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Antimicrobial Effects of Some Diazoaminobenzene Derivatives

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Abstract

Seven diazoaminobenzene derivatives:1,3-Diphenyltriazene (diazoaminobenzene), 1,3-Bis(2-tolyl)-1-triazene, 1,3- Bis (4-tolyl)-1-triazene, 1,3- Bis (4-phenylcarboxylic acid)-1-triazene, 1,3- Bis (4-phenylsulphonamide)-1-triazene, 1,3- Bis (3-chlorophenyl)-1-triazene and 1,3-Dinaphtyl-1-triazene were prepared, purified and spectroscopically identified with IR, UV, NMR and Ms spectroscopy measurements. These derivatives were tested for their fungicidal and bactericidal effects against phytoparasitic microorganisms. It was found that substitution with 2-CH_3 significantly increased the effect of 1,3-diphenyl-1-triazezene against *A. solani*, while 4-CH_3, 3-Cl and 4-COOH reduced it. Substitution of 1, 3-dinaphthyl- instead of 1,3-diphenyl- increased the activity against *B. cinerea*, *R. solani* and *S. rolfsii* on contrary to *F. oxysporum*. 1,3-Diphenyl-, 1,3-dinaphthyl- and 1,3-bis(2-tolyl)-1-triazene were more effective on *E. carotovora* than *A. tumefaciens*.

Keywords: Diazoaminobenzene, Bactericidal effects, Fungicidal effects

1. Introduction

Diazoaminobenzene (triazene) derivatives attracted several scientists in different respects even other than antimicrobial action as in solar cells manufacturing [1], colorimetric detection [2-4] or other many uses

Triazenes are characterized by diazoamino moiety (-N=N-NH-) and synthesized by different ways, most widely by coupling of diazonium salts to amines and addition of organometallic reagents to alkyl azides [5-7]. Triazenes showed a wide range of applications as anticancer drugs, DNA alkylating agents in tumor therapy, polymer and oligomer synthesis, optical data storage, photo responsive systems, protecting groups in natural product synthesis and forming heterocycles [8-10]. 3-Hydroxy-3-*p*-tolyl-1-*m*-chloro-phenyltriazene showed insecticidal effect against the one day old male *Drosophila melanogaster* Meig fly. Among some triazene derivatives, 3-hydroxy-3-*n*-propyl-1-*m*-chlorophenyl triazene was the most active with LC_{50} value 0.9847 ppm [11], whereas the least compound was 3-hydroxy-3-*n*-propyl-1-*p*-methylphenyltriazene with LC_{50} value 16.52 ppm [12]. Hydroxytriazenes also showed antibacterial and antifungal activities with MIC values ranging from < 12.5 to 50 µg/ml against five bacteria: *Streptococcus faecalis, Klebsiella pneumoniae, E. coli, P. aeruginosa,* and *S. aureus* and five fungi (*Candida albicans, Cryptococcus neoformans, Sporotrichum schenckii, Trichophyton mentagrophytes* and *Aspergillus fumigates*) [13]. Hydroxytriazenes and their cobalt (II) complexes affected *E. coli*, *K. peneumoniae* and *Bacillus sp* [14]. Anti-microbial activity of some substituted *p*-aminoazobenzene with thymol moiety was proved *in vitro* against *B. subitillis, S. aureus* and *E. coli* [7]. The azo compounds exhibited strong antibacterial activities towards gram positive *S. enterica*, *M. luteus* and *B. subtilis* bacteria and weak activity against gram negative *S. enterica* and *P. aeruginosa* bacteria [15].

This work aimed to study the effect of certain diazoaminobenzene (triazene) derivatives against some plant pathogenic fungi and bacteria. So seven diazoaminobenzene (triazene) derivatives: 1,3-diphenyl-; 1,3-bis(2-tolyl)-; 1,3-bis(4-tolyl)-; 1,3-bis(4-phenylcarboxylic acid)-; 1,3-bis-(4-phenyl-sulphonamide)-; 1,3-bis-(3-chlorophenyl)- and 1,3-dinaphtyl-1-triazene (s) were synthesized, structurally confirmed and tested for their effects on *Alternaria solani, Botrytis cinerea*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* plant pathogenic fungi and *Agrobacterium tumefaciens* and

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Erwinia carotovora **economic phytoparasitic bacteria.**

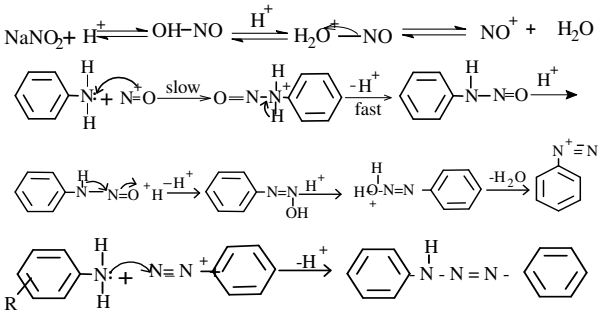
Materials and methods

1. Instruments and chemicals

Melting points were determined on Kofler block and uncorrected. Elemental micro-analysis (C, H, N, X) were done. I.R spectra were measured on Shimadzu FT/IR 4100 Instrument. U.V measurements were carried out on an instrument of UV-1600 serious. ^{1}H NMR spectra were recorded on DELTA2-500 NMR Spectrometer in DMSO-d6 using tetramethylsilane (TMS) as a standard. Mass spectra (EI-MS) were recorded on DI analysis, Shimadzu Qp-2010 plus at 70 eV. I.R and ^{1}H NMR analysis were done at Micro-analytical Center, Faculty of Science, Alexandria University, Egypt. Elemental analysis, U.V and EI-MS measurements were carried out at Micro-analytical Center, Faculty of Science, Cairo University, Egypt. All chemicals and solvents were purchased from El-Gomhouria Drug Company, Egypt.

