Asian Journal of Chemistry; Vol. 27, No. 10 (2015), 3579-3585

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.18872

Antimicrobial and Biochemical Activities of Some Arylazomorpholine Derivatives

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Received: 22 November 2014; *Accepted*: 3 March 2015; *Published online*: 22 June 2015; AJC-17307

Ten arylazomorpholines were prepared and characterized. Their fungicidal activity against *Fusarium oxysporum, Rhizoctonia solani, Macrophomina phasoli, Helminthosporum sp.* and *Trichoderma harzianum* and antibacterial effects against *Erwinia amylovora* were examined. Derivatives with persuasive *in vitro* antifungal effects, were checked *in vivo* on polyphenoloxidase, peroxidase DNA, RNA and sugar contents of *Rhizoctonia solani* as biomarkers searching their main target. 4-(4-Chlorophenyl)-, 4-(3-chlorophenyl)-, 4-(2-chlorophenyl)- and 4-(4-hydroxy-3,5-dichlorophenyl)azomorpholines highly inhibited all the tested fungi comparing with the standard fungicide metalaxyl. 4-(4-Chlorophenyl)azomorpholine and 4-(2-chlorophenyl)azomorpholine were more effective than metalaxyl inhibiting the hyphal growth of *R. solani* in liquid media. 4-(Phenyl)azomorpholine and 4-(3-chlorophenyl)azomorpholine completely stopped *E. amylovora* growth with MIC 4×10^{-5} M similar to streptomycin. 4-(4-Chlorophenyl)azomorpholine and 4-(4-hydroxy-3,5-dichlorophenyl)azomorpholine stimulated polyphenoloxidase and peroxidase. The tested arylazomorpholine derivatives were not specific against DNA and RNA contents. 4-(4-Chlorophenyl)-azomorpholine proved to be more potent than metalaxyl in reducing total soluble sugars and reduced sugars, whereas metalaxyl was near to it on non-reducing sugars.

Keywords: Azomorpholine, Fungicidal, Antibacterial, Polyphenoloxidase, Peroxidase, DNA, RNA, Sugars.

INTRODUCTION

The morpholino moiety has been utilized extensively by the pharmaceutical industry in drug design[1]. Five known morpholine fungicides have been applied world-wide in agriculture[2]. These fungicides are Fenpropimorph (*cis*-4-[(*RS*)-3-(4-*tert*-butylphenyl)-2-methylpropyl]-2,6-dimethylmorpholine), Tridemorph (2,6-dimethyl-4-tridecylmorpholine), Dodemorph (4-cyclododecyl-2,6-dimethylmorpholine) as dodemorph acetate, Flumorph (4-[3-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)- acryloyl]morpholine) and Dimethomorph {(*EZ*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxy-phenyl)-acryloyl]morpholine} that are systemic foliar fungicides with protective and curative action controlling several fungal species in several crops. *In vitro* excellent antibacterial activity of N-(morpholinoacetyl)-3,5-dimethyl-2,6-*bis*(*p*-methoxylphenyl)-piperidine-4-one against *S. aureus, E. coli, P. aeruginosa* and *Salmonella taphi* and potent antifungal activity against *Rhizopus sp.*, while N-morpholinoacetyl-3,5-dimethyl-2,6-*bis*(*p*-chlorophenyl)piperidine-4-one exhibited excellent antifungal activities against *C. albicans* and *A. flavus* were exhibited[3]. Morpholine moiety was required for enhancing antimicrobial activities[4]. N-[4,6-*bis*(4-flourophenyl)pyrimidin-2-yl]-2-morpholinoacetamide affected *E. coli* and *Pseudomonas* at MIC of 6.25 µg/mL. Compounds N-[4-(4-fluorophenyl)-6-phenylpyrimidin-2-yl]-2-morpholino-acetamide, N-[4-phenyl-6-(4-flourophenyl)pyrimidin-2-yl]-2-morpholinoacetamide and N-[4-(4-fluorophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl]-2-morpholinoacetamide inhibited *Aspergillus flavus* with MIC of 6,25 µg/mL. Compound N-[4-methylphenyl)-6-(4-fluorophenyl)pyrimidin-2-yl]-2-morpholinoacetamide inhibits *Microsporum gypsuem* with MIC of 12.5 µg/mL[5]. 4-(4-Hydroxybenzylideneiminophenyl)-morpholine was very effective with minimum inhibitory concentration (MIC) of 25, 19, 21, 16, 29, 20 and 40 µg/mL against *S. aureus, S. epidermides, B. cereus, M. luteus, E. coli, C. albicans* and *A. riger*, respectively[6]. The percentage of germinated spores incubated on sterol biosynthesis inhibitor (SBI) fungicides like fenpropimorph [4-[3-(4-*tert*-butyl-phenyl)-2-methylpropyl]-2,6-dimethylmorpholine) and fenhexamide *N*-(2,3-dichloro-4-hydroxyphenyl)-1-methyl-cyclo-hexanecarboxamide and the length of the germ tubes decreased with increasing their concentrations[7].

In this study, fungicidal and bactericidal effects of some arylazomorpholine derivatives were examined. Ten aryl-azomorpholines derivatives were prepared and tested for their activity against *Fusarium oxysporum, Rhizoctonia solani, Macrophomina phasoli, Helminthosporum sp.* and *Trichoderma*

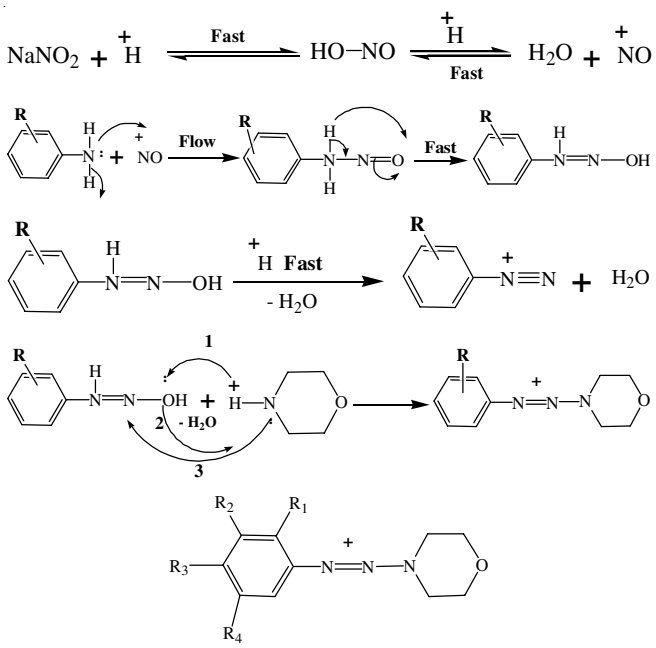
harzianum as well as against *Erwinia amylovora* bacteria. Derivatives, which showed persuasive *in vitro* antifungal effects, were checked *in vivo* for their inhibitory effects on polyphenoloxidase, peroxidase, DNA, RNA and sugar contents as biomarkers in the treated fungi searching their main target.

