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Phytocidal and molluscicidal activity of some diazoaminobenzene derivatives

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Abstract

Seven diazoaminobenzene derivatives were prepared, and tested for their phytocidal activities against wheat (*T. aestivum*) and squash (*Cucumber pepo*) seeds germination, seedling growth and dry weigh of the grown seedlings. Their molluscicidal effect on the terrestrial snail, *Theba pisana* was also studied. The tested triazene derivatives inhibited the seed germination, the produced seedlings growth and the dry weight increasingly with increasing the concentration and differently based on the derivative structure. Substitution with 2-CH_3 increased the germination inhibition with non-significant differences on shoot and root systems growth compared with 1,3- diphenyl-1-triazene. Vice Versa, 4-CH_3 substituent decreased the effect. The structure activity relationship of the tested compounds against the seed germination, the produced seedlings growth and the dry weight was illustrated. Regarding the molluscicidal effect, 1,3-Bis(2-tolyl)-1-triazene caused moderate lethal effect, while the other derivatives required to use higher concentrations than 10% to be significantly active against the treated snail.

Keywords:, Diazoaminobenzene, Triazene, Molluscicidal, Phytocidal effect, Dry weight

Introduction

Diazoaminobenzene (triazene) derivatives are characterized by diazoamino moiety (-N=N-NH-) and can be synthesized with different ways, most widely by the coupling of the diazonium salts to amines and the addition of organometallic reagents to alkyl azides (Unsalan *et al*. 2011; Chauhan *et al*. 2010; Piste *et al*., 2012). Diazoaminobenzene (triazene) derivatives showed a wide range of applications such as solar cells manufacturing (Shabzendedara *et. al*., 2020) and analytical detection (Liu, *et al*. 2019; Caterina *et al*. (2022); Mohamad *et al*. (2019). They were used 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 (Rouzer *et al*., 1996; Morigaki *et al*., 2003; Lazny *et al*, 2001). 3-Hydroxy-phenyltriazene derivatives exhibited insecticidal effect against *Drosophila melanogastar* Meig fly differently according to the structure differences (Rezaie *et al*., 1997; Kumar *et al*. 2009); antibacterial and antifungal activities against *Streptococcus faecalis, Klebsiella pneumoniae, E. coli, P. aeruginosa, Bacillus sp* and *S. aureus* bacteria and *Candida albicans, Cryptococcus neoformans, Sporotrichum schenckii, Trichophyton mentagrophytes* and *Aspergillus fumigates*) fungi (Goswami and Purohit 2001; Singh *et al.* 2008; Piste *et al*., 2012; Mohammadi, 2014)**.** Triazo derivatives such as benzotriazole derivatives showed significant herbicidal capacity on both monocotyledons and dicotyledons (Abdel-Aty**,** 2011; Caramazza *et al*., 1990), while Triazolinones displayed potential herbicidal activities exceeding the commercial product sulfentrazone (Luo *et al*., 2008). Amitrole (ATz, 3-amino-1H-1,2,4-triazole) is a widely employed herbicide (Watanabe *et al*., 2005). Phytocidal activities of certain pyrazole derivatives have been reported by Abdel-Aty (2007).

Therefore, seven triazene 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 tested for their, phytocidal and molluscicidal activities.

Materials and methods

1. Tested compounds

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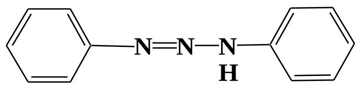
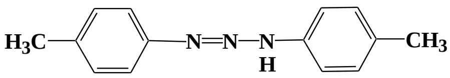
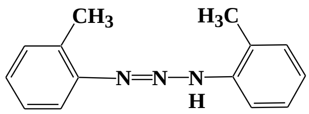
As Shown in Figure (1), seven triazene derivatives were tested. These compounds are 1,3-diphenyltriazene (diazoaminobenzene); 1,3-bis(2-tolyl)-1-triazene; 1,3- bis (4-tolyl)-1-triazene; 1,3-bis (4-phenylcarboxylic acid)-1-triazene; 1,3- bis (4-phenylsulphonamide)-1-triazene; 1,3- bis (3-chlorophenyl)-1-triazene and 1,3-dinaphtyl-1-triazene were synthesized, structurally confirmed by determining their melting points, elemental micro-analysis (C, H, N, X), I.R spectra , UV- analysis, ^{1}H NMR and Mass spectroscopy (EI-MS) measurements (Abdel-Aty *et al*. (2023). All chemicals and solvents were purchased from El-Gomhouria Drug Company, Egypt.