2. Preparation of the tested compounds

According to [16] with modifications, Water (75 ml), concentrated HCl (20 ml, 24 gm) and the aniline derivative (0.15 mole) were vigorously shacked and crushed ice (50 gm) was added. Sodium nitrite (5.2 gm in 12 ml water) was added in portions and the mixture was left aside with frequent shacking. After 15 minutes, aqueous sodium acetate (53 %) was added in 5 minutes. The product was allowed to stand with shaking for 45 minutes at a temperature as high as 20° C, filtered off, washed with cold water, recrystallized from light petroleum (60-80°) and dried over phosphorus pentoxide. The general reaction mechanism of the synthesized derivatives and their structures are shown in Figure **(1)**.



Sodium acetate was added to liberate the remaining aniline from its acid conjugate for coupling to the diazoaminobenzene derivative and prevent the rearrangement to C- coupling products, which needs an acid media.

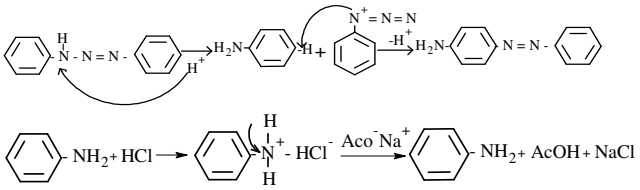
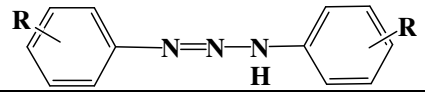


Figure (1): The mechanism of the diazoaminobenzene preparation and the prepared derivatives

The seven prepared derivatives are as follows:



	R	The chemical structure
1	H	1,3-Diphenyltriazene (diazoaminobenzene)
2	2-CH_3	1,3-Bis(2-tolyl)-1-triazene
3	4- CH_3	1,3- Bis (4-tolyl)-1-triazene
4	4-COOH	1,3- Bis (4-phenylcarboxylic acid)-1-triazene
5	4-SO_2NH_2	1,3- Bis (4-phenylsulphonamide)-1-triazene
6	3-Cl	1,3- Bis (3-chlorophenyl)-1-triazene
7		1,3-Dinaphtyl-1-triazene

3. Fungicidal activity measurements

3.1. Tested fungi

Alternaria solani, Botrytis cinerea, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* plant pathogenic fungi were firstly provided from Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt. The pathogenic fungi were grown on potato dextrose agar (PDA) medium at 27° C for 7 days before using in the test.

3.2. Fungicidal activity measurement

The sterilized well-known potato dextrose agar (PDA) medium was used for evaluation by the poison food technique. The tested compounds were added to the medium at 2×10^{-4}, 5×10^{-4}, 10^{-3}, 2×10^{-3} and 10^{-2} molar in dimethylsulfoxide (DMSO) that was used at as high as 1% of the poisoned medium. Control in presence of 1% DMSO was concurrently conducted. The poison medium was inoculated with each treated fungus individually and incubated at 27° C with three replicates for each treatment. All the mentioned

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additions or mixing were conducted under sterile conditions. The results were recorded and the percentage inhibition was calculated when the hyphal growth of control filled the Petri-dish. The average of the hyphal growth ±SD was calculated [17-18] and IC_{50} values in molar were determined [19]. The obtained results were compared with that of metalaxyl (methyl-N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alaninate) as a standard fungicide Figure (2).

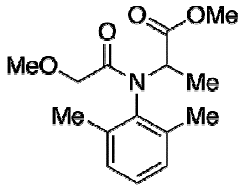
4. Bactericidal activity measurements:

4.1. Tested species

Agrobacterium tumefaciens and *Erwinia carotovora* were provided by Microbiology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Alexandria University and maintained on nutrient agar medium.

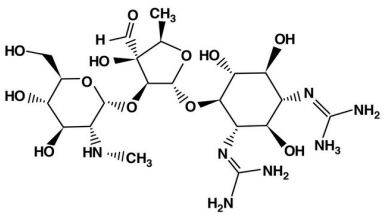
4.2. Bactericidal effect measurement

Agar dilution method was used, as recommended by European Society of Clinical Microbiology and Infection Disease [20], for determination of minimum inhibitory concentration (MIC) [21]. The tested compounds were used at 2×10^{-4}, 5×10^{-4}, 10^{-3}, 2×10^{-3} and 10^{-2} molar in Petri dishes. After solidifications, 6 µl of approximately 10^8 CFU/ml bacterial culture grown in nutrient broth for 12 hours was spotted (three spots per each plate) using 2µl standard loop and incubated at 35° C for 24 hrs. Three replicates were used for each treatment and control was concurrently conducted. Visible bacterial growth in the agar plates was noticed and data were compared with that 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) as a standard bactericide Figure (**2 A &B**).