EXPERIMENTAL

Melting points were determined on Kofler block and were uncorrected. Elemental micro-analysis (C, H, N, X) were conducted at Microanalytical Center, Faculty of Science, Cairo University, Giza, Egypt. The infrared spectra were recorded on Shimadzu FT/IR 4100 Instrument, ultra violet measurements were conducted on Shimadzu spectrophoto-meter. Mass spectra (EI-MS) were recorded on DI analysis, Shimadzu Qp-2010 plus at 70 eV. IR, UV and Ms spectra were recorded in Micro-analytical Center, Faculty of Science, University of Cairo, Giza, Egypt. NMR spectra were recorded on DELTA2-500 NMR Spectrometer with DMSO-d_6 solution using tetramethylsilane (TMS) as a standard, Faculty of Science, University of Alexandria, Alexandria, Egypt.

Preparation of the tested compounds: The tested aryl azomorpholine derivatives were prepared *via* diazotization of an arylamine followed by nucleophilic addition[8,9] which could be summarized as follows:

A mixture of the used aryl amine derivative (87 mmol) and hydrochloric acid (6 N, 36.4 mL, 210 mmol) were placed in a 500 mL Erlenmeyer flask equipped with electrical stirring bar and the mixture was warmed on a water bath to reach a clear solution. The mixture was cooled to 0 °C to produce a thick precipitate. An aqueous solution of sodium nitrite (6.3 g, 91 mmol) (10 mL) was added dropwise over 10 min, with continuous stirring at 0 °C for 20 min to obtain a clear solution. Morpholine (8.3 g, 9.0 mL, 96 mmol) was added dropwise to the solution over 10 min, then water (100 mL) was added followed by the dropwise addition of an aqueous sodium bicarbonate solution (10 %, 130 mL), CO_2 was evolved. The mixture was stirred for 1 h. The precipitated solid was filtered, washed and air dried. The air dried solid precipitate was recrystallized from hot light petroleum ether (60-80, 80 mL) and treated with activated charcoal (1.5 g), then the hot mixture was filtered and the resulted filtrate was concentrated to about 40 mL. Upon cooling to room temperature, the pure compounds were filtered, died and checked for their melting points and elemental analysis (Table-1). Absorption spectral identification (IR, UV, NMR and MS) were also determined. The reaction mechanism was indicated in Fig. 1.



No	R_1	R_2	R_3	R_4	The chemical structure
1	H	H	H	H	4-Phenylazomorpholine
2	H	H	**Br**	H	4-(4-Bromophenyl)azomorpholine
3	H	H	**Cl**	H	4-(4-Chlorophenyl)azomorpholine
4	H	**Cl**	H	H	4-(3-Clorophenyl)azomorpholine
5	**Cl**	H	H	H	4-(2-Chlorophenyl)azomorpholine
6	H	**OH**	H	H	4-(3-Hydroxyphenyl)azomorpholine
7	H	H	CH_3	H	4-(4-Methylphenyl)azomorpholine
8	CH_3	H	H	H	4- (2-Methylphenyl)azomorpholine
9	H	**Cl**	**OH**	**Cl**	4- (3,5-Dichloro-4-hydroxyphenyl)-azomorpholine
10	H	H	**NO_2**	H	4- (4-Nitrophenyl)azomorpholine

Fig. 1. Preparation scheme and the total tested arylazomorpholine derivatives

4-Phenylazomorpholine: White crystals, m.w. 191, m.p. 29 °C (ref. 29-30 °C), Found C 61.8, H 8.1, N 21.57, Calc. C 62.8, H 6.8, N 21.9. ^{1}H NMR spectrum explained the aromatic ring protons distribution at 7.1-7.46 δ. *Para* proton peak appeared at 7.19 δ (1 H, t, J = 7.65, 7.60 Hz), *ortho* protons at 7.37 δ (2 H, t, J = 7.65, 6.1 Hz) and *meta* protons at 7.46 δ (2 H, dd, J = 6.85, 5.3 and 9.2 Hz) with 0.51, 1.0 and 1.26

TABLE-1
PHYSICAL AND ANALYTICAL DATA OF THE PREPARED ARYLAZOMORPHOLINE DERIVATIVES

Azomorpholine derivatives	m.w.	m.p.: Found (Reported)	Elemental analysis (%): Calcd. (Found)			
			C	H	N	X
4-Phenylazomorpholine	191	29 (29-30)	62.8 (61.8)	6.8 (8.1)	22.0 (21.6)	–
4-(2-Chlorophenyl)azomorpholine	270	89 (89.5-90)	44.4 (44.1)	4.4 (5.1)	15.6 (15.6)	29.6 (31.2)
4-(3-Chlorophenyl)azomorpholine	225	54 (54-55)	53.2 (52.9)	5.3 (5.3)	18.7 (18.7)	15.7 (14.8)
4-(4-Chlorophenyl)azomorpholine	225	Oil (-)	53.2 (51.2)	5.3 (5.4)	18.7 (17.9)	15.7 (14.0)
4-(4-Bromophenyl)azomorpholine	225	22 (20-22)	53.2 (52.9)	5.3 (5.3)	18.7 (18.4)	15.7 (17.9)
4-(3-Hydroxyphenyl)azomorpholine	207	160 (-)	58.0 (57.3)	6.3 (5.2)	20.3 (22.6)	–
4-(4-Methylphenyl)azomorpholine	205	50 (49.5-50.5)	64.4 (63.5)	7.3 (7.3)	20.5 (20.0)	–
4- (2-Methylphenyl)azomorpholine	205	33 (32-33)	64.4 (63.2)	7.3 (7.3)	20.5 (20.3)	–
4- (4-Hydroxy-3,5-dichloro-phenyl)azomorpholine	275	160 (-)	43.7 (38.1)	4.0 (4.3)	15.2 (14.3)	25.7 (28.6)
4- (4-Nitrophenyl)azomorpholine	236	138 (137.5-138.5)	50.4 (50.2)	5.0 (5.9)	24.4 (23.7)	–