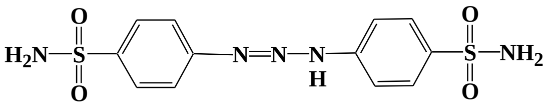
1,3-Diphenyltriazene (diazoaminobenzene)



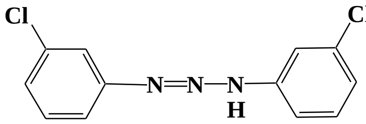
1,3- Bis (4-tolyl)-1-triazene



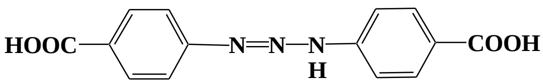
1,3-Bis(2-tolyl)-1-triazene



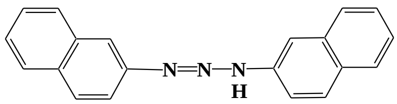
1,3- Bis (4-phenylsulphonamide)-1-triazene



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



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



1,3-Dinaphtyl-1-triazene

Figure (1): Chemical structure of the tested diazoaminobenzene derivatives

2. Phytocidal activity measurements

2.1. Preliminary seed treatments

The prepared compounds were tested for their phytotoxicity on wheat seeds using the cotton plug technique (Grodzinsky & Grodzinsky, 1973) at 50, 100, 200, 500 and 1000 µg/ml in dimethylformamide (DMF) at as high as 1% of the solution volume. Three replicates were considered as a treatment. Control was concurrently conducted. After 10 days, the number of germinated seeds, the height of seedlings and their inhibition percents were recorded. Effective concentration caused 50% inhibition (EC_{50}) was calculated for each compound (Finney, 1971).

2.2. Effect on pre-germinated seeds

This test was conducted using plain agar solution (1.5%) (Zemanek, 1963) in test tubes at 5×10^{-2}, 2×10^{-2}, 1×10^{-2} and 2×10^{-3} molar in DMSO at as high as 1%. The homogeneous pre-germinated seeds of either wheat (*Triticum aestivum*) or squash (*Cucumber pepo*) were selected and used. Three replicates were considered as a treatment. Control was concurrently conducted. The growth inhibition of root and shoot systems were calculated and EC_{50} values were determined.

2.3. Effect on dry weight

In pre emergence treatment, the grown wheat plants were collected at the soil surface and weighed. The fresh weight was air dried for 10 days and followed by drying at 105 ^{O}C. Oven dry matter content **(**DMC) was determined and compared at different concentrations.

3. Molluscicidal activity measurements:

Theba pasiana terrestrial snails were collected from the gardens of the Faculty of Agriculture, University of Alexandria, adapted and treated with the tested compounds at 1, 2, 5 and 10 % (W/W) (Miller *et al*., 1988). The number of alive snails was recorded after 3, 7, 10, 15 and 17 days. Mortality percents and LC_{50} values were determined.

Results and discussion

Phytocidal activity

Treatment of wheat (*T. aestivum*) seeds with the tested triazenes inhibited its germination and the produced seedlings growth increasingly with increasing the concentration. This effect differed based on the derivative structure. Substitution with 2-CH_3 increased the germination inhibition with non-significant differences on shoot and root systems growth compared with 1,3- diphenyl-1-triazene. Vice Versa, 4-CH_3 substituent decreased the effect. Substitution with 1,3-bis(4-phenylcarboxylic acid) increased the effect significantly on seed germination. This effect was increased on seed germination, root system and shoot system growth in case of treatment with 1,3-bis(4-phenylsulphonamide)-1-triazene. Substitution with 3-chloro on the phenyl rings in 1,3-bis(3-chlorophenyl)-1-triazene reduced its activity on germination significantly in comparison with the non- substituted 1,3-diphenyl-1-triazene and non-significantly on root and shoot systems growth. 1,3-Dinaphthyl-1-triazene harshly increased the inhibition of seed germination and both root and shoot systems growth (Table **1**)