Methyl N-(2,6-dimethylphenyl)-N- (methoxyacetyl)-DL-alaninate (**Metalaxyl**)

Figure (2A): The used standard fungicide



o-2-Deoxy-2-methylamino-α-L-glucopyranosyl-(1 → 2)-*o*-5-deoxy-3-C-formyl-α-L-lyxofuranosyl(1 →4-N1,N3-diamidino-D-streptamine (**Streptomycin**)

Figure (2B): The used standard bactericide

Results and discussion

1. Identification of the prepared compounds

1.1. Melting point, elemental analysis, ^{1}H NMR and MS spectroscopy measurements

The obtained results of the prepared triazene derivatives identification regarding melting point, elemental analysis, ^{1}H NMR and Mass spectroscopy measurements could be summarized as follows

1,3-Diphenyltriazene

Yellow crystalline powder, **Mw** 197, 40 gm (87%), melting point 97° C. **Found** C 73.3, H 5.2, N 21.2; **Calc**. C 73.1, H 5.6, N 21.3. **^{1}H NMR**: The triazene N-H proton at 12.42 δ (singlet). The aromatic protons at 7.1–7.5 δ as the anilino moiety protons gave their peaks at 7.11 δ (broad singlet, 2H, *ortho* protons), 7.14 δ (d, *J* = 7.65 Hz, *para*-H proton) and 7.4 δ (t, *J*= 6.85, 7.35 Hz, *meta*-2H). Benzene diazo moiety protons at 7.41 δ (broad singlet, conjugation with -N=N- group**). Mass spectrum**, the parent ion at m/z 197 ($M^{\cdot+}$, 9.9%). Diphenyl-aniline ion at m/z 169 (4.58%, M- N_2), phenyl ion at m/z 77 (100%) and phenyltriazene ion at m/z 120 (11.58%) that gave aniline ion at m/z 93 (36.2%) and phenyl-diazo ion at m/z 105 (23.0%), which loses N_2 to phenyl ion at m/z 77. Aniline losses a neutral HCN followed by a hydrogen atom to m/z 66 (11.3%) and 65 (38.1%), respectively. The phenyl ring exposed by fission to $C_4H_3^{\cdot+}$ ion at m/z 51 (34.5%).

1,3-Bis(2-tolyl)-1-triazene

Yellowish brown powder, **Mw** 225, 46.5 gm (46.6%), melting point 46-47° C. **Found** C 75.4, H 6.6, N 17.1; **Calc**. C 74.7, H 6.7, N 18.6. Mass spectrum, the parent ion at m/z 225 ($M^{\cdot+}$, 7.97%) lost N_2 to bis-(2-tolyl-)-aniline at m/z 196 (7.07%), which was fragmented to 2-tolylaniline ion at m/z 106 (100%) and 2-tolyl ion at m/z 91 (67.4%) that loses CH_3 to phenyl ion at m/z 77 (56.8%). The parent ion gave 1-phenyl-3-(2-tolyl)-1-triazene ion at m/z 210 (4.9%) by loss methyl radical to 2-tolyldiazo ion at m/z 119 (8.1%), 2-tolylaniline ion at m/z 106 (100%) and a tropylium ion at m/z 91 (67.4%), which subsequently loses C_2H_2 to m/z 65 (42.4%) or ethyl benzene ion at m/z 106 (100%). The phenyl ion exposed to fission to $C_4H_3^{\cdot+}$ at m/z 51 (34.6%).

1,3-Bis(4-tolyl)-1-triazene

Yellow crystalline powder, **Mw** 225, 28.8 gm (28.35%), melting point 117-118° C. **Found** C 71.1, H 6.1, N 17.7, **Calc**. C 74.6, H 6.7, N 18.6. ^{1}H NMR: Aliphatic protons at 2.25 δ (s, 4-CH_3, aniline moiety) and 2.47 δ (s, 4-CH_3, diazo moiety) due to N-H and -N=N- groups effects. The anilino moiety protons peaks at 7.13 δ (2H, d, *J* = 7.65 Hz, *ortho*-2H protons) and at 7.15 δ (2H, d, *J* = 7.65 Hz, *meta*-2H protons), referring to different methyl groups shielding effect. Direct bonding to -N=N- group, overlapped the diazo moiety protons to 7.27 δ as a broad singlet. N-H singlet peak was down-field to 12.2 δ. Mass spectrum was identical to that of 1,3-bis (4-tolyl-)-1-triazene.

1,3-Bis(4-phenylcarboxylic acid)-1-triazene

Yellow powder, **Mw** 285, 118.1 gm (91.71%), melting point 181-182° C. **Found** C 60.2, H 3.2, N 12.0, **Calc**. C 59.0, H 3.8, N 14.3. **^{1}H NMR**: The carboxylic groups down fielded the benzene diazo moiety peaks at 7.93 δ (2H, dd, *J* = 8.4, 8.4 Hz, *meta*-2H) and at 7.95 δ (2H, dd, *J* = 8.4, 8.4 Hz, *ortho*-2H) than in 1,3-bis-(4-tolyl)-1-triazene (the effect of the two substituted CH_3). N-H non shared electrons slightly up-field the aniline protons at 7.49 δ (2H, dd, *J* = 8.5, 8.5 Hz, *meta*-2H) and at 7.51 δ (2H, dd, *J* = 8.5, 8.5 Hz, *ortho*-2H), respectively. **Mass spectrum**: the parent ion at m/z 285 (38.2%), m/z 241 (40.1%, M-COOH) or m/z 198 (48.4%, M-2COOH) ions. The parent ion loses N_2 molecule to m/z 258 (40.1%),142 (48.1%) and 120 (70.06%), which lost a COOH, HCN followed by H to m/z 65 ion (100%). 4-Diazophenylcarboxlic acid loses N_2 and COOH to benzene ion at m/z 78 (44.59%).

