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IN VITRO ANTIFUNGAL ACTIVITY OF SOME SCHIFFBASES DERIVED FROM *ORTHO*-HYDROXYBENZALDEHYDE AGAINST *FUSARIUM*

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Abstract

Schiff bases derivatives from *ortho*-hydroxybenzaldehyde (salicylaldehyde) were prepared and characterized by infrared spectroscopy and elemental analysis and by measurement of their melting points. Indeed, the mode of ultraviolet absorption of Schiff bases has carried out in five solvents of different polarity (cyclohexane, toluene, ethanol, DMSO and DMF). The tests antifungal activity *in vitro* of the Schiff bases obtained were performed in DMSO at concentrations: 01, 02, 04 and 08 mg / ml against *F. culmorum*, *F.graminearum* and *F. verticillioides*, using the agar dilution method. The results revealed that the inhibitory power of Schiff bases seems to be proportional to the concentration. The inhibition highest of the *N*-salicylidene-3-methoxyaniline and *N*-salicylidene-4-chloroaniline at 08 mg / ml against *F. culmorum* was 100%. In the case of N-salicylidene-3-methylaniline, *N*-salicylidene-4-methoxyaniline, *N*-salicylidene-3-chloroaniline and *N*-salicylidene-4-chloroaniline at 08 mg / ml against *F. graminearum* reaches 100% with the exception of the *N*-salicyliden-2-nitroaniline at 08 mg / ml was 31.53, 40.92 and 38.29%, proves weakly active against *F. culmorum*, *F. graminearum* and *F. verticillioides* respectively. However, the mode of absorption of *N*-salicylidene-2-nitroaniline in DMSO and DMF indicated the presence of bands above 400 nm; these bands characterize the existence of tautomer keto-amine. Indeed, the action of the substituent's a donor or acceptor (R = CH_3-, CH_3O-, Cl or NO_2 on the aniline at the *ortho*, *meta* or *para*) and the effect of the solvent influenced the process of the tautomerism and thus the antifungal activity *in vitro*.

Keywords: Agar dilution method, antifungal activity, *Fusarium*, Schiff bases, substituted aniline, electronic absorption.

Nomenclatures

SA	*N*-salicylideneaniline

Greek Symbols

ε	Molar coefficient, L mol^{-1} cm^{-1}.
λ_{max}	The Wavelength of maximum absorption, nm.

Abbreviations

DMF	Dimethylformamide.
DMSO	Dimethylsulfoxide.
F	*Fusarium.*
GM	Gentamycin.
IR	Infra-Red
M.p	Melting points, °C
PDA	Potato Dextrose Agar

1. Introduction

All compounds contents of imine function (-C=N-) are appointed Schiff bases, these compounds were represented an important class of diversity of properties and applications. The studies of the Schiff bases are rapidly developed, because they have excellent characteristics such as structural similarities with natural biological substances [1].

The Schiff bases have been showed the evaluation of antibacterial [2], antifungal [3-6] and cancer [7].The diversity presence of the substituent in the phenyl rings of aromatic Schiff bases are responsible for the antifungal activity, which can be changed depending on the substituent present on the aromatic rings [8]**.** Schiff bases may contain various substituents with various electron-withdrawing or electron-donating groups, and may have interesting chemical structural properties. They cause a specific interest of their biological activities [9].

2-Hydroxy Schiff bases were realised to a equilibrium between two tautomeric forms a enol-imine and form keto-amine (OH...N and O...HN), these forms belong to the existence of intramolecular hydrogen bond in the presence of the hydroxyl group in the α imine function [10-14].

The studies of spectroscopic in UV-visible absorption of 2-hydroxy Schiff bases have allowed a hypothesis on the nature of the forms existed in certain conditions. Namely: the nature of the solvent and the substituent. A purely, enol form due to the presence of bands of absorption below 400 nm, while those adopting the keto form (in equilibrium with the enol form) absorb at above 400 nm [15-17].

