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STRUCTURE ACTIVITY RELATIONSHIP OF NEW ARYL ISOTHIOCYANATE DERIVATIVES AS PESTICIDES III- FUNGICIDAL AND BACTERICIDAL EFFICACY OF PHENYLISOTHIOCYANATES

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ABSTRACT

The relationship between chemical stracture and biocidal activity of new derivatives of phenylisothiocyanate . was evaluated against fungi: Rhizoctonia. solani; Fuarium. oxysporum ; Botryodiopioda theobromae and bacteria: Pseudomonas. solanacearum and Agrobacterium.tumefaclneseas as well as their inhibitory power to enzyme systems : dehydrogenase (from bacteria), PME and Mg - ATPaeses (from Fungus). These derivaties had different levels and modes of toxicity on the fungi and bacteria growth as well as enzymes inhibition. Obviously tne analog 3-nitro-4-amino was very active as biocide and it was as toxic as the recommended pesticides followed by 2-amino-5-nitro analog while 2-nitro-4-amino was the lowest one. The inhibitory power of the compounds was paralliel to their toxicity to fungi and bacteria. The ATPase activity was less snesitive than the other enzyme systems.

INTRODUCTION

The toxicity of several analogous of isothiocyanate compounds were used as fungicides or bactericides depending on their chemical structure. and as active ingredient in many compounds produced by thiocarbamates breakdown[1] Methylisothiocyanate was toxic to R. solani growth as well as inhibitor to glucosemetabolism[2] The derivatives of phenylisothocyanate. containing methyl. methoxy, chloro and nitro groups were active as fungicides. while benzyl and napnthylisothiocyanate derivatives were active as bactericides[2,3] The antimicrobial activity of aryl or alkyl isothiocyanate derivatives were studied by several investigators[4,5,6,7]

On the other hand. different biochemical effects of isothiocyanate compounds were studied . where the allyl derivatives have affected significantly the activity of succinic dehydrogenase and amino oxidase in rats [8], while aryl alkyl derivatives decreased adenine and leucine incorporation in bacteria [9]. The oxygen uptake and cytochrome oxidase activity in yeasts and bacteria have been inhibited by these compounds.[10] In the present study. we concerned to evaluate the biocidal activity of new arylisothiocyanate analogs against fungi and bacteria as well as their inhibitory effect to enzyme systems.

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MATERIALS AND METHODS

Tested chemicals:

Three derivatives of phenylisothiocyanate were prepered and analysed to confirm their structure by elementa. micro analysis and spectral techniques (IR, UV and NMR)". The derivatives are. 3-nitro-4-aminophenylisothiocyanate:2-nitro-4-aminophenylisothiocyanate , and 2-amino-5-nitrophenylisothiocyanate Standard recommended pesticides: the fungicide. vitavax-200 and the bactericide. streptomycine were also used.

Tested organism:

The following fungi,bacteria and enzyme systems were used to evaluate the biocidal activity of the compounds: Fungi, Fusariuem oxysporum: Rhizoctonia solani and Botryodiplodia theobromae. The used medium was Czapeck-Dox medium. Bacteria: Pseudomonas solancearum and Agrobacterium tumefaciens and the used medium was glycerol nutrient broth medium.

Enzyme systems:

a. Pectin methyl esterase (PME) derived from R. solani. The enzyme activity was determind by titrating the reaction mixture [illegible] NaOH [2,3,14,15]

b. Dehydrogenases. derived from F.solanacearum. The enzyme activity was determined by measuring the time required to give anaerobic reduction of methyl blue [16]

c. Mg ATPase isolated from mitochondria and cytosol of F.oxysporum. The activity was determined according to convenient methods used to measure the inorganic phosphates produced from the hydrolyzing ATP [17,18]. The cytosol fraction collected after the isolation of mitochondiral pellets.

Fungitoxicity and bacteritoxicity studies:

The toxicity to fungi (fungitoxic or fungistatic effects) was assayed by using the poisoned food techniques (solid and liquid media) by measuring the radial growth as well as spore germination and the time required to give complete disk formation [19,20]

The toxicity to bacteria was assayed by using the poisoned food technique and determining the density of bacterial suspension colourmetry [21]
The tested concentrations were: 0.5, 1.0, 10, 25, 50 and 100 ppm, in water.