1,3-Bis(4-phenylsulphonamide)-1-triazene

Yellow powder, **Mw** 335, 96.5 gm (50.89%), melting point 109° C. **Found** C 38.6, H 3.4, N 17.9, S 18.1, **Calc**. C 40.5, H 3.7, N 19.1, S 18.1. **^{1}H NMR**: The two sulfonamide groups deshielded the aromatic protons. NH non-shared electron pair slightly up field shifted the anilino moiety protons as 2-H and 6-H protons at 7.49 δ as doublet (d, *J*= 8.45 Hz, *ortho*-2H) and 7.51 δ (2H, d, *J* = 8.45 Hz, *meta*-2H), respectively. Benzene diazo moiety protons peaks distributed at 7.93 δ (2H, d, *J* = 8.4 Hz, *meta*- 2H) and at 7.95 δ (2H, d, *J* = 8.4 Hz, *ortho*-2H), respectively. The aliphatic N-H proton appeared as singlet at 2.47 δ due to conjugation with SO_2 group. **Mass spectrum**: (M-H) at m/z 354 (43.15). It may loses N_2 followed by one or two sulfonamide groups to m/z 327 (43.15), 247 (37.67) and m/z 168 (53.42), respectively. Its fission gave ion at m/z 172 (36.99), which loses a neutral HCN molecule to m/z 135 (55.48) m/z 184 that loses N_2 to fragment ion at m/z 157 (36.99). Fragments at m/z 64 (100) and its protonated fragment at m/z 65 (37.67) are due to phenyl ring fission. Loss of a sulfonamide amino group gave m/z 259 (37.67) ion.

1,3-Bis(3-chlorophenyl)-1-triazene

Yellow crystalline powder; **Mw** 266, 83.7 gm (52.82%), melting point 87-88°C. **Found** C 52.8, H 4.0, N 16.2, Cl 26.7, **Calc**. C 54.1, H 3.4, N 15.8, Cl 29.1. **Mass spectrum**: m/z 267 ion (75.86, MH^+). It loses a neutral N_2 to m/z 239 (2.30). Its fission gave fragments at m/z 126 (20.69) that loses a neutral HCN molecule to m/z 89 (39.08) ion and m/z 112 (14.99) that gives ion at m/z 96 (65.52, ring fission). Fragments at m/z 165 (100) was due to ring fission of the parent molecular ion.

1,3-Dinaphthyl-1-triazene

Orange powder, **Mw** 297, 42.7 gm (45.35%), melting point 183.5-185° C. **Found** C 79.6, H 4.2, N 13.8, **Calc**. C 80.8, H 5.1, N 14.1. **Mass spectrum**: m/z 297 (26.8%, M^+), dinaphthyl amine ion at m/z 268 (19.1%, MH^+ - N_2), which was consequently fragmented by fission into naphthyl amine ion at m/z 142 (47.8%) and naphthyl ion at m/z 127 (40%) that loses a neutral HCN giving indene ion at m/z 115, (100%).

1.2. UV and I.R Identification of the prepared compounds:

I.R and UV spectra helped the identification [22-23]. U.V spectra of diazoaminobenzene (triazene) derivatives undergo n — σ^*, π — π^* and n — π^* transitions. Conjugation of the azo group (chromophoric group) and –NH- (secondary amine) revealed the n — π^* absorption band near 350 - 360 nm with low absorptivity. π — π^* transition occurs in the far ultraviolet. Direct attachment of the chromophore to the benzene ring causes a strong bathochromic shift of **B-**band and the appearance of **K-** band at 250-260 nm. Naphthyl ring attached to the -N=N-NH- gave very similar absorption bands at 221 and 312 nm in **E_1**– and **B**- bands, respectively. Substitution of CH_3 on the benzene ring produces a bathochromic shift (red shift) of the B-band due to hyper conjugation of σ- electron of H_2C-H bond in resonance with the ring.

Substituted *ortho* methyl absorbs at the shortest wavelength, 266 nm whereas *para*- isomer absorbs at the longest wavelength, 293 nm, which expressed as σ — π^* transition due to steric interaction of *o*-substitution that effectively reduced hyper conjugation. Substitution of COOH and SO_2NH_2 affected n — π^* band due to their electronegativity (COOH is more electronegative) shifting them at 213.5 nm and 239.5 nm, respectively. Their π—π^* transitions were found at 371.5 and 364.5 nm, respectively. Substitution of an

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auxochromic group (-Cl) on benzene ring shifts **E**- and **B**- bands to longer wave length, so, n — π^* transition appeared at 261 nm.

IR spectroscopic results ascertained the chemical structures of the prepared derivatives and due to their similarly, the obtained results are summarized in Table (**1**).