In this study, Schiff bases of substituted aniline in position *ortho*-, *meta*-, or *para*-, with *ortho*-hydroxybenzaldehyde have been prepared and characterized to investigate the electronic absorption behaviour and the antifungal activity of the Schiff bases against plant pathogenic fungus like *Fusarium* for three species *F. culmorum*, *F. graminearum* and *F.verticillioides.*

2. Materials and methods

2.1. Chemistry

All chemicals and solvents used were obtained commercially from Sigma-Aldrich. The melting points of solid Schiff bases were determined by electro thermal m.p apparatus model BUCHI-540. Infra-Red spectra were recorded as KBr pellets in the range (4000-400cm^{-1}) on a Shimadzu FT-IR-8400S Spectrophotometer. Elemental analyses were obtained using an atomic emission spectrometer (Inductively Coupled Plasma). The electronic spectra of the solution were investigated in various solvents of different polarities. The electronic spectra were recorded on a Shimadzu UV-1240 spectrophotometer using 1cm quartz cell at room temperature with concentration 10^{-4}mol /L.

2.2. Preparation of Schiff bases (1-12)

A stirred mixture of *ortho*-hydroxybenzaldehyde (0.01mol) with *o*-methyl, *m*-methyl, *p*-methyl, *o*-methoxy, *m*-methoxy, *p*-methoxy, *o*-chloro, *m*-chlore, *p*-chloro, *o*-nitro, *m*-nitro, or *p*-nitroaniline (0.01mol) dissolved in ethanol (15 ml) were heated to reflux temperature to 70 °C for 2 hours. The mixture is cooled to room temperature follows then concentrated by evaporation of the solvent. The solid product obtained was purified by re-crystallization from ethanol [18-19]. The physical, analytical and spectroscopic data for the synthesized Schiff bases are summarized in Tables 1 and 2.

Hadj et al. [20] showed that the presence of bands in the vicinities of 1613 cm^{-1} and 3340cm^{-1} corresponding to the azomethine and phenolic hydroxyl respectively, these they is find in the Schiff base unsubstituted (*N*-salicylideneaniline), is an analogue of our Schiff bases. The position of *ν*(C=N) changes with changes in the molecular structure of the Schiff base; the position of this band varies depending on the type of substitution in the aromatic ring. Furthermore, the elemental analysis of the SA (Calculated C79.19;H5.58; N7.10%; Found; C79.25;H5.55; N7.18%) consisting of the same order of the values of our compounds.

Table 1. Physical and infra-red spectral data of Schiff bases.

Schiff bases	Colour	%Yield	M.p.°C	IR (cm^{-1})	
				ν (OH)	*ν*(C=N)
1	Yellow	85	52	3418	1618
2	Yellow	75	54	3450	1619
3	Yellow	80	91-93	3450	1619
4	Yellow-green	80	58-60	3420	1618
5	Yellow	70	62	3440	1603
6	Grisâtre	85	90	3419	1621
7	Yellow	78	90	3438	1595
8	Yellow	88	97	3424	1620
9	Yellow	85	123	3421	1595
10	Orange	60	65	3430	1630
11	Yellow	65	119	3480	1604
12	Yellow-orange	70	146	3440	1629

Table 2. Elemental analysis data of Schiff bases.

Schiff bases	Calculated (Found)		
	% C	% H	% N
1	79.52 (78.84)	5.15 (5.19)	6.63 (6.6)
2	79.52 (78.81)	5.15 (5.15)	6.63 (6.57)
3	79.52 (78.79)	5.15 (5.17)	6.63 (6.59)
4	73.99 (73.12)	5.77 (5.89)	6.16 (6.12)
5	73.99 (73.10)	5.77 (5.85)	6.16 (6.09)
6	73.99 (73.13)	5.77 (5.87)	6.16 (6.11)
7	67.39 (66.97)	4.35 (4.31)	6.05 (6.00)
8	67.39 (66.99)	4.35 (4.28)	6.05 (6.03)
9	67.39 (66.95)	4.35 (4.32)	6.05 (6.01)
10	62.8 (62.2)	3.93 (3.81)	12.02 (11.97)
11	62.8 (62.24)	3.93 (3.85)	12.02 (11.99)
12	62.8 (62.22)	3.93 (3.83)	12.02 (11.98)

2.3. Fungal strains and culture medium

Three fungal strains to the genus *Fusarium* (phytopathogenic fungus) such as: *F. culmorum, F.graminearum* and *F.verticillioides* were collected from the laboratory of Phytopathology and molecular Biology, National High School of Agronomy (El Harrach, Algiers, Algeria). Using PDA like culture medium for the mycelia growth of the fungal strains to be tested. The PDA was prepared in the laboratory by composition of 200 g potato, 20 g dextrose, 15 g agar and 1000 ml distilled water, this medium selectivity fungus by adding an antibiotic (GM). The medium was sterilized in an autoclave at 120 °C for 30 min.