As shown in Table (**2**), The treatment of the pre-germinated wheat (*T. aestivum*) and squash (*Cucumber pepo*) seedlings with the tested triazene derivatives. 1,3-Dinaphtyl- and 1,3-diphenyl-1-triazene were more effective to inhibit the wheat shoot system with EC_{50} values equaled 0.46 and 0.65 mM, respectively. The substitution of different groups on the phenyl ring relatively decreased the activity on shoot growth. Therefore, 1,3-bis(3-chlorophenyl)-, 1,3-bis(2-tolyl)-, 1,3-bis(4-tolyl)-, 1,3-bis(4-phenylsulphonamide)- and 1,3-bis(4-phenylcarboxylic acid)-1-triazene caused EC_{50} values equaled 2.0 mM, 2.8 mM, 2.97 mM, 5.36 mM and 13.7 mM, respectively in descending order. Dinaphthyl derivative inhibited wheat shoot system growth with 1.43 times more than diphenyl derivative. The substitution on phenyl decreased the effects of the diphenyl-1-triazene with 3.07, 4.4, 4.75, 8.2 and 21.0 times in case of 1,3-bis(3-chlorophenyl)-, 1,3-bis(2-tolyl)-, 1,3-bis(4-tolyl)-, 1,3-bis(4-phenylsulphon-amide)- and 1,3-bis(4-phenylcarboxylic acid)-1-triazene against wheat shoot system, respectively. 1,3-Diphenyl-1-triazene and 1,3-bis(3-chlorophenyl)-1-triazene inhibited the growth of the wheat root system with EC_{50} values of 0.51 and 0.71 mM, respectively. The other derivatives moderately affected the roots with EC_{50} values equal 1.29 mM, 1.31mM, 2.2 mM, 2.61 mM and 14.5 mM for 1,3-bis(2-tolyl)-, 1,3-dinaphtyl-, 1,3-bis(4-phenylsulphonamide)- and 1,3-bis(4-phenylcarboxylic acid)-1-triazene, respectively. The substitution of different groups on the phenyl ring relatively reduced the phytoxicity against the root system. Hence, 3-chloro-, 2-methyl-, 4-methyl-, 4-sulphonamide and 4-carboxylic acid decreased the inhibition effect of the non-substituted diphenyl with 1. 54, 2.2, 2.55, 5.1 and 28.2 times, respectively. Dinaphtyl-1-triazene showed EC_{50} value of 2.2×10^{-3} M which was less toxic than diphenyl derivative with 4.28 times against the wheat root system1,3-Bis(4-tolyl)-1-triazene was highly toxic against the squash shoot system with EC_{50} value equaled 0.98 mM. The other derivatives moderately inhibited it with EC_{50} values equaled 1.15 mM, 6.55 mM, 11.3 mM, 13.3 mM, 13.3 mM and 18.7 mM, which were caused by 1,3-dinaphtyl-, 1,3-bis(3-chlorophenyl)-, 1,3-bis(2-tolyl)-, 1,3-diphenyl-, 1,3-bis(4-phenylcarboxylic acid)- and 1,3-bis(4-phenylsulphonamide)-1-triazene, respectively in descending order. However, 4-tolyl derivative was the highest toxic. On the other hand, the other derivatives effects were reduced by 1.2, 6.7, 11.5, 13.6, 13.6 and 19.1 times in case of 1,3-dinaphtyl-, 1,3-bis(3-chlorophenyl)-, 1,3-bis(2-tolyl)-, 1,3-diphenyl-, 1,3-bis(4-phenylcarboxylic acid)-, and 1,3-bis(4-phenylsulphonamide)-1-triazene, in comparison with highest toxic one, 1,3-bis(4-tolyl)-1-triazene.

1,3-Dinaphtyl-1-triazene caused the highest inhibitory effect on its root system with the EC_{50} value equaled 0.36 mM. 1,3-Bis(4-tolyl)-1-triazene and 1,3-Bis(3-chlorophenyl)-1-triazene were also highly toxic with EC_{50} values equaled 0.68 mM and 0.83 mM, respectively. The other derivatives were moderately toxic on squash root system with EC_{50} values of 2.19 mM, 12.8 mM, 17.8 mM and 88.0 mM by 1,3-bis(4-phenylcarboxylic acid)-, 1,3-bis(2-tolyl)-, 1,3-diphenyl- and 1,3-bis(4-phenylsulphonamide)-1-triazene, which were less phytotoxic than the highest two compounds 6.1, 35.9, 49.9 and 246.5 times, respectively.