RESULTS AND DISCUSSION

The relation between chemical structure of phenyliosthocyanate compounds and their toxic effect to fungi , bacteria and enzyme systems, were evaluated and conducted in the followings remarkes.

A- The fungitoxicity:

Fusarium oxysporum: Strong fungitoxic effect to the mycelial growth was displayed by 3-nitro-4-amino derivative, since the growth retardation percentage at 100 ppm was 87% with EC50 value 3.0 ppm, while 2-nitro-4-amino and 2-amino-5- nitro derivatives were less effective where the EC50 values were 60 and 58.0 ppm, respectively (Table 1). The analog 3-nitro-4-amino was more active as the recommeded fungicide "Vitavax-200) by about thirteen fold increase where the EC50 values was 39 ppm

Rhizoctonia solani: The fungus myclial growth was greatly affected by the treatment with 3-nitro-4-amino derivative, followed by 2-amino-5-nitro and 2-nitro-4-amino analogs since they prohibited the growth by 100, 100 and 84.7% at 100 ppm , respectively (Table 1). On the other hand the three analogs had less fungitoxicity than fungicide vitavax 200 (EC50 0.88 ppm), where their EC50 values were 4.9, 22.0 and 35.0 ppm respectively in the same order.

Botryodiplodia theobromae: 3- nitro-4-amino phenylisothiocyanate was highly toxic to the myclial growth, as much as the fungicide vitavax-200 , since the EC50 value was 6.6 ppm for both. The other tested compounds were less effective as growth retardants (Table 1). The results recorded in Table 2 showed the effect against spore germination of the fungus and it revealed that 3-nitro-4-amino derivative displayed as fungitoxicant , while the other tested compounds have displayed as fungistatic agents, since the spore pretreated with the compounds started to germ on new fresh medium (Table 2.3).

B - The bacteritoxicity:

Pseudomonas solanacearum: The bacteritoxic effect of the tested compounds , which recorded in Table 1, have proved that the analogs 3-nitro-4-amino and 2-amino-5-nitro revealed a dramatic bacteritoxic effects against the bacteria, since they inhibited 90.4 and 85.3% of the growth at 50 ppm while the antibiotic streeptomycin was 86.7%, the EC50 values for the two analogs were 6.3 and 8.0 ppm and there was no significant differences between them and the antibiotic. The last derivative was less effectivenss.

Agrobacterium tumefaciens: A low bacteritoxic effect was observed with the three thiocyanate derivatives, since the EC50

values were more than 100 ppm. for each one, while for the antibiotic was 16.0 ppm. So, it was obviously that the bacteria P. solanacearum was more sensitive than A. tumefacines.

C - Interference with enzyme systems:

The potency of phenylisothiocyanate analogs to inhibit the different enzyme systems was recorded in Table 1.

P. solanacearum dehydrogenases: The potent inhibitory effect to the enzyme activity was observed by 2-amino-5-nitro derivative followed by 3-nitro-4-amino analog , while the least inhibitor was 2-nitro-4-amino. The I50 values were 1.4, 2.8 and 18 ppm for the derivates , respectively. These observations indicated that there was a corelation between the bacteritoxicity and enzyme inhibition.

Pectin Methyl Esterase, derived from R. solani: The derivative 3-nitro-4-amino displayed a most potant inhibitor to PME activity, since 90% of the activity about 20 fold than the other compounds. The I50 values were 4.8, 6.8 and 100 ppm respectively as mentioned. The enzymatic inhibition of the tested compounds was highly correlated with their fungitoxicity as well as their structure activity behavior.

Mitochondrial and cytosol Mg-ATPases, from F. oxysporum: The activity of mitochondrial ATPase was moderately inhibited by the tested compounds where 90 and 75% of activity was inhibited by 2-amino-5-nitro and 3-nitro-4-amino deratives at 100 ppm. The analog 2-nitro-4-amino was inactive as potent inhibitor to enzyme activity.

On the other hand , the cytosol Mg-ATPase activity was less sensitive than the mitochondrial one. A significant differences were observed between the three compounds where the I50 values were 40, 100 and > 100 ppm for 3-nitro-4-amino; 2-amino-5-nitro and 2-nitro-4-amino respectivly. The structure enzyme inhibition relationship was so far resemble with the structure fungitoxicity relationship.