Table (1)
IR identification of the prepared triazene derivatives

Wave number (cm^{-1})	Assignment
1503–1466	-N=N-NH- (three nitrogen atoms conjugation)
1440	Ar-N-H- stretching.
3438 – 3382	Secondary amine NH- stretching (single weak band)
1598.7	C – C stretching (phenyl rings, sharp band)
1241	C–H deformation (in–plane, sharp)
	C–H deformation (out–of–plane, sharp weak bands)
756 - 685	Compound **1**, 3-adjacent atoms
757	Compound **2**, 4-adjacent atoms
813	Compound **3**
744 – 849 820 – 890	Compounds **4** and **5**, respectively (low frequency) due to carboxylic and sulfonamide substituents effect
896 – 681	C-H out–of–plane bending (three weak bands) (compound **7**)
1591 and 1524	C-H deformation of CH_3 group (splitting sharp bands) compound **2** and compound **3**, respectively
3434	O-H stretching (slightly broadened, COOH)
1689 – 1606	C–O stretching -O–H deformation
1151	$-SO_2-NH_2$ (strong sharp band)
753- 716	C-Cl stretching (compound **6**)

2. Fungicidal activity

As shown in Table (**2**). The hyphal growth was differently inhibited in a function of concentration, tested derivative and the treated fungus. All compounds affected increasingly with increasing the concentration. The used standard fungicide metalaxyl caused low inhibition with $IC_{50} > 10^{-2}$ M. Against *A. solani* hyphal growth, 1,3-bis(4-tolyl)-, 1,3-bis(4-phenylcarboxylic acid)- and 1,3-bis(3-chlorophenyl)-1-triazene derivatives were similar to the standard fungicide with $IC_{50} > 10^{-2}$ M. 1,3-Bis(2-tolyl)-, 1,3-Bis(4-phenylsulphonamide)- ,1,3-diphenyl- and 1,3-dinaphthyl-1-triazene were more effective with IC_{50} values equaled 0.56×10^{-3} M, , 0.54×10^{-2} M, 0.23×10^{-2} M and 0.58×10^{-2} M, respectively. Against *B. cinerea*, 1,3-bis(4-phenylcarboxylic acid)-1-triazene inhibited its hyphal growth with less than 50% at the concentration range. The other derivatives inhibited its hyphal growth with IC_{50} values of 0.65×10^{-3} M, 0.8×10^{-3} M, 0.74×10^{-2} M, 0.57×10^{-3} M, 0.45×10^{-3} M and 0.25×10^{-4} M in case of 1,3-diphenyl-, 1,3-bis(2-tolyl)-, 1,3-bis(4-tolyl)-, 1,3-bis(4-phenylsulphonamide)-, 1,3-bis(3-chloro phenyl)- and 1,3-dinaphthyl-1-triazene in the same array.

Against *F. oxysporum*, 1,3-Bis(2-tolyl)-1-triazene and the used standard fungicide (metalaxyl) weakly inhibited the hyphal growth with IC_{50} value more than 10^{-2} molar. Both 1,3-bis(4-phenylsulphonamide)- and 1,3-diphenyl-1-triazene were highly effective with IC_{50} values equaled 2.9×10^{-4} M and 4.8×10^{-4} M, respectively. On the other hand, 1,3-dinaphthyl-, 1,3-bis(4-tolyl)-, 1,3-bis(4-phenyl-carboxylic acid)- and 1,3-bis(3-chlorophenyl)-1-triazene moderately inhibit it with IC_{50} values equaled 1.3×10^{-3} M, 2.1×10^{-3} M, 3×10^{-3} M and 4.0×10^{-3} M, respectively. On *R. solani*, while all the tested derivatives were as weak as the standard fungicide metalaxyl with IC_{50} values more than 10^{-2} molar, 1,3-dinaphthyl-1-triazene exceeded metalaxyl and the other derivatives in its effect with IC_{50} equaled 2.9×10^{-3} M. The *S. rolfsii* hyphal growth was inhibited with less than 50% except when treated with 1,3-diphenyl-, 1,3-bis(4-tolyl)-, 1,3-bis(3-chlorophenyl)-, and 1,3-dinaphthyl-1-triazene as they achieved IC_{50} values of 0.26×10^{-2} M, 0.24×10^{-2} M, 0.16×10^{-2} M and 0.35×10^{-3} M, respectively.

Generally, it was found that 1,3-dinaphthyl-1-triazene was the most effective against both *B. cinerea* and *S. rolfsii* followed by 1,3-bis(3-chlorophenyl)-, 1,3-bis(4-phenylsulphonamide)-, 1,3-diphenyl- and 1,3-bis(2-tolyl)-1-triazene against *B. cinerea* and followed by 1,3-bis(3-chlorophenyl)-, 1,3-bis(4-tolyl)- and 1,3-diphenyl-1-triazene against *S. rolfsii*, respectively. Worth mentioning, the activity was affected by the structure differences. Substitution with 2- CH_3 significantly increased the effect of 1,3-diphenyl-1-triazezene against *A. solani*, while 4-CH_3, 3-Cl and 4-COOH reduced it.

Substitution of 1,3-dinaphthyl- instead of 1,3-diphenyl- multiply the activity against *B. cinerea*, *R. solani* and *S. rolfsii* on contrary to *F. oxysporum*.

Table (2)
Fungicidal effects of the tested triazene derivatives on hyphal growth of some plant pathogenic fungi, shown as IC_{50} values