The chosen active compounds differently affect the wheat seedling fresh, 10 days air dried and oven dry weights at low concentration range (50 – 100 µg/ml).While the untreated wheat seedlings were 0.69 ± 0.02, 0.30 ± 0.01 and 0.20 ± 0.002 gm fresh, 10 days air dried and oven dried seedling weights, 1,3- bis(2-tolyl)-1-triazene was less effective reducing the weight from 0.58 ± 0.02 to 0.39± 0.01, 0.23 ± 0.014 to 0.18± 0.008 and 0.15± 0.009 to 0.17± 0.013 gm, respectively with EC_{50} equaled >100 µg/ml in all cases.

1,3-Bis(4-phenylcarboxylic acid)- and 1,3-dinaphthyl-1-triazene were more effective without significant differences between each other with EC_{50} values equaled 46.3, 35.6 and 55.3 µg/ml, in comparison to 38.0, 27.0 and 55.3 µg/ml against the fresh, 10 days air dried and oven dried seedling weights. 1,3 Bis (4-phenyl sulfonamide)-1- triazene was the most effective with 12.3, 17.4 and 13.8 µg/ml EC_{50} values (Table **3**).

Table (1): Preliminary phytocidal effects of the tested triazene derivatives on *T. aestivum* seeds.

Effect on	Compound	EC$_{50}$ values (in mmolar) (mM) EC$_{50}$	95% C.L.	Slope± SE	χ^2
Seed germination	1,3-Diphenyl-1-triazene	1.2	1.0 – 1.4	1.6 ± 0.02	2.3
	1,3-Bis(2-tolyl)-1-triazene	0.46	0.40 – 0.53	2.6 ± 0.046	14
	1,3-Bis(4-tolyl)-1-triazene	5.0	3.4 – 7.2	1.3 ± 0.03	3.6
	1,3-Bis(4-phenylcarboxylic acid)-1-triazene	0.57	0.48 – 0.67	1.8 ± 0.023	17
	1,3-Bis(4-phenylsulphonamide)-1- triazene	0.36	0.31 – 0.43	1.9 ± 0.03	9.9
	1,3-Bis(3-chlorophenyl)-1-triazene	1.1	0.83 – 0.13	1.2 ± 0.017	2.6
	1,3-Dinaphthyl-1-triazene	0.55	0.47 – 0.64	1.9 ± 0.024	8.6
Shoot system	1,3-Diphenyl-1-triazene	0.42	0.33 – 0.53	1.5 ± 0.032	3.3
	1,3-Bis(2-tolyl)-1-triazene	0.33	0.29 – 0.38	2.9± 0.079	6.9
	1,3-Bis(4-tolyl)-1-triazene	3.2	2.0 – 5.1	2.4 ± 0.84	22
	1,3-Bis(4-phenylcarboxylic acid)-1-triazene	0.34	0.29 – 0.40	2.0 ± 0.032	4.4
	1,3-Bis(4-phenylsulphonamide)-1- triazene	0.057	0.03 – 0.11	1.18 ± 0.034	4.6
	1,3-Bis(3-chlorophenyl)-1-triazene	3.6	0.9 - 12	3.4 ± 0.057	19
	1,3-Dinaphthyl-1-triazene	0.38	0.34 – 0.43	3.1 ± 0.065	5.1
Root system	1,3-Diphenyl-1-triazene	0.29	0.25 – 0.34	2.9± 0.11	1.6
	1,3-Bis(2-tolyl)-1-triazene	0.28	0.25 – 0.33	3.3 ± 0.131	0.3
	1,3-Bis(4-tolyl)-1-triazene	2.1	1.5 – 3.0	0.87 ± 0.016	13
	1,3-Bis(4-phenylcarboxylic acid)-1-triazene	0.39	0.33 - 0.45	2.1 ± 0.031	3.4
	1,3-Bis(4-phenylsulphonamide)-1- triazene	0.18	0.14 - 0.24	1.8 ± 0.042	24
	1,3-Bis(3-chlorophenyl)-1-triazene	0.70	0.6 - 0.81	1.91 ± 0.023	12
	1,3-Dinaphthyl-1-triazene	0.004	0.003 - 0.005	2.43 ± 0.043	14