X = the substituted halogen

integration indicating 1, 2 and 2 protons, respectively. Multicipility was due to the coupling among these protons. The hetero-aromatic (morpholine) ring gave their peaks as double doublet at 3.69 δ (4 H, d, J = 4.55 Hz) for C_2-2H and C_6-2H protons, while C_3-2H and C_5-2H protons at 3.71 δ (4 H, d, J = 6.15 Hz). This multiplicity was referred to coupling of these groups each other. Mass spectrum showed the parent molecular ion at *m/z* 191 ($M^{\bullet+}$, 74 %), morpholine ion at *m/z* 86 (15.9) and phenylazo ion at *m/z* 105 (100 %), which was fragmented into phenyl ion at *m/z* 77 (81.6 %) and the nitrogen molecule. The phenyl ion was exposed to fission by loss –HC≡CH– group to give $C_4H_3^+$ ion at *m/z* 51 (78.5 %). On the other hand, morpholine molecular ion moiety may loss -C_2H_5 fragment by fission and H^+ transfer giving molecular ion peak at *m/z* 56 (68.6 %) for $^+$O≡C-CH=NH. IR ν_{max} (nujol) (cm^{-1}): 1435-1350 (-CH_2-O alicyclic), 3062-3000 (CH_2-N alicyclic), 1150-1060 (C-O-C), 1500-1429 (N=N) and 870-840 (C-N).

4-(4-Bromophenyl)azomorpholine: Yellow crystals, m.w. 270, m.p. 89 °C (ref. 89.5-90 °C), Found C 44.1, H 5.1, N 15.2, Br 31.2 Calc. C 44.4, H 4.5, N 15.6, Br 29.6. ^{1}H NMR spectrum explained that the aromatic protons were distributed up-field due to the bromine atom as *ortho* protons appeared at 7.29 δ (2 H, d, J = 8.4 Hz) up-field *meta* protons at 7.49 δ (2 H, d, J = 8.4 Hz) due to the bromine atom. Multicipility was due *ortho* coupling between the two signals. The hetero-aromatic (morpholine) ring gave their peaks as triplet at 3.36 d (4 H, t, C_3-2H and C_5-2H) up-field to that of C_2-2H and C_6-2H protons at 3.71 δ (4 H, t). because of the oxygen atom. This multiplicity was referred to coupling each other. Mass spectrum showed the parent molecular ion at *m/z* 270 ($M^{\bullet+}$, 74 %), which by cleavage of the carbon nitrogen bond adjacent to the azo group produced bromophenyl moiety at *m/z* 77 (11.2 %), which loses the bromine atom to phenyl ion at *m/z* 77 (11.2 %) that by subsequent loss of acetylene gave $C_4H_3^+$ at *m/z* 51. The second fragment was morpholino-azo ion at *m/z* 114 (8 %), which may loss -N≡N and -C_2H_5 fragments by fission and hydrogen transfer producing 55, 56 for $^+$ O≡C-CH=NH (92.3 %). In the parent molecular ion, cleavage of N≡N bond to 4-bromophenylazo ion at *m/z* 184, which losses N≡N to 4-bromophenyl ion at *m/z* 156 and The second moiety was morpholino molecular ion at *m/z* 86, may loses C_2H_5 fragment by fission and hydrogen ion transfer to give $^+$O≡C-CH=NH $\rightleftharpoons$ O=C=C=$^+$NH + $H^{\bullet}$ ion at *m/z* 56-55 (92.3 %). IR ν_{max} (nujol) (cm^{-1}): 600-500 (bromophenyl-).

4-(4-Chlorophenyl)azomorpholine: Yellow crystals, m.w. 225, m.p. 54 °C (ref. 54-55 °C), Found C 52.9, H 5.3, N 18.7, Cl 14.8 Calc. C 53.2, H 5.3, N 18.6, Cl 15.7. ^{1}H NMR spectrum explained that the aromatic protons at 7.34 δ (2 H, C_2-H and C_6-H superimposing on C_3-H and C_5-H protons) due to downfield shifting of C_3-H and C_5-H protons peaks owing to substitution with Cl atom comparing with methyl group in case of 4-(4-methylphenyl)azomorpholine. The hetero-aromatic (morpholine) ring gave their eight protons as triplet centered at 3.96 δ with coupling constant 9.15 and 4.6 Hz referring to coupling each other. Mass spectrum showed that the parent molecular ion at *m/z* 225 (4.5 %) fragmented into morpholinoazo ion at *m/z* 114 (6.6 %) and chlorophenyl ion at *m/z* 111 (32.1 %), which loses the chlorine atom to the phenyl ion at *m/z* 77 (43.3 %), subsequent loss of acetylene resulted in $C_4H_3^+$ ion at *m/z* 51 (19.6 %). Morpholineazo fragment cleavage nitrogen molecule to morpholine ion which then loses C_2H_5 molecule with hydrogen transfer to $^+$O≡C-CH=N $\rightleftharpoons$ O=C=C=NH ion at *m/z* 56 (41.6 %) and 55 (95.2 %). On the other hand, the parent molecular ion may be fragmented into 4-chlorophenylazo ion at *m/z* 139 (17.6 %) that loses the nitrogen molecule to chlorophenyl ion at *m/z* 111 (32.1 %), subsequent loss of chlorine atom produces the phenyl ion at *m/z* 77 (42.3 %), which finally gives $C_4H_3^+$ ion at *m/z* 51 (19.6 %) by losing acetylene. The other moiety is morpholino ion at *m/z* 86 (9.9 %), which was fragmented as seen above. IR ν_{max} (nujol) (cm^{-1}): 750-700 (chlorophenyl-).

4-(3-Clorophenyl)azomorpholine: Yellow oil, m.w. 225 (ref. orange oil), Found C 51.2, H 5.4, N 17.9, Cl 14.0 Calc. C 53.2, H 5.3, N 18.7, Cl 15.7. ^{1}H NMR spectrum showed that substitution of chlorine atom on meta position of aromatic ring differently affected its protons as their peaks were superimposed as triplet with J = 10.7, 5.4 and 5.4 Hz at 7.29 δ owing to *ortho* and *para* positions effect of –N=N- and Cl, its multiplicity is referred to meta coupling with C_2-H and each other as well as *ortho* coupling with C_5-H protons. C_5-H proton at meta position of both Cl and –N=N- groups was up-field shifted to 7.16 δ as quartet with J = 10.7, 5.1 and 2.3 Hz. The hetero-aromatic (morpholine) ring protons distributed as two doublets at 3.5 and 3.7 δ for (C_3-2H and C_5-2H, 4H, d) and (C_2-2H and C_6-2H, 4H, d), respectively. Mass spectrum of 4-(3-chlorophenyl)azomorpholine is similar to the spectrum of its isomer of 4-(4-chlorophenyl)azomorpholine. IR ν_{max} (nujol) (cm^{-1}): 750-700 (chlorophenyl-).