2.4. Commercial fungicide used

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

2.5. Antifungal activity (*In vitro*)

The Schiff bases (1-12) synthesized were tested on three fungal species viz: *F.culmorum, F. graminearum* and *F. verticillioides*, the test was determined by the calculating the percentage of inhibition *I (%*) by using agar dilution method [21-22] and hymexazol was used as a standard fungicide. The test consists of preparing solutions of Schiff bases in DMSO at concentration of 01 mg/ml, 02 mg/ml, 04 mg/ml and 08 mg/ml, these solutions were incorporated into 20ml of culture medium sterilized molten state at a 45 °C temperature in Petri dishes. In parallel a control (DMSO) was prepared in the PDA medium sterilized without Schiff bases. After solidification of the mixture, the performing seeding by a levy of a mycelium disc (5 mm diameter) of each yellow and pure culture, this disc was placed in the center of each Petri dish. The test was repeated three times for each concentration. By measuring the average of two perpendicular diameters of the mycelia growth in millimetres (mm) after seven days of incubation at 27±2°C. The percentage inhibition of fungal growth was calculated as follows:

$$I = \left[\frac{C-T}{C}\right] \times 100$$

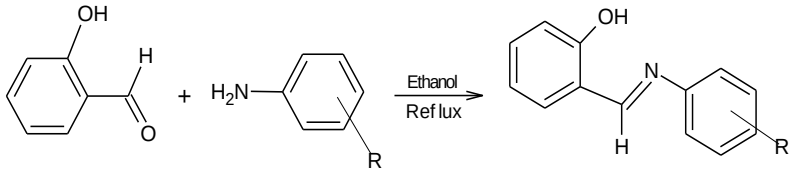
where I is the percentage of inhibition (%), C is the diameter of the mycelia growth (mm) of the control plate and T is the diameter mycelia growth (mm) of the test plate.

2.6. Statistical analysis

To better visualize the effects Schiff bases on three strains *Fusarium*. A statistical method of data by principal component analysis (PCA), using the software of PAST version 2.17 [23].This multivariate method displays the projection of products and the direction of variable vectors on the plane defined by the first two composents.

3. Results and Discussion

Schiff bases (1-12) were obtained in good yields by condensation reaction of *ortho*-hydroxybenzaldehyde and the substituted aniline as shown in Scheme.1



R= *o*-CH_3 = *o*-methyl-SA **(1)**	R= *o*-Cl = *o*-chloro-SA **(7)**
R= *m*-CH_3 = *m*-methyl-SA **(2)**	R= *m*-Cl = *m*-chloro-SA **(8)**
R= *p*-CH_3 = *p*-methyl-SA**(3)**	R= *p*-Cl = *p*-chloro-SA **(9)**
R= *o*-OCH_3 = *o*-methoxy-SA **(4)**	R= *o*-NO_2 = *o*-nitro-SA **(10)**
R= *m*-OCH_3 = *m*-methoxy-SA **(5)**	R= *m*-NO_2 = *m*-nitro-SA **(11)**
R= *p*-OCH_3 = *p*-methoxy-SA **(6)**	R= *p*-NO_2 = *p*-nitro-SA **(12)**

Scheme.1. Synthesis reaction of Schiff bases 1 to 12.

The Schiff bases are soluble in organic solvents such as ethanol, the DMF, the DMSO, cyclohexane and toluene but insoluble in water

The IR spectra data of all the compounds formation contains a band in the region 1595-1630 cm^{-1} which is attributed to azomethine group (C=N) stretching vibration confirms the formation of the Schiff base [20, 24].The characteristic phenolic (O-H) band, due to the presence of a hydroxyl group at *ortho* position in the Schiff bases was observed at 3418-3480 cm^{-1}.