Degree of freedom = 3 95% C.L., 95% Confidence limit

Table (2): Phytotocidal activity of the tested triazene derivatives on wheat *(T. aestivum*) and squash (*C. pepo*) seedlings

Plant	Compound	EC$_{50}$ values (in mmolar) (mM) Root system EC$_{50}$	95% C. L.	χ^2	Shoot system EC$_{50}$	95% C. L.	χ^2
Wheat (*T. aestivum*)	1,3-Diphenyl-1-triazene	0.51	0.27 – 0.88	0.3	0.65	0.30 – 1.38	13
	1,3-Bis(2-tolyl)-1-triazene	1.29	0.40 – 3.88	1.4	2.83	1.92 – 4.10	17
	1,3-Bis(4-tolyl)-1-triazene	1.31	0.58 – 2.90	2.4	2.97	1.80 – 4.74	1.6
	1,3-Bis(4-phenylcarboxylic acide)-1-triazene	15.4	12.0 – 18.0	6.9	13.7	12.0 – 15.7	3.1
	1,3-Bis(4-phenylsulphon amide)-1- triazene	2. 61	1.53– 4.40	1.0	5.36	3.20 – 8.92	0.8
	1,3-Bis(3-chlorophenyl)-1-triazene	0.71	0.20 – 3.0	1.7	2.0	1.40 – 3.84	4.9
	1,3-Dinaphthyl-1-triazene	2.20	1.46 – 3.30	8.6	0.46	0.14 – 1.20	11
Squash (*C. pepo*)	1,3-Diphenyl-1-triazene	17.8	14.6 – 22.0	22	13.3	12.0 – 15.0	1.9
	1,3-Bis(2-tolyl)-1-triazene	12.8	11.0 – 15.3	27	11.13	10.0 – 12.7	2.6
	1,3-Bis(4-tolyl)-1-triazene	0.68	0.13 - 3.30	6.6	0.98	0.49 – 1.9	9.3
	1,3-Bis(4-phenylcarboxylic acide)-1-triazene	2.19	1.21 - 3.90	5.6	13.3	12.0 – 14.5	0.3
	1,3-Bis(4-phenylsulphon amide)-1- triazene	88.0	65 – 120	13	18.7	16.0 – 21.4	0.7
	1,3-Bis(3-chlorophenyl)-1-triazene	0.83	0.33 - 2.0	7.7	6.55	5.55 – 7.70	5.3
	1,3-Dinaphthyl-1-triazene	0.36	0.01 - 1.3	3.8	1.15	0.7 - 1.9	21

Degree of freedom = 3 95% C.L., 95% Confidence limit

Table (3): Effect of the tested compounds on wheat seedlings dry weight.

Comp.	Weight (gm)	Average weight at different concentrations µg/ml 0	5	10	20	50	100	EC$_{50}$ 95% C.L	Slope ± SE	χ^2
1,3-Bis(2-tolyl)-1-triazene	Fresh	0.69 ± 0.02	0.58 ± 0.02	0.54 ± 0.10	0.50 ± 0.05	0.48 ± 0.02	0.39 ± 0.01	> 100		
	Air dried	0.30 ± 0.01	0.23 ± 0.014	0.20 ± 0.008	0.19 ± 0.007	0.19 ± 0.005	0.18 ± 0.008	> 100		
	Oven dried	0.20± 0.002	0.15 ± 0.009	0.14 ± 0.006	0.12 ± 0.008	0.16 ± 0.005	0.17± 0.013	> 100		
1,3-Bis(4-phenyl-carboxylic acid)-1-triazene	Fresh	0.69 ± 0.02	0.64 ± 0.02	0.62 ± 0.03	0.44 ± 0.02	0.31 ± 0.03	0.24 ± 0.01	**46.3** 37.5–57.3	1.5± 0.020	6
	Air dried	0.30 ± 0.01	0.29 ± 0.005	0.20 ± 0.006	0.18 ± 0.01	0.13 ± 0.01	0.09 ± 0.01	**35.6** 28.8 – 44.1	1.4 ± 0.018	15
	Oven dried	0.20 ± 0.002	0.19 ± 0.003	0.17 ± 0.005	0.16 ± 0.003	0.10 ± 0.006	0.07 ± 0.007	**55.3** 44.4 – 69.2	1.6 ± 0.023	2
1,3-Bis(4-phenylsulphonamide)-1-	Fresh	0.69 ± 0.02	0.44 ± 0.01	0.34 ± 0.10	0.30 ± 0.05	0.28 ± 0.03	0.08 ± 0.01	**12.3** 9.0 – 16.9	0.93 ± 0.016	12