4-(2-Chlorophenyl)azomorpholine: Light brown crystals, m.w. 225, m.p. 22 °C (ref. 20-22 °C). Found C 52.9, H 5.3, N 18.4, Cl 18.0 Calc. C 53.2, H 5.3, N 18.7, Cl 15.7. ^{1}H NMR spectrum showed that chlorine atom affected protons peaks distribution as protons on position *ortho* and *para* to chlorine atom were down-field shifted to 7.41 δ as triplet (2H, C_4-H and C_6-H, t, J = 7.65 and 8.4 Hz) owing to *ortho* coupling with C_4-H and C_6-H protons that were less affected with Cl atom and gave their signals at 7.21 δ (1H, t, J = 7.65 and 5.9 Hz) and 7.24 δ (1H, t, J = 7.65 and 7.65 Hz), respectively due to the different effect of azo group on them, their multiplicity is due to coupling each other. Mass spectrum of 4-(2-chlorophenyl)azomorpholine is similar to the spectra of both 4-(4-chlorophenyl)azomorpholine and 4-(3-chloro-phenyl)-azomorpholine isomers with some differences in relative abundances. IR ν_{max} (nujol) (cm^{-1}): 750-700 (chlorophenyl-).

4-(3-Hydroxyphenyl)azomorpholine: Black crystals, m.w. 207, m.p. 160 °C (decomposition), Found C 57.3, H 5.2, N 22.6, Calc. C 58.0, H 6.3, N 20.3. Mass spectrum showed the parent molecular ion at *m/z* 207 (0.48 %) indicating that the compound was highly fragmented under electron impact. It gave ions at *m/z* 114 (1.9 %) and 94 (5.77 %) of morpholinoazo and 3-hydroxyphenyl that may tautomeride with its keto-form, which loses carbon monoxide to cyclopentdiene ion at *m/z* 66 (9.0 %). The morpholinoazo ion loses nitrogen molecule to morpholine ion at *m/z* 86 (2.0 %), which in turn loses C_2H_5 with hydrogen transfer to give $^+$O≡C-CH=NH $\rightleftharpoons$ O=C=C=NH ion at *m/z* 56 (9.4 %) and 55 (14.4 %). The parent molecular

ion maybe fragmented into 3-hydroxyphenylazo ion at *m/z* 121 (3.1 %), which loses a nitrogen molecule to hydroxyphenyl and morpholino ions that were fragmented as mentioned above. IR ν_{max} (nujol) (cm^{-1}): 750-650 (OH out of plane).

4-(4-Methylphenyl)azomorpholine: Tan crystals, m.w. 205, m.p. 50 °C (ref. 49.5-50.5 °C). Found C 63.5, H 7.3, N 20.0, Calc. C 64.4, H 7.3, N 20.5. ^{1}H NMR spectrum showed that comparing with bromine atom, the substituted 4-methyl increased the shielding effect of the aromatic protons as their peaks were arranged as *ortho* protons (2H, d, *J* = 7.7 Hz) and *meta* protons (2H, d, *J* = 8.4 Hz) at 7.27 and 7.12 δ, respectively. Heteroaromatic protons gave their peaks at 3.64 δ (2H, C_3-2H and C_5-2H, t) and 3.72 δ (4H, C_2-2H and C_6-2H, t). Substituted methyl group gives its singlet peak at 3.38 δ. Mass spectrum showed the parent molecular ion at *m/z* 205 (7.4 %). It was fragmented to 2-methylphenylazo ion at *m/z* 119 (13.4 %), which loses the nitrogen molecule to give tolyl molecular ion at *m/z* 91 (34.7 %) following by loss of a hydrogen atom and rearrangement producing tropylium molecular ion at *m/z* 91. The observed peak at *m/z* 65 (12.7 %) resulted from the neutral acetylene molecule (CH≡CH) elimination from the tropylium ion. The second fragments was a morpholino ion at *m/z* 86 (10.6 %), which loss a C_2H_5 molecule with hydrogen transfer to give $^+$O≡C-CH=NH $\rightleftharpoons$ O=C=C=NH ion at *m/z* 56 (5.4 %) and 55 (8.3 %). The parent molecular ion may tends to undergo cleavage of C-N bond to give *m/z* 91 ion (34.7 %), which loses a hydrogen atom with rearrangement to tropylium ion at *m/z* 91 that eliminates a neutral acetylene (CH≡CH) to ion at *m/z* 65 (12.7 %). It gives morpholinoazo ion at *m/z* 114 (5.6 %), which by loss of a nitrogen molecule gives morpholine molecular ion at *m/z* 86 (10.6 %), which in turn loses a C_2H_5 molecule with hydrogen transfere gave $^+$O≡CCH=NH at *m/z* 56 (48 %) and 55 (93.2 %). IR ν_{max} (nujol) (cm^{-1}): 1574-1272 (C-H of CH_3 group).

4-(2-Methylphenyl)azomorpholine: Yellow crystals, m.w. 205, m.p. 33 °C (ref. 32-33 °C). Found C 63.2, H 7.3, N 20.3, Calc. C 64.4, H 7.3, N 20.5. ^{1}H NMR spectrum showed that the singlet peak at 2.37 d is owed to the substituted aliphatic methyl group, the heteroaromatic ring protons were distributed as two doublets at 3.65 δ (4H, d, C_3-2H and C_5-2H, *J* = 2.3 Hz) and 3.71 δ (4H, d, C_2-2H and C_6-2H, *J* = 3.3 Hz) due to coupling each other. Substitution of 2-methyl affected the aromatic protons differently. Its *ortho* and *para* protons gave their peaks up-field at δ 7.07 (1H, t, C_5-H, *J* = 6.1 Hz) and 7.11 (1H, d, C_3-H, *J* = 7.65 Hz), respectively than C_6-H and C_4-H protons at δ 7.18 (1H, d, C_6-H, *J* = 6.9 Hz) and 7.32 (1H, d, C_4-H, *J* = 5.35 Hz), respectively. Mass spectrum, mass spectral data are nearly as discussed for the previously mentioned 4-(4-methylphenyl)azomorpholine derivative. IR ν_{max} (nujol) (cm^{-1}): 1574-1272 (C-H of CH_3 group).