Fung	Tested derivative	IC_{50} values (in molar)			
		IC_{50}	95% C. L.	Slope ± SE	χ^2
A. solani	1,3-Diphenyl-1-triazene	0.23×10^{-2}	$(0.17 – 0.33)\times10^{-2}$	1.34 ± 0.02	4
	1,3-Bis(2-tolyl)-1-triazene	0.56×10^{-3}	$(0.45– 0.68)\times 10^{-3}$	1.3 ± 0.01	9
	1,3-Bis(4-tolyl)-1-triazene	$>1\times10^{-2}$			
	1,3-Bis(4-phenylcarboxylic acid)-1-triazene	$>1\times10^{-2}$			
	1,3-Bis(4-phenylsulphonamide)-1- triazene	0.54×10^{-2}	$(0.2 – 1.5)\times10^{-2}$	0.71± 0.013	0.3
	1,3-Bis(3-chlorophenyl)-1-triazene	$>1\times10^{-2}$			
	1,3-Dinaphthyl-1-triazene	0.58×10^{-2}	$(0.25 – 1.4)\times10^{-2}$	0.87± 0.017	1.6
	The used standard fungicide (metalaxyl)	**$>1\times10^{-2}$**			
B. cinerea	1,3-Diphenyl-1-triazene	0.65×10^{-3}	$(0.54 – 0.8)\times 10^{-3}$	1.5± 0.014	9.3
	1,3-Bis(2-tolyl)-1-triazene	0.8×10^{-3}	$(0.64 – 1.0)\times 10^{-3}$	1.4± 0.013	8.9
	1,3-Bis(4-tolyl)-1-triazene	0.74×10^{-2}	$(0.32 – 1.0)\times10^{-2}$	0.9± 0.016	1.2
	1,3-Bis(4-phenylcarboxylic acid)-1-triazene	$>1\times10^{-2}$			
	1,3-Bis(4-phenylsulphonamide)-1- triazene	0.57×10^{-5}	$(0.39 – 0.83)\times10^{-5}$	1.01± 0.12	4.3
	1,3-Bis(3-chlorophenyl)-1-triazene	0.45×10^{-3}	$(0.38– 0.53)\times10^{-3}$	1.5± 0.013	9.2
	1,3-Dinaphthyl-1-triazene	0.25×10^{-4}	$(0.12– 0.51)\times10^{-4}$	0.66± 0.01	1.0
	The used standard fungicide (metalaxyl)	**$>1\times10^{-2}$**			
F. oxysporum	1,3-Diphenyl-1-triazene	4.8×10^{-4}	$(3.1– 7.4)\times 10^{-4}$	0.7 ± 0.01	15
	1,3-Bis(2-tolyl)-1-triazene	$>1\times10^{-2}$			
	1,3-Bis(4-tolyl)-1-triazene	2.1×10^{-3}	$(1.6 – 2.8)\times 10^{-3}$	0.97± 0.02	47
	1,3-Bis(4-phenylcarboxylic acid)-1-triazene	3.0×10^{-3}	$(1.2 – 6.9)\times 10^{-3}$	0.37± 0.01	6.2
	1,3-Bis(4-phenylsulphonamide)-1- triazene	2.9×10^{-4}	$(9.3 – 8.9)\times 10^{-4}$	0.33± 0.01	14
	1,3-Bis(3-chlorophenyl)-1-triazene	4.0×10^{-3}	$(3.34 – 4.9)\times 10^{-3}$	2.1± 0.030	14
	1,3-Dinaphthyl-1-triazene	1.3×10^{-3}	$(1.1 – 1.6)\times 10^{-3}$	1.72± 0.02	6.8
	The used standard fungicide (metalaxyl)	**$>1\times10^{-2}$**			
R. solani	1,3-Diphenyl-1-triazene	$>1\times10^{-2}$			
	1,3-Bis(2-tolyl)-1-triazene	$>1\times10^{-2}$			
	1,3-Bis(4-tolyl)-1-triazene	$>1\times10^{-2}$			
	1,3-Bis(4-phenylcarboxylic acid)-1-triazene	$>1\times10^{-2}$			
	1,3-Bis(4-phenylsulphonamide)-1- triazene	$>1\times10^{-2}$			
	1,3-Bis(3-chlorophenyl)-1-triazene	$>1\times10^{-2}$			
	1,3-Dinaphthyl-1-triazene	2.9×10^{-3}	$(2.5 – 3.4)\times 10^{-3}$	2.36±0.33	15
	The used standard fungicide (metalaxyl)	**$>1\times10^{-2}$**			
S. rolfsii	1,3-Diphenyl-1-triazene	0.26×10^{-2}	$(0.17 – 0.4)\times10^{-2}$	1.0 ± 0.013	0.6
	1,3-Bis(2-tolyl)-1-triazene	$>1\times10^{-2}$			
	1,3-Bis(4-tolyl)-1-triazene	0.24×10^{-2}	$(0.16 – 0.34)\times10^{-2}$	1.3 ± 0.019	3
	1,3-Bis(4-phenylcarboxylic acid)-1-triazene	$>1\times10^{-2}$			
	1,3-Bis(4-phenylsulphonamide)-1- triazene	$>1\times10^{-2}$			
	1,3-Bis(3-chlorophenyl)-1-triazene	0.16×10^{-2}	$(0.12 – 0.22)\times10^{-2}$	1.2 ± 0.02	5
	1,3-Dinaphthyl-1-triazene	0.35×10^{-3}	$(0.25 – 0.5)\times 10^{-3}$	0.7 ± 0.09	2
	The used standard fungicide (metalaxyl)	**$>1\times10^{-2}$**			

DF, Degree of freedom = 3

Economically, the *B. cinerea* fungus causes gray mold on tomato, strawberry and other crops while *A. solani* causes early blight disease of potato and tomato. Southern blight as a serious disease of field crops, vegetable, fruits and ornamentals is causing by *S. rolfsii*. The soil borne fungi *F. oxysporum* and *R.*

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solani caused welt and damping off. So based on the obtained good effects of the prepared tested compounds on these dangerous fungi, the active tested triazene derivatives could enter the fungicides clique.