Infrared spectroscopy and elemental analysis are good for identifying the structures of our compounds because of the flexibility and simplicity of the method for synthesis these Schiff bases derivatives. However, the results obtained are consistent with those reported in the literature for analogous compounds [20].

3.1. Electronic absorption spectra.

Electronic absorption spectra of Schiff bases were recorded between 200 and 500 nm in solvents of different polarities namely ethanol, DMSO, DMF, cyclohexane and toluene. The results of spectra data are summarized in Table 3.

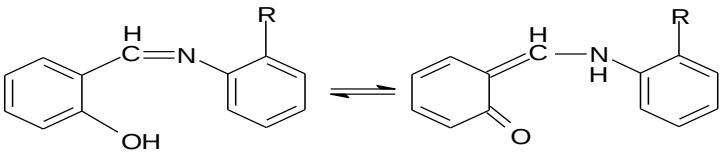
Table 3. Electronic absorption bands of Schiff bases

Schiff bases	[λ_{max} (nm), ε (L mol^{-1} cm^{-1})]				
	Cyclohexane	**Toluene**	**Ethanol**	**DMSO**	**DMF**
1	268, (7800) 340, (5200)	345, (6500)	265, (5400) 335, (4200)	340, (4800)	343, (6800)
2	268, (7200) 340, (6000)	345, (6000)	266, (5200) 338, (4400)	343, (4800)	343, (4600)
3	268, (7700) 340, (6200)	343, (5000)	260, (6000) 339, (5600)	343, (5400)	343, (4800)
4	265, (9200) 345, (6400)	345, (4800)	260, (9000) 338, (7200) 445, (0800)	338, (4800)	330, (5400)
5	265, (8800) 340, (4600)	340, (5800)	260, (8200) 335, (6200)	339, (5600)	340, (6200)
6	265, (9400) 345, (5600)	345, (6700)	260, (8800) 338, (6000)	338, (6000)	330, (6600)
7	270, (9200) 345, (5600)	345, (5800)	275, (7200) 345, (5400)	345, (1400)	345, (6200)
8	270, (6200) 345, (4000)	345, (4600)	255, (9000) 330, (4200)	345, (4200)	340, (5400)
9	270, (6200) 345, (4600)	345, (3200)	255, (6200) 345, (5000)	345, (5800)	345, (6200)
10	270, (6200) 365, (5400)	375, (4000)	275, (6000) 405, (5600)	415, (5500)	410, (6000)
11	270, (8900) 348, (4200)	348, (4800)	255, (7200) 330, (1800)	345, (3400)	340, (4000)
12	280, (6800) 310, (7200) 360, (6000)	360, (4200)	255, (4800) 370, (6200)	365, (5200)	360, (5800)

The molecular structure of the compound and the physico-chemical properties of the medium involved on changes in the intensity and the displacement of the absorption band whatsoever to blue or the red [25]. The bands in the range 210 to 280 nm are assigned to the excitation of π- electrons of the aromatic system [26].

The bands appear in the range from 330 to 375 nm attributed to the charge transfer to the intramolecular interaction at the inside of the entire molecule [27]. The charge transfer bands are more sensitive to variations solvent and the substituent [27].Indeed, the mode of absorption Schiff bases **(1), (2)** and **(3)** in cyclohexane and ethanol revealed two bands absorption; the first located about 260-288 nm and 335-340 nm located toward second characterize the enol-imine tautomer. It is also obtained in the solvents toluene, DMF and DMSO and this by the appearance of a single band situated around 343-345 nm. Is thus obtained under these conditions to a single tautomer form constituted by a purely enol form and reflected by absorption below 400 nm. Furthermore, noting us also for Schiff bases **(5), (6), (7), (8), (9), (11)** and **(12),** their absorption (265-280 nm, 310-360 nm), (340-360 nm), (265-275nm, 330-370nm), (338-365 nm) and (330-360 nm) corresponding to the cyclohexane solvent, toluene, ethanol, DMSO and DMF, respectively, they are characterized by the same enol form. The stability of this

form is probably due to the establishment of the intramolecular hydrogen bond entre les nonbinding doublet nitrogen and the phenolic hydroxyl group. Contrary to what type of shape; the case of Schiff bases **(10)** showed an absorption band of above at 400 nm and this due to the presence of *o*-nitro electron withdrawing which promotes the formation of the tautomer keto-amine. This results in the bands at 405 nm, 410 nm and 415 nm, observed in polar solvents; DMF and DMSO respectively, with the exception of ethanol, the tautomer keto-amine in equilibrium with the tautomer enol-imine (Scheme.2). While in cyclohexane and toluene exclude this form and lead to the absorptions below 400 nm (enol form).