4-(3,5-Dichloro-4-hydroxyphenyl)azomorpholine: Brown crystals, m.w. 275, m.p. 160 °C (decomposition). Found C 38.1, H 4.3, N 14.3, Cl 28.6, Calc. C 43.7, H 4.0, N 15.2, Cl 25.7. ^{1}H NMR spectrum showed the aromatic protons signal at 8.23 δ downfield due to substitution with chloro atoms. Hetero aromatic protons gave their peaks at 3.36 δ (4H, d, C_2-2H and C_6-2H) and 2.45 δ (4H, d, C_3-2H and C_5-2H), respectively. Mass spectrum showed the parent molecular ion at *m/z* 275 (0.05 %), which exhibited that this compound was nearly full fragmented under electron impact. Fragmentation by N-N cleavage produced 4-(3,5-dichloro-4-hydroxyphenyl)azo and morpholino molecular ions at *m/z* 190 (11.3 %) and 86 (1.79 %), respectively. 4-(3,5-Dichloro-4-hydroxyphenyl)azo ion loses the nitrogen molecule to give 3,5-dichloro-4-hydroxyphenyl ion at *m/z* 162 (22.9 %), which in turn loses a hydrochloride molecule to produce 2-chlorophenol ion at *m/z* 125, (0.22 %), 2-chlorophenol will be in tautomerism with its quinone form as 2-chlorobenzoquinone ion at *m/z* 125 which exposed to rearrangement with elimination of carbon monoxide forming $C_5H_4Cl^{+\bullet}$ at *m/z* 99 (33.78 %). The frequently observed peak at *m/z* 97 (100 %) results from elimination of hydrogen molecule to produce $C_5H_2Cl^{+\bullet}$ molecular ion. The morpholino molecular ion fragmented at *m/z* 86 (1.79 %) loses a C_2H_5 molecule with hydrogen transfer producing $^+$O≡C-CH=NH $\rightleftharpoons$ -H^+ O=C=C=NH molecular ion peak at *m/z* 55. IR ν_{max} (nujol) (cm^{-1}): 3600 - 3450 (OH adjacent to Cl- atom).

4-(4-Nitrophenyl)azomorpholine: Yellow to orange crystals, m.w. 236, m.p. 138 °C (ref. 137.5-138.5 °C). Found C 50.2, H 5.9, N 23.7, Calc. C 50.0, H 5.0, N 24.4. ^{1}H NMR spectrum explained the deshielding effect of nitro group as it shifted the signals of aromatic protons to 7.5 δ (2H, d, C_2-H and C_6-H, *J* = 9.15 Hz) and 8.18 δ (2H, d, C_3-H and C_5-H, *J* = 8.4 Hz) due to coupling each other. Heteroaromatic protons gave their peaks at 3.36 δ (4H, d, C_3-H and C_5-H, *J* = 9.15 Hz) and 3.85 δ (4H, d, C_2-H and C_6-H, *J* = 8.4 Hz). This downfield may be referred to the conjugation among nitro, aromatic bonds, diazo group and nitrogen lone pair of electrons. Mass spectrum revealed the parent molecular ion at *m/z* 236, which was fragmented by the fission into 4-nitrophenylazo at *m/z* 150 (34.34 %) that undergo cleavage of C-N bond to nitrophenyl ion at *m/z* 122 (26.7 %) and a nitrogen molecule. The nitrophenyl ion loses NO molecule to form phenoxide at *m/z* 92 (29.8 %), which may be exposed to C-O bond cleavage by elimination of an oxygen atom to produce the phenyl ion at *m/z* 76 (100 %) or it will be in tautomerism with its keto-form (quinone) molecular ion at *m/z* 92, which in turn loses carbon monoxide to give cyclopentadiene ion at *m/z* 64 (26.95 %). The second fragment of the parent ion was morpholino molecular ion at *m/z* 86 (6.87 %), which loses a C_2H_5 with hydrogen transfer to give $^+$O≡CCH=NH at *m/z* 56 (67.19 %). IR ν_{max} (nujol) (cm^{-1}): 1365-1335 (aromatic NO_2).

UV spectroscopy measurements of the prepared derivatives: Benzene ring showed bands at 204 and 256 nm due to π-π* transitions. Substitution of methyl on the benzene ring causes B-band bathochromic as a result of hyperconjugation to 261 nm with σ-σ* transition while, substitution with auxochromic groups (-OH, Cl:, Br:) shifts the E and β bands to longer wavelengths with n-π* transition at 210 nm. Substitution with nitro group (NO_2) shows weak absorption in the near ultraviolet region resulting from n-π* transition at 252 nm, whereas the effect of conjugation upon the absorption of the nitro group appeared strong K band with π-π* transition at 280 nm. Direct attachment of -N=N- (chromophore) which has a double bond and two lone pairs of electrons caused π-π* transition at 350 nm. CH_2 in morpholine ring absorbs in the region σ-σ* but the strain of both oxygen atom (n- and nitrogen atoms caused it absorption at longer wave lengths.

Materials: Morpholine, the standard fungicide, metalaxyl, *N*-(2,6-dimethylphenyl-*N*-methoxyacetyl)-DL-alanine-methylester were provided by El-Helb Pesticides & Chemicals Company, Egypt. Streptomycin (*O*-2-deoxy-2-methylamino-α-L-glucopyranosyl-(1→2)-*O*-5-deoxy-3-*C*-formyl-α-L-lyxofuranosyl-(1→4)-N^1,N^3-diamidino-D-streptamine) was brought from the drug store. Other used chemicals and solvents were purchased from El-Gomhouria Drug Company, Egypt. Both enzymatic activity and nucleic acids contents were measured using Nicolet 100 UV-VIS spectrophotometer, Thermo Electron Corporation. Sugar contents were measured on Unico-1200 Spectrophotometer.

Fungicidal activities of the tested compounds

Tested fungi: Five economic plant pathogenic fungi: *Fusarium oxysporum, Rhizoctonia solani, Trichoderma harzianum, Machrophomina phaseoli* and *Helminthosporum* sp. These fungi were firstly provided from Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt and allowed to grow on Czapeck-dox agar medium for 7 days before using in the test.