triazene	Air dried	0.30 ± 0.01	0.18 ± 0.006	0.17 ± 0.002	0.16 ± 0.007	0.15 ± 0.007	0.05 ± 0.002	**17.4** 12.1 – 24.8	0.74 ± 0.015	17
	Oven dried	0.20 ± 0.002	0.15 ± 0.003	0.10 ± 0.010	0.08 ± 0.010	0.06 ± 0.006	0.03 ± 0.004	**13.8** 10.8 – 17.6	1.2 ± 0.018	4.3
1,3-Di-naphthyl-1-triazene	Fresh	0.69 ± 0.02	0.59 ± 0.05	0.63 ± 0.02	0.40 ± 0.05	0.26 ± 0.02	0.23 ± 0.02	**38.0** 31.0 – 46.7	1.5 ± 0.020	8.3
	Air dried	0.30 ± 0.01	0.23 ± 0.003	0.17 ± 0.006	0.16 ± 0.003	0.13 ± 0.011	0.10 ± 0.005	**27.0** 19.3 – 37.8	0.79 ± 0.016	3.2
	Oven dried	0.20 ± 0.002	0.18 ± 0.005	0.15 ± 0.006	0.12 ± 0.010	0.10 ± 0.008	0.09 ± 0.006	**55.3** 39.5 – 78.3	0.99 ± 0.017	5.6

Degree of freedom = 4 95% C.L., 95% Confidence limit

1. Molluscicidal activity:

Mollusicidal effects of the tested triazenes on *Theba pisana* snails showed that 1,3-bis(2-tolyl)-1-triazene caused moderate lethal effect, while the other derivatives required to use higher concentrations than 10% to be significantly active against the treated snail (Table **4**).

From the obtained results, the tested triazene derivatives showed several fungicidal, phytocidal, bactericidal and molluscicidal effects. Regarding to the environmental effects, Rouzer *et al.* (1996) exhibited their use as cancer chemotherapeutic agents in a number of biological systems. Rezaei-Seresht *et al*., 2017 revealed that series of azo compounds with extended p-conjugated systems are very active against breast cancer adenocarcinoma (MCF-7), cervix adenocarcinoma (HeLa) and human embryonic kidney (HEK 293) cell lines with potent *in vitro* anti-proliferative activity against MCF-7 and HeLa cell lines with inhibitory effects in varieties of cancers such 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 (Boon *et al*., 2017). 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 (Singh *et al*., 2008). Also, Shabzendedara *et al*. (2020) exhibited using the diazoaminobenzene nanomaterials with high efficiency in solar cells fabrication would provide a promising platform to discover clean energy sources and preserve the environment

Table (4): Molluscicidal activities of the tested triazene derivatives

Compound	Molluscicidal effect on *T. pisana*				
	LC_{50}	95% C. L	Slope ± SE	χ^2	DF
1,3-Diphenyl-1-triazene	>10				
1,3-Bis(2-tolyl)-1-triazene	3.13	2.78 – 3.54	2.64± 0.03	19	4
1,3-Bis(4-tolyl)-1-triazene	>10				
1,3-Bis(4-phenylcarboxylic acide)-1-triazene	>10				
1,3-Bis(4-phenylsulphon amide)-1- triazene	>10				
1,3-Bis(3-chlorophenyl)-1-triazene	>10				
1,3-Dinaphthyl-1-triazene	>10				

Degree of freedom = 3 95% C.L., 95% Confidence limit

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