Enol-imine keto-amine

Scheme.2: Keto-enol tautomerism of *N*-salicylidene-*o*-nitroaniline and *N*-salicylidene-*o*-methoxyaniline in ethanol (R= NO_2-, CH_3O-).

On the other, the compound **(4)** shown in ethanol the existence of an equilibrium between two tautomer forms: imine enol / keto-amine (Scheme.2), where the dominant enol form, this equilibrium and reflected by the absorptions 260, 338 and 445 nm (λ_{max} = 445 nm showed very low intensity: ε = 800 L mol^{-1} cm^{-1}) due to electron donating group (*ortho*-methoxy). As for the other remaining solvents result the absorptions below 400 nm and this mean that the predominant form enol-imine.

The charge transfer band shifts to longer wavelength (bathochromic) with a change of substituent in aniline ring in the order CH_3- < Cl < CH_3O- < NO_2, this is due to the electron-withdrawing nature of the substituent [25]. The substituted group position in the compounds leads to an inductive effect ordered as: *para*<*meta*<*ortho.*

3.2. Evaluation of antifungal activity.

All the Schiff bases were studied against *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium verticillioides*, for determining *in vitro* antifungal activity. The results obtained are presented in Figs.1 to 6. (Comparing the test results of the antifungal activity by the commercial fungicide: Hymexazol).

3.2.1. Antifungal activities of Schiff bases against *F. culmorum*

As shown in Figs. 1 and 2, the Schiff bases were shown antifungal activities at all the tested concentrations against *F. culmorum.* The *m*-methoxy-SA showed better antifungal activity than hymexazol at 01 mg / ml with an inhibition percentage of 25.95%. The inhibition percentages of *m*-methoxy-SA, *p*-chloro-SA enhanced

with increasing their concentrations, and they reach up to 100% at a concentration of 08 mg / ml.

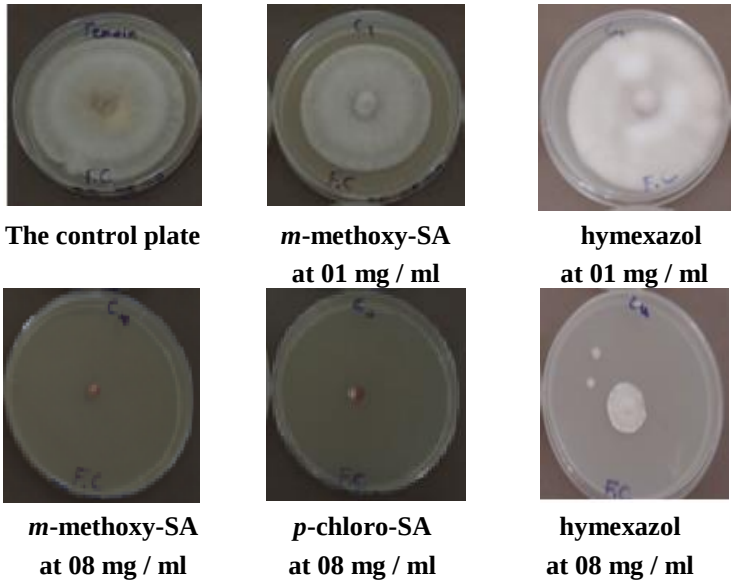


Fig. 1. Inhibitory effect of compounds on *F. culmorum* strain tested.