***in vitro* Fungitoxic effects (Radial growth method):** This test was done as reported by Desheesh *et al.*[10], a definite volume of triplet well-known Czapek-Dox medium (13.3 mL) containing agar (1.5 g/100 mL water) was sterilized in each conical flask. Citrate-phosphate buffer solution was separately autoclaved; both solutions were mixed in the conical flask. The calculated volume of distilled water was also autoclaved and added to the flask. The tested compounds were dissolved in dimethyl-formamide (DMF) and used at 10^{-4}, 1.25×10^{-4}, 2.5×10^{-4}, 5×10^{-4}, 10^{-3}, 1.25×10^{-3}, 2.5×10^{-3}, 5×10^{-3}, 10^{-2} molar. Dimethyl-formamide was used at as high as 1 % of the poisoned medium. Control was concurrently conducted. Each flask content (40 mL) of the poisoned medium was poured in three sterilized Petri-dishes as three replicates of one treatment. After solidification of the medium, a disk inoculum (5 mm in diameter) was located in the center of the Petri-dish and incubated at 27°C. The results were recorded measuring two vertical radii of the growth in each Petri-dish every day. The percentage inhibition was calculated at the completion of the untreated fungi hyphal growth filled the Petri-dish. Inhibition percentages in growth of the treated fungi were recorded and IC_{50} values were calculated[11]. Metalaxyl was used as a standard fungicide for comparison at the same concentration range in dimethyl formamide (DMF). The average hyphal growth and its standard deviation (SD) were calculated[12].

***in vivo* Biochemical effects (Liquid media):** *Rhizoctonia solani* was selected for testing in liquid media method by using conical flasks (100 mL) at the same tested concentrations. The inoculum was fungal culture grown on Czapek-Dox agar medium seven days old. The inoculation was located in the center of the liquid medium surface (the tested Czapek-Dox without agar). Three replicates were used. When the hyphal growth of untreated fungus filled the surface of the liquid media, the hyphal growth was separated and left at room temperature for 1 h. The fresh weight of the hyphal growth was recorded for all replicates and the averages were calculated. The percentages of inhibition of the hyphal growth for each treatment and IC_{50} values were determined. The treated and untreated solutions of liquid medium of fungal growth were used for protein determination and enzymes activity measurements.

Total protein determination was carried out by known method[13]. While specific activity of polyphenoloxidase (PPO) was carried out by the method of Broesch[14]. The resulted colour was measured at 575 nm and the optical density (OD) values were determined. Peroxidase activity was measured[15]. The percents of enzymes inhibition were recorded by the comparison between the treated and untreated results. Specific activity of the tested enzymes were calculated. Determination of DNA and RNA contents was done using perchloric acid method at 270 and 290 nm[16]. Determination of reduced and non-reduced sugars as well as total soluble sugars were conducted[17].

Bactericidal activity of the tested compounds: Bactericidal effects of the synthesized arylazomorpholine were evaluated against *Erwinia amylovora* bacteria at (2.5×10^{-5}, 3.3×10^{-5}, 4×10^{-5}, 5×10^{-5}, 5.7×10^{-5}, 6.6×10^{-5}, 8×10^{-5}, 10^{-4}, 1.25×10^{-4}, 1.66×10^{-4}) by determining the minimum inhibitory concentration (MIC). This method was carried out[18,19]. With some modification in presence of streptomycin (*O*-2-deoxy-2-methylamino-α-L-glucopyranosyl-(1→2)-*O*-5-deoxy-3-*C*-formyl-α-L-lyxofuranosyl-(1→4)-N^1,N^3-diamidino-D-streptamine) was used for comparison as standard bactericide.

Statistical analysis: The obtained results (inhibition percents) in hyphal growth were analyzed using the analysis of variance (ANOVA) and Student-Newman-Kules Test. EC_{50}, IC_{50} and 95 % confidence limit, were determined using probit analysis method[20].

RESULTS AND DISCUSSION

Fungicidal activities of arylazomorpholines derivatives (*in vitro*):

Fungicidal effects by radial growth method (solid media): Results of the fungicidal effects of arylazomorpholine derivatives on the hyphal growth of the tested fungi are recorded as IC_{50} values in Table-2. All the prepared arylazomorpholine derivatives proved powerful effect in inhibiting the fungal growth of *Helminthosporum sp* more than the standard fungicide metalaxyl. IC_{50} values of metalaxyl against this fungus was 16.2×10^{-4} M, whereas it was 0.51×10^{-4} M- 6.56×10^{-4} M in range for all the tested azomorpholine derivatives. *Fusarium oxysporum* was found to be more inhibited with 4-(4-hydroxy-3,5-dichlorophenyl)azomorpholine (1.61×10^{-4} M) in comparison with the standard fungicide metalaxyl (5.35×10^{-4} M). 4-(4-Bromophenyl)azomorpholine (0.48×10^{-4} M), 4-(3-chlorophenyl)azomorpholine (0.85×10^{-4} M), 4-(4-chlorophenyl)azomorpholine (0.9×10^{-4} M), 4-(4-hydroxy-3,5-dichlorophenyl)azomorpholine (0.99×10^{-4} M), 4-(2-chlorophenyl)azomorpholine (1.2×10^{-4} M) were more potent to inhibit the fungal growth of *M. phaseoli* fungus than the standard fungicide, metalaxyl (7.52×10^{-4} M). *Rhizoctonia solani* effectively inhibited by 4-(4-hydroxy-3,5-dichlorophenyl)-, 4-(4-chlorophenyl)-, 4-(3-chlorophenyl)- and 4-(2-chlorophenyl)azomorpholine derivatives with IC_{50} values of 0.84×10^{-4} M, 3.9×10^{-4} M, 4.6×10^{-4} M), 6.4×10^{-4} M, respectively in comparison with metalaxyl (10.4×10^{-4} M). *Trichoderma harzianum* highly inhibited with 4-(4-hydroxy-

TABLE-2
in vitro EFFECTS OF THE PREPARED AZOMORPHOLINE COMPOUNDS AGAINST THE TREATED PATHOGENIC FUNGI; SHOWN AS IC_{50} VALUES