3. Bactericidal activity

The synthesized triazenes exhibited their bactericidal activities *in vitro* in comparison with streptomycin against both *A. tumefaciens* and *E. carotovora* bacteria are recorded in minimum inhibition concentration (MIC) values in Table (**3**). Streptomycin completely prevents the bacterial growth of *A. tumefaciens* and *E. carotovora* at 2×10^{-4} M and 2×10^{-3} M, respectively. 1,3-Bis(4-phenylcarboxylic acid)-, 1,3-diphenyl- and 1,3-bis(3-chlorophenyl)-1-triazene were active against *A. tumefaciens* with MIC equaled 1.0×10^{-3}, 2.0×10^{-3} and 2.0×10^{-3} M, respectively. 1,3-bis(2-tolyl)-, 1,3-bis(4-tolyl)-, 1,3-bis(4-phenylsulphonamide)- and 1,3-dinaphthyl-1-triazene gave similar effects on *A. tumefaciens* with MIC equaled 1.0×10^{-2} M. Concerning *E. carotovora*, streptomycin, 1,3-bis(4-tolyl)- and 1,3-bis(3-chlorophenyl)-1-triazene achieved the same MIC value (2.0×10^{-3} M). On the other hand, 1,3-diphenyl-, 1,3-bis(4-phenylcarboxylic acid)- and 1,3-dinaphtyl-1-triazene were effective inhibiting its growth with MIC value of 1×10^{-3} M. Both 1,3-bis(2-tolyl)- and 1,3-bis(4-phenylsulphonamide)-1-triazene exhibited MIC value equaled 1.0×10^{-2} M for each. These produced effects may be referred to the mutagenic effect on bacteria [24] as he exhibited that diazoaminobenzene was mutagenic in some *Salmonella typhimurium* strains.

Table (3)
Bactericidal activities of the tested triazene derivatives

Compound	MIC in Molar	
	A. tumefaciens	***E. carotovora***
1,3-Diphenyl-1-triazene	2×10^{-3}	1×10^{-3}
1,3-Bis(2-tolyl)-1-triazene	1×10^{-2}	1×10^{-2}
1,3-Bis(4-tolyl)-1-triazene	1×10^{-2}	2×10^{-3}
1,3-Bis(4-phenylcarboxy-lic acid)-1-triazene	1×10^{-3}	1×10^{-3}
1,3-Bis(4-phenylsulphon--amide) -1-triazene	1×10^{-2}	1×10^{-2}
1,3-Bis(3-chlorophenyl)-1-triazene	2×10^{-3}	2×10^{-3}
1,3-Dinaphthyl-1-triazene	1×10^{-2}	1×10^{-3}
Streptomycin	$\mathbf{2\times10^{-4}}$	$\mathbf{2\times10^{-3}}$

From the obtained results, the tested triazene derivatives showed several fungicidal and bactericidal effects in agreement with [25-26]. Regarding to the environmental effects, [8] showed that some triazenes are effective cancer chemotherapeutic agents in a number of biological systems as [27] found that series of azo compounds with extended p-conjugated systems were very active against breast cancer adenocarcinoma (MCF-7), cervix adenocarcinoma (HeLa) and human embryonic kidney (HEK 293) cell lines. They added that some azo products exhibited potent *in vitro* anti-proliferative activity against MCF-7 and HeLa cell lines with inhibitory effects in varieties of cancers as good potent drug candidates. It was also proved that 4-[(E)-(Fluorophenyl)diazenyl]phenol showed the highest anticancer activity against nasopharyngeal cancer (NPC) HK-1 cell lines, while 4-[(Halophenyl) diazenyl]phenyl aspirinate showed better anticancer activity than aspirin alone [28]. Both legands and their vanadium complexes showed very significant anti-inflammatory activity up to an hour in comparable to diclofenac sodium as a standard drug [14]. Also, it was exhibited that using high efficiency diazoaminobenzene DAAB nano-materials in solar cells fabrication would provide a promising platform to discover clean energy sources and preserve the environment [1].

Conflicts of interest

"There are no conflicts to declare".

References

[1] Shabzendedara S., Modarresi-Alama, A. R., Bahrpeymac, A. , Noroozifard, M. and Kermand, K.. **(2020).** Novel conductive multi-walled polymeric nanotubes of poly (diazoaminobenzene) for single-layer polymer solar cell. *Reactive and Functional Polymers*, 149: 104529**.**

[2] Liu, X.; Huang, D.; Lai, C.; Qin, L.; Zeng, G.; Xu, P.; Li, B.; Yi, H. and Zhang, M. (2019). Peroxidase-like activity of smart nanomaterials and their advanced application in colorimetric glucose biosensors. Small, **15**, 1900133.

[3] Caterina S., A. Fantoni, C. B. Elisabete, A. Alegria and Vieira, M. (2022). Hybrid nanocomposites of plasmonic metal nanostructures and 2D nanomaterials for improved colorimetric detection. Chemosensors, 10: 237.

[4] Mohamad, A.; Teo, H.; Keasberry, N.A. and Ahmed, M.U. Recent developments in colorimetric mmunoassays using nanozymes and plasmonic nanoparticles. Crit. Rev. Biotechnol. **2019**, 39, 50–66.