The *m*-methyl-SA and *p*-methoxy-SA exhibited much better antifungal activities than *o*-methyl-SA, *p*-methyl-SA, *o*-methoxy-SA, *o*-chloro-SA and *m*-chloro-SA. However, their antifungal activities against *F. culmorum* did not reveal apparent improvement than hymexazol at 08 mg / ml**.** (The study of such equilibrium by electron spectroscopy (UV-Visible) of these 2-hydroxy Schiff base in a series of solvent, in order to highlight the structural form that will react during the process of antifungal activity [25, 28]).

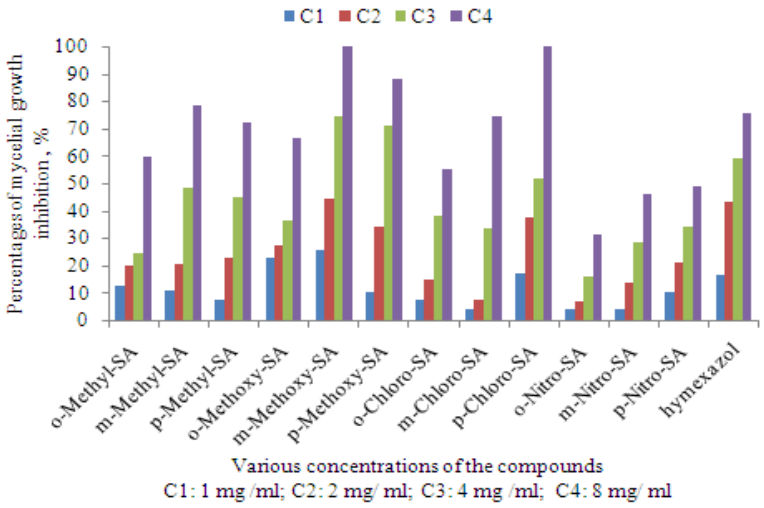


Fig. 2. Antifungal activities of Schiff bases against *F. culmorum*.

3.2.2. Antifungal activities of Schiff bases against *F. graminearum*.

The Schiff bases were shown of good antifungal activities against *F. graminearum*. The inhibition percentage of *p*-methoxy-SA at 01 mg / ml was 39.28 % whereas the inhibition percentages of *m*-methyl-SA, *p*-methoxy-SA, *m*-chloro-SA and *p*-chloro-SA at 08mg/ml were 100% which is the same of hymexazol. However, the inhibition percentages of *o*-chloro-SA, *o*-methyl-SA, *p*-methyl-SA, *o*-methoxy-SA, *m*-methoxy-SA, *m*-nitro-SA and *p*-nitro-SA, were greater than 50 % at 08 mg / ml (Figs.3 and 4).

