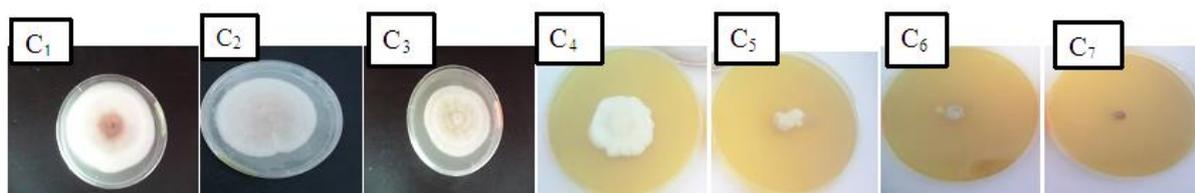


Fig. 5: Effect of N-salicylidene-2-nitroaniline on inhibition of *F.culmorum* growth

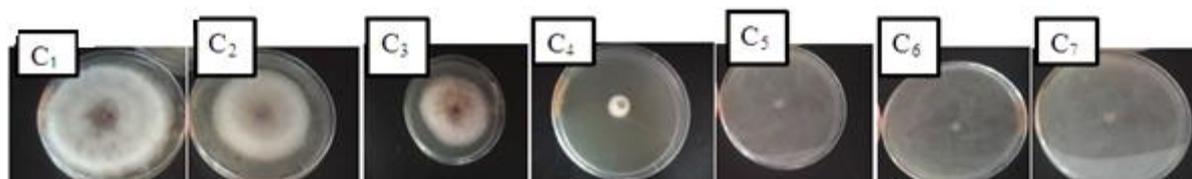


Fig.6: Effect of N-salicylidene-2-methoxyaniline on inhibition of *F.graminearum* growth

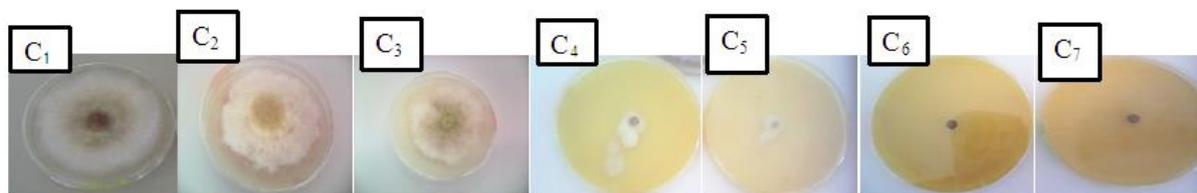


Fig.7: Effect of 2- N-salicylidene-2-nitroaniline on inhibition of *F.graminearum* growth

Chen et al 1996, Vinita et al, 2013 [15]-[16] showing that the Schiff bases may contain various substituent (groups of electron-withdrawing or electron-donating) and may have interesting structural chemical properties.

IV .CONCLUSION.

The present study examines the effect of the pH of the culture medium on mycelial growth and the inhibitory activity of two Schiff bases (**I** and **II**) against three species of

Fusarium. Our results indicate that the inhibitory activity *in vitro* depends on the molecular structure of these compounds. However, the highest activity reported for the compound (**I**) on the strains of *F.culmorum* and *F.graminearum*. We recorded the pH = 5.8 standard medium was promoted good growth for three strains while density of mycelial remains low when the alkalinity and acidity of the medium is very important.

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