Azomorpholine derivative	Probit analysis	IC_{50} values on the treated fungi (Molar) × 10^{-4}				
		Helminthosporum sp	*F. oxysporum*	*M. phaseoli*	*R. solani*	*T. harizanum*
4-Phenyl-	IC_{50} (95 % C.L)	0.85 (0.61-1.2)	29.2 (23.3-36.5	5.4 (4.4-6.7	12.3 (10-14.8)	20.6 (14.8-29)
	Slope ±SE	1.27 ± 0.014	1.53 ± 0.02	1.53 ± 0.02	1.3 ± 0.011	0.83 ± 0.01
	γ^2	14.7	8.3	21.3	24.7	7.42
4-(2-Chlorophenyl)-	IC_{50} (95 % C.L)	0.57 (0.34-0.67)	12.9 (6.1-28)	0.48 (0.12-1.1)	6.4 (3.8-10.6)	10.5 (3.8-32)
	Slope ±SE	0.21 ± 0.007	0.3 ± 0.005	0.15 ± 0.006	0.39 ± 0.004	0.2 ± 0.005
	γ^2	0.9	4.6	7.2	2.8	0.68
4-(3-Chlorophenyl)-	IC_{50} (95 % C.L)	0.51 (3.0-8.6)	4.9 (3.9-6.3	0.9 (0.8-1.0)	4.6 (3.9-5.4)	1.1 (1.08-1.2)
	Slope ±SE	3.7 ± 1.5	0.9 ± 0.006	5.8 ± 0.013	1.4 ± 0.011	12.6 ± 2.9
	γ^2	2.5	7.7	1.5	17.1	8.2
4-(4-Chlorophenyl)-	IC_{50} (95 % C.L)	0.56 (0.35-0.88)	5.4 (4.0-7.4)	0.85 (0.7-1.07)	3.9 (3.0-5.0)	0.7 (0.54-0.92)
	Slope ±SE	4.1 ± 1.6	0.67 ± 0.005	2.9 ± 0.14	0.9 ± 0.005	2.5 ± 0.13
	γ^2	1.5	17	4.3	20.1	4.3
4-(4-Bromophenyl)-	IC_{50} (95 % C.L)	0.85 (0.74-0.98)	15.5 (12.5-19)	1.2 (1.1-1.3)	14.7 (12.5-17)	1.9 (1.4-2.6)
	Slope ±SE	5.5 ± 1.28	1.13 ± 0.007	11.7 ± 3.7	1.3 ± 0.007	0.9 ± 0.007
	γ^2	1.7	23	8.8	16.4	15.5
4-(3-Hydroxyphenyl)-	IC_{50} (95 % C.L)	3.4 (2.6-4.4)	24.4 (20.7-28.8)	10.3 (8.6-12.4)	10.2 (8.7-12)	8.4 (6.9-10.2)
	Slope ±SE	1.09 ± 0.008	1.64 ± 0.001	1.4 ± 0.013	1.3 ± 0.007	1.52 ± 0.02
	γ^2	20	14.7	19.9	21.2	23.1
4-(4-Methylphenyl)-	IC_{50} (95 % C.L)	6.6 (5.5-7.8)	18.0 (15.5-20.9)	14.6 (10-19.9)	14.9 (13-18)	14.4 (11.6-17.9)
	Slope ±SE	1.58 ± 0.001	2.52 ± 0.007	0.87 ± 0.01	1.2 ± 0.006	1.03 ± 0.006
	γ^2	9.8	25	1.2	17.3	10.6
4-(2-Methylphenyl)-	IC_{50} (95 % C.L)	2.5 (2.0-3.2)	14.7 (11.9-18)	5.6 (4.6-6.9)	10.7 (8.5-13.5)	5.9 (4.7-7.2)
	Slope ±SE	1.3 ± 0.011	1.2 ± 0.007	1.3 ± 0.01	0.88 ± 0.005	1.1 ± 0.008
	γ^2	10.9	8.1	12.2	14.2	3.3
4-(4-Hydroxy-3,5-dichloro-phenyl)-	IC_{50} (95 % C.L)	1.1 (1.0-1.2)	1.6 (1.5-1.8)	0.99 (0.91-1.1)	0.84 (0.7-1.0)	0.1 (0.09-0.12)
	Slope ±SE	7.6 ± 1.5	3.4 ± 0.08	7.1 ± 1.9	2.4 ± 0.094	3.5 ± 0.18
	γ^2	0.2	2.7	0.4	10.3	1.5
4-(4-Nitrophenyl)-	IC_{50} (95 % C.L)	4.3 (2.7-5.9)	31.4 (21.8-45.3)	>1.0 x 10^{-4}	14.2 (11-18.6)	26 (9.5-45)
	Slope ±SE	0.55 ± 0.005	0.7 ± 0.007		0.76 ± 0.004	0.3 ± 0.004
	γ^2	6.1	13.1		11.2	4.3
Metalaxyl	IC_{50} (95 % C.L)	16.2 (13.5-19.3)	5.4 (3.8-7.5)	7.5 (6.5-8.8)	10.4 (8.4-12.8)	0.15 (0.03-0.7)
	Slope ±SE	1.2 ± 0.006	0.6 ± 0.005	1.64 ± 0.015	1.29 ± 0.017	0.3 ± 0.005
	γ^2	19.9	0.9	19.9	24.8	1.7

3,5-dichlorophenyl)azomorpholine derivative (0.1×10^{-4} M), which was nearly the same as the used standard fungicide, metalaxyl (0.15×10^{-4} M).

Fungicidal effects on *R. solani* in liquid media: The fresh weight of hyphal *Rhizoctonia solani* in liquid media was highly inhibited with 4-(2-chlorophenyl)azomorpholine and 4-(4-chlorophenyl)azomorpholine with 0.58×10^{-5} M and 2.9×10^{-5} M IC_{50} values, respectively in comparison to the standard fungicide, metalaxyl with IC_{50} value of 7×10^{-5} M, whereas the other tested azomorpholine derivatives were less effective than the standard fungicide in this respect (Table-3).

Bactericidal activities of azomorpholine derivatives: The synthesized arylazomorpholine derivatives in addition to the standard antibacterial streptomycin differently prevented the growth of the treated bacteria, *Erwinia amylovora* depending on their minimum inhibition concentration (MIC) values. Streptomycin (3.3×10^{-5} M), 4-phenylazomorpholine (4×10^{-5} M) and 4-(3-chlorophenyl)azomorpholine (4×10^{-5} M) relatively prevented the bacterial growth of the tested bacteria with nearly the same MIC (Table-4). The other tested azomorpholine derivatives were less effective against *E. amylovora* comparing with streptomycin.