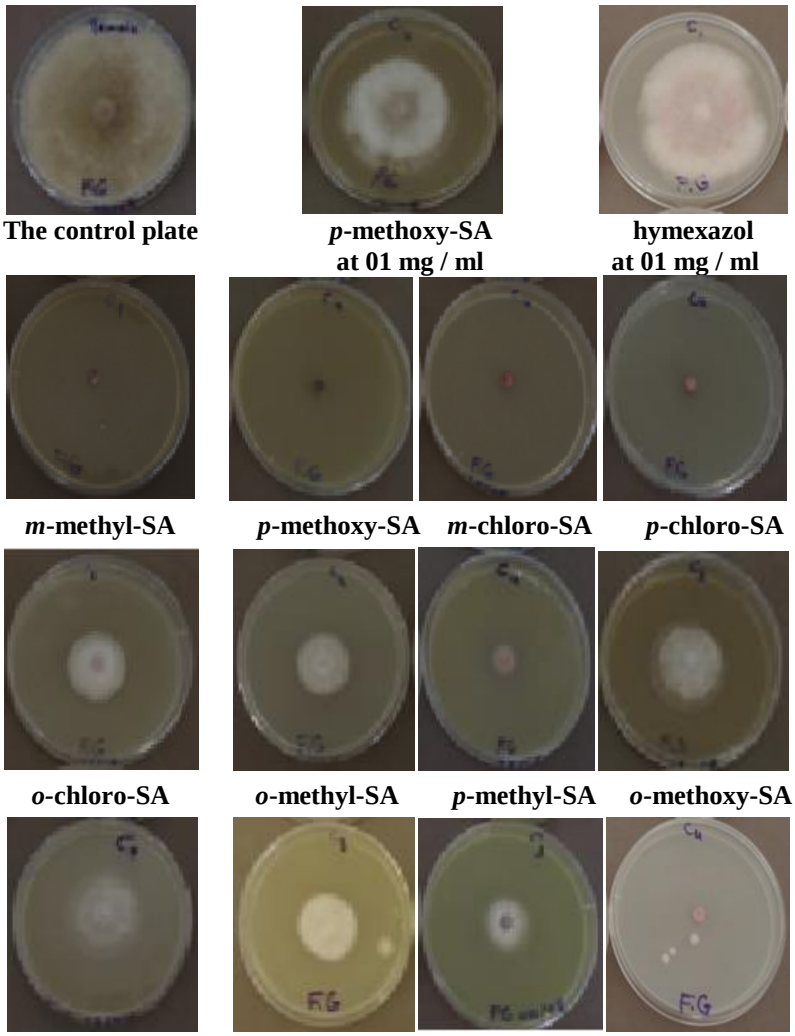


Fig. 3. Inhibitory effect of compounds on *F. graminearum* strain tested.

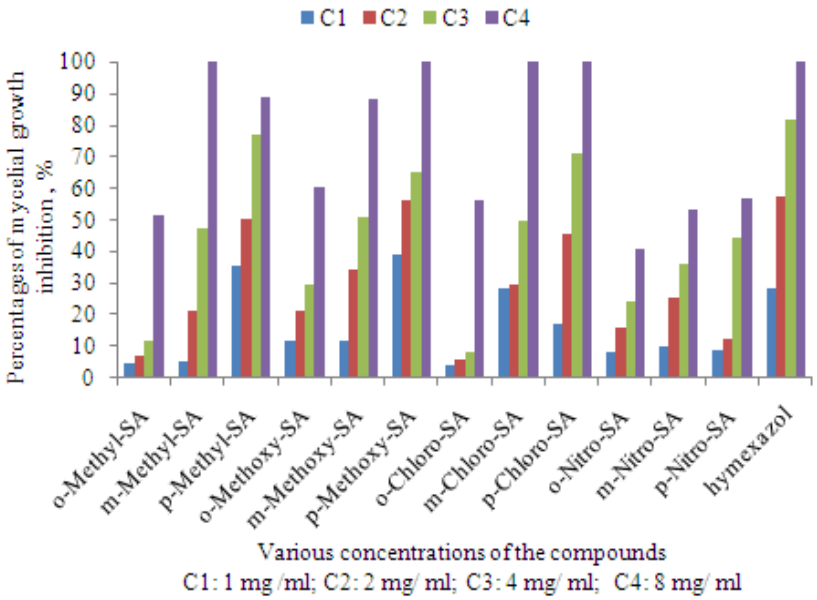


Fig. 4. Antifungal activities of Schiff bases against *F. graminearum.*

3.2.3. Antifungal activities of Schiff bases against *F. verticillioides*.

The Schiff bases had moderated antifungal activities against *F. verticillioides* and the maximum inhibition percentage was 61.13% of *p*-chloro-SA at 08 mg / ml. It indicates that the antifungal activity of hymexazol was better than that of *p*-chloro-SA at the same concentration (Figs. 5 and 6).