[5] Unsalan, S., Cikla, P., kucukguzel, S. G., Rollas, S., Sahin, F. and Bayrak, O.F. (2011). Synthesis and characterization of triazenes derived from

sulfonamides. Marmara Pharmaceutical Journal, 15: 11-17.
[6] Chauhan, L.S., Jain, C.P., Chauhan, R.S. and Goswam, A.K. (2010). Synthesis and antimicrobial activity of some substituted hydroxytrizenes. Journal of Chemical and Pharmaceutical Research, 2 (**4**): 979 - 983.
[7] Piste, P.B., Indalkar, D.P., Zambare, D.N. and Mundada, P.S. (2012). Synthesis and antimicrobial activity of substituted *p*-amino azobenzene with thymol moiety- A green protocol. International Journal of Chemistry Research, 3 (**2**): 25 -29.
[8] Rouzer, C.A., Sabourin, M., Skinner, T.L.,Thompson, E.J., Wood T.O. et *al*. (1996). Oxidative metabolism of 1-(2-chloroehyl)-3-alkyl-3-(methylcarbamoyl) triazenes: formation of chloroactaldehyde and relevance to biological activity. Chemical Research Toxicology, 9: 172 - 178.c
[9] Morigaki, K., Schonerr, H., Frank, C.W. & Knoll, W. (2003). Langmuir19: 6994-7002. C.f: Mohammadi, A. (2014). Novel triazene dyes based on N-phenyltriazene: synthesis, anti - bactrial activity and solvatochromic properties. Journal of Molecular Liquids, 193: 69 -73.
[10] Lazny, P., Sienkiewicz, M. and Brase, S. (2001). Tetrahedron, 57: 5825-5832. C.f: Mohammadi, A. (2014). Novel triazene dyes based on N-phenyltriazene: synthesis, anti - bactrial activity and solvatochromic properties. Journal of Molecular Liquids, 193: 69 -73.
[11] Rezaie, B., Ressalan, S., Chauhan, R.S., Goswami, A.K.and Purohit, N.D. (1997). Synthesis and insecticidal studies of hydroxytriazenes. Asian Journal of Chemistry, 9 (**4**): 891-892.
[12] Kumar, S., M. Garg, M., Jodha, J.S., Singh, R.P., Pareek, N., Chauhan, R. S. and Goswami, A. K. (2009). Studies in insecticidal activity of some hydroxytriazene derivatives. Electronic Journal of Chemistry, 6 (**2**): 466-468.
[13] Goswami, A. K. and Purohit, D. N. (2001). Synthesis and antimicrobial activities of some hydroxytriazenes: A new class of biologically active compounds. Analytical Sciences, 17 (**I**): 789-791.
[14] Singh, K., Patel, P. and Goswami, A.K. (2008). Anti-inflammatory activity of hydroxytriazenes and their vanadium complexes. Electronic Journal of Chemistry, 5 (**52**): 1144-1148.
[15] Mohammadi, A. (2014). Novel triazene dyes based on N-phenyltriazene: synthesis, anti - bactrial activity and solvatochromic properties. Journal of Molecular Liquids, 193: 69 -73.
[16] Vogel, A. I. (1989). A text Book of Practical Organic Chemistery 5th Edition, page 852 Longmans, Green and Co. , London, Newyork. Tornonto.
[17] Cohort Software Inc. (1986). Costa Users Manual, Version 3.03 Berkeley. Califoronia, USA.
[18] Topps, W. & Wain, R.L. (1957). Investigation fungicides III. The fungitoxicity of 4- and 5-alkylsalicylanilide and p-chloroanilines. Ann. Appl. Biol., 45 (**3**): 506 - *511*.
[19] Finney, D. J. (1971). Probit Analysis.3rd edition Cambridge University Press, London, Page: 138.
[20] European Society of Clinical Microbiology and Infection Diseases (ESCMID), (2000). Determination of minimum inhibitory concentrations (MLC) of antibacterial agents by agar dilution. Clin. Microbiol. Infect, **6**: 509 – 515.
[21] Badawy, M. E. I.,Ahmed, S.M. & Rabea, E.I. (2006). Bactericidal and fungicidal activity of different molecular weight chitosan samples. Journal of Pest Control & Environmental Sciences. 14 (**2**): 19-34.
[22] Dyke, S.F., Floyd, A.J., Sainsbury, M. & Theobold, R.S. (1978). Organic Spectroscopy,2nd edition, Longman, London and New York.
[23] Silverstein, R. M. and Bassler, G.C. (1967). Spectrometric identification of organic compounds, John Wily and Sons, Inc., New York, London, Sydney.
[24] Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., and Speck, W. (1987). *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. Environ. Mutagen. 9 (Suppl. 9), 1-110.
[25] Abdel-Aty, A. S. (2009). Pesticidal activity of some imidazole and oxazole derivatives. World Journal of Agricultural Sciences, 5 (1): 105-113.
[26] Abdel-Aty, A. S. (2010). Fungicidal activity of certain indole derivatives against some plant pathogenic fungi. J. Pestic. Sci., 35 (**4**), 431–440.
[27] Rezaei-Seresht, E., Mireskandari, E., Kheirabadi, M, Cheshomi, H., Rezaei-Seresht, H.*et al*. (2017). Synthesis and anticancer activity of new azo compounds containing extended p-conjugated systems. Chem. Pap., 71: 1463 – 1469.
[28] Boon, K.H., Ngaini, Z., Neilsen, P.M., Hwang, S.S., Linton, R.E. *et al*. (2017). Synthesis and Anticancer Activities of 4-[(Halophenyl)di-azenyl]phenol and 4-[(Halophenyl)-diazenyl]-phenyl Aspirinate Derivatives against Nasopharyngeal Cancer Cell Lines. Journal of Chemistry, https://doi.org/10.1155/2017/6760413