TABLE-3
EFFECT OF THE SCREENED ACTIVE COMPOUNDS ON *Rhizoctonia solani* FRESH WEIGHT

Tested compounds	Effect on *Rhizoctonia solani* fresh weight	
	IC_{50} (95 % C.L) × 10^{-5} (molar)	Slope ± SE
4-(4-Chlorophenyl)azomorpholine	2.9 (2.4-3.5)	1.3 ± 0.9
4-(3-Clorophenyl)azomorpholine	9.6 (8.4-10.9)	1.9 ± 1.4
4-(2-Chlorophenyl)azomorpholine	0.6 (0.3-1.1)	0.7 ± 0.8
4-(3,5-Dichloro-4-hydroxy-phenyl)azomorpholine	22.2 (17.9-27.6)	1.3 ± 1.1
Metalaxyl	7.8 (5.6-11.1)	0.6 ± 0.65

Biochemical measurements

Effect on sugar contents: As shown in Table-5, 4-(4-chlorophenyl)azomorpholine derivative proved to be the most effective derivative to decrease the total soluble sugars and reduced sugars contents in *R. solani* with effective concentration of 50 % (EC_{50}) value equal to 0.14×10^{-5} M and 0.37×10^{-5} M, respectively in comparison to the other tested azomorpholine derivatives and metalaxyl, which exhibited 0.7×10^{-5} M and 7.8×10^{-5} M EC_{50} values in the same array. On the other hand, the content of non-reduced sugars in *R. solani* was decreased by

TABLE-4
EFFECT OF THE SYNTHESIZED AZOMORPHOLINE DERIVATIVES ON *Erwinia amylovora*; SHOWN AS MIC IN MOLAR

Tested compounds	MIC (molar)
4-Phenylazomorpholine	4.00×10^{-5}
4-(4-Bromophenyl)azomorpholine	1.66×10^{-4}
4-(4-Chlorophenyl)azomorpholine	1.66×10^{-4}
4-(3-Clorophenyl)azomorpholine	4.00×10^{-5}
4-(2-Chlorophenyl)azomorpholine	1.66×10^{-4}
4-(3-Hydroxyphenyl)azomorpholine	1.00×10^{-4}
4-(4-Methylphenyl)azomorpholine	1.66×10^{-4}
4-(2-Methylphenyl)azomorpholine	1.66×10^{-4}
4-(4-Hydroxy-3,5-dichlorophenyl)azomorpholine	5.70×10^{-5}
4-4-Nitrophenyl)azomorpholine	8.00×10^{-5}
Streptomycin	3.30×10^{-5}

TABLE-5
EFFECT OF THE SCREENED ACTIVE COMPOUNDS ON *Rhizoctonia solani* SUGAR CONTENTS; SHOWN AS EC_{50} VALUES

Tested compounds	Effect on *Rhizoctonia solani* sugar contents EC_{50} values (molar)		
	RS	Non RS	TSS
4-(4-Chlorophenyl)azomorpholine	0.37	0.15	0.14
4-(3-Clorophenyl)azomorpholine	3.5	1.8	6.0
4-(2-Chlorophenyl)azomorpholine	7.0	14.0	11.0
4-(4-Hydroxy-3,5-dichlorophenyl)-azomorpholine	6.4	6.2	8.0
Metalaxyl	7.8	0.11	7.0

EC_{50} values: Effective concentration reducing 50 % of sugar contents in *R. solani* are $\times 10^{-5}$; RS = reduced sugars; Non-RS = Non-reduced sugars; TSS = Total soluble sugars.

relatively equal EC_{50} values of metalaxyl (0.11×10^{-5} M) and 4-(4-chlorophenyl)azomorpholine (0.15×10^{-5} M), which more potant than the other tested arylazomorpholine derivatives.

Effect on enzyme activities: It was found that 4-(4-chlorophenyl)azomorpholine and 4-(4-hydroxy-3,5-dichlorophenyl)azomorpholine derivatives highly stimulated the activity of both polyphenoloxidase and peroxidase enzymes in *R. solani in vivo* comparing with the used standartd fungicide, metalaxyl, which caused slight stimulation for peroxidase but it was very weak to stimulate or reduce the activity of polyphenoloxidase enzyme in *R. solani* indicating the high effect of the prepared azomorpholine derivatives.

Effect on DNA and RNA contents: All the tested compounds weakly affected the contents of RNA and DNA in *R. solani* in comparison with metalaxyl, It could be said that, the tested azomorpholine derivatives may be were not specific against DNA and RNA of *R. solani*.

Conclusion

A series of arylazomorpholine derivatives have been prepared and structurally elucidated. Their fungicidal and biochemical effects were a function of the tested structure and concentration. *M. phaseoli* and *Helminthosporum sp.* were sensitive to all derivatives. *T. harzianum* was highly affected by 4-(3-chlorophenyl)-, 4-(4-hydroxy-3,5-dichlorophenyl)-, metalaxyl and 4-(2-chlorophenyl)azomorpholine in descending order. *R. solani* was highly inhibited by 4-(4-hydroxy-3,5-dichlorophenyl)azomorpholine, whereas 4-(3-chlorophenyl)-, 4-(4-chlorophenyl)azomorpholine derivatives moderately inhibited it in descending order. The other tested derivatives in addition to metalaxyl slightly affected it. 4-(4-Hydroxy-3,5-dichlorophenyl)azomorpholine highly inhibited *F. oxysporum* but 4-(4-chlorophenyl)- and 4-(3-chlorophenyl)-azomorpholine derivatives in addition to metalaxyl moderately affected it, whereas slightly or weakly affected by other compounds. *R. solani* hyphal fresh weight was highly inhibited with 4-(2-chlorophenyl)azomorpholine and 4-(4-chlorophenyl)azomorpholine in comparison with metalaxyl. 4-(4-chlorophenyl) and 4-(4-Hydroxy-3,5-dichlorophenyl)-azomorpholine derivatives highly stimulated polyphenoloxidase and peroxidase activities. All the tested derivatives weakly affected DNA and RNA contents in *R. solani*. The effect on sugar contents were differed according to the tested compound, tested concentration and the sugar type. However, the presence of hydroxyl group and chloro atoms substituents may be enhanced the fungicidal activity of azomorpholine derivatives. In addition, these effects maybe due to the diverse effect of morpholino moiety which involved in four different commercial fungicides. The synthesized azomorpholines were prevented the growth of *Erwinia amylovora* bacteria with different MIC values.

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