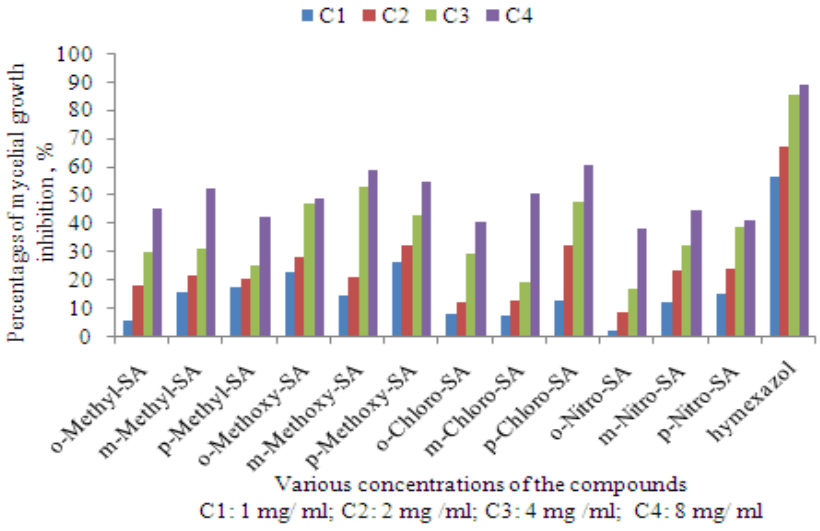


Fig. 5. Antifungal activities of Schiff bases against *F. verticillioides.*

The control plate | ***p*-chloro-SA at 08 mg / ml** | **hymexazol at 08 mg / ml**

Fig. 6. Inhibitory effect of compounds on *F. verticillioides* strain tested.

The above results indicate that the *N*-salicylidene-2-nitroaniline was showed low antifungal activity, due to the presence of electron-withdrawing o-nitro group at higher concentration (08 mg / ml). Furthermore, the presence of the electron-donating groups (CH_3-, CH_3O- and Cl-) improved antifungal activity of the compounds. (The effect mesmeric and inductive of the substituents and the relations dose-response of the test compounds play a very important role in the antifungal activity [25]).

3.3. Statistical analysis

The scores of activities of our Schiff bases tested (the scores of activities belong to the concentration C4=08mg/ml). The principal component analysis is presented graphically of scores (Fig. 7).The two components of the PCA include 95.22% of the total variance of the cloud of points with respective partial contributions of 79.62% and 15.60%. This relatively sufficient value to discrimination the factorial axes of this type of PCA. The graph is obtained shows three distinct areas:

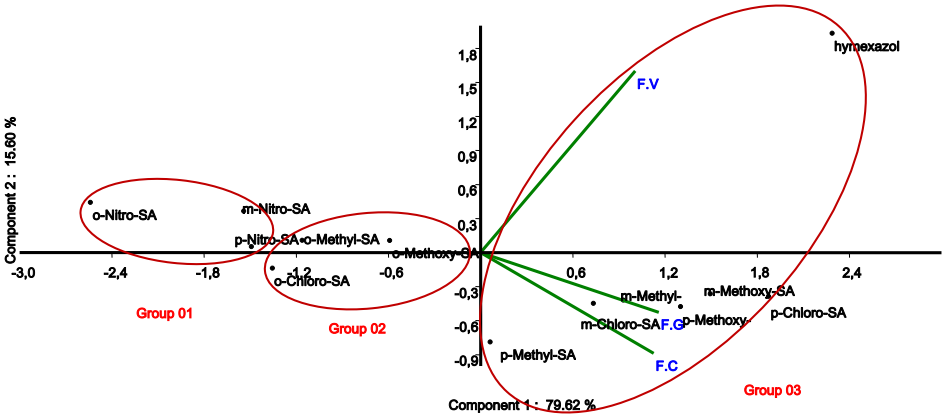


Fig. 7. Projected of the compounds to the concentration C4 = 08 mg/ml on plans CP1- CP2 after principal component analysis (PCA).

Group 1: is located in the left of the projection. It consists of *o*, *m* and *p*-nitro-SA. This group of compounds is weakly active on three strains of *Fusarium* due to the

remoteness of this group contributed to strains and its position in parallel with the component 1 (15.60%) in the negative side.

Group2 is located in the middle of the projection. It groups the compounds possess moderate activity like *o*-methyl-SA, *o*- methoxy-SA and *o*-chloro-SA.

Group 3 gather the rest of the compounds. These compounds are located in the right side of composent1 represent better activity due to their location near the strains of *Fusarium.*

4. Conclusions

Twelve Schiff bases were prepared and characterized by elemental analysis and spectral data.

- The electronic absorption spectra suggests that the keto-amine form which exists in DMSO of the *N*-salicylidene-2-nitroaniline is less active compared to the other compounds indicate the enol-imine form at same solvent.
- The results show that the compounds ability to inhibit mycelia growth of the *F. culmorum, F. graminearum* and *F. verticillioides.*
- The antifungal activities of Schiff bases depend on the concentrations of the compounds and the nature of substituent group on the aniline.

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