Moerover , the derivatives of isothiocyanate had affected the oxidation pathways and inhibited denydrogenases activity (in rats). Which was suggested the site of their action[9,22]. Also they inhibited energy production (ATP production)[23]. So, these results agree with that observed in this study , and that could explain the site of action of this group of compounds to bacteria and fungi and also the dithiocarbamate compound which could decomposed or release isothiocyanate structures[24].

In conclusion, the structure, biocidal potency relationships of phenyliso thioayanates revealed that the nature and position of the substituents played an important role in fungal and bacterial toxicity. The position of nitro and amino group in

phenyl moiety exhibited different activities , since the position 3 for nitro and 4 for amino proved a highly effectivenss structure and configrueration against fungi, bacteria as well as enzyme systems. Many studied have revealed that the arylisothiocyanates were more active than the corresponding aliphatic cycloaliphatic and arylaliphatic compounds. In addition , the para position in aromatic ring moiety exhibited better activity than meta position [2,3,4,7,24].

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Table 1: Biocidal activity of the phenylisothiocyanate derivatives against fungi, bacteria and enzyme systems. The data represented as inhibition percnetages EC50 and I50 ppm.

Tested Compounds	Tested organisa								
	Fungi			Bacteria		Enzymes			
	R. solani	F.oxy	Botryo. sp.	P. solani	Agto.tumef.	PME	Mg ATP cyt.	Mg ATP mit.	Beh.
$3\text{-}NO_2\text{-}4\text{-}NH_2$									
0.5 ppm	0.00	16.33	19.89	0.00	0.00	13.70	26.84	0.58	5.51
1	1.89	33.11	28.89	6.53	0.00	27.40	31.58	8.31	25.93
5	66.44	61.44	45.22	46.12	0.00	42.47	33.68	3.47	81.04
10	68.56	73.89	50.56	64.89	4.38	45.21	34.74	4.78	82.73
25	87.41	81.67	68.33	83.06	7.73	82.19	36.84	14.45	90.47
50	94.00	86.44	75.78	90.41	21.91	93.15	44.21	26.12	91.59
100	100.00	87.22	84.11	91.84	85.31	100.00	56.32	75.72	94.30
EC50	4.90	3.00	6.60	6.30	>100.00	4.80	40.00	>100.0	2.80
$2\text{-}NO_2\text{-}4\text{-}NH_2$									
0.5 ppm	0.00	5.22	15.33	0.00	9.79	0.00	0.00	5.2	0.00
1	0.00	16.11	18.56	0.00	10.05	4.11	0.00	10.98	0.00
5	0.00	19.78	26.89	0.00	12.89	13.70	0.00	17.19	27.70
10	11.67	28.00	34.22	31.43	13.14	21.92	1.58	22.54	44.95
25	49.67	38.11	36.11	34.69	13.40	35.62	5.26	24.28	55.06
50	57.00	66.11	73.56	62.24	22.42	41.10	14.74	24.86	74.58
100	84.67	80.22	80.89	75.92	29.38	49.32	65.26	31.21	78.84
EC50	35.00	60.00	54.00	27.00	>100.00	100.00	>100.00	>100.00	18.00
$2\text{-}NH_2\text{-}5\text{-}NO_2$									
0.5 ppm	0.00	0.00	0.00	0.00	0.00	4.11	12.11	16.76	30.64
1	0.00	1.44	1.89	21.84	0.00	17.81	13.16	23.12	42.03
5	5.22	8.44	8.56	39.39	6.19	35.62	14.74	32.95	85.76
10	32.56	5.33	10.78	54.49	7.73	45.21	20.00	38.15	90.43
25	56.33	33.33	53.87	82.04	21.91	82.19	25.26	49.71	90.93
50	68.11	49.22	54.22	85.31	30.15	90.41	31.05	72.83	92.61
100	100.00	78.33	86.67	87.35	33.25	100.00	58.42	90.17	98.90
EC50	22.00	52.00	29.00	8.00	>100.00	6.80	>100.00	30.00	1.40
EC50 values for									
Vitavax-200	0.88	39.00	6.00						
Streptomycin				1.40	16.00				

Table 2: Effect of phenylisothiocyanate derivatives on spore germination and hyphal growth of Botryodioplodia sp. after 11 days in liquid medium (The data repesented as inhibition percentages).

Compounds	Concentrodion (ppm) 0.5	1	5	10	25	50	100
3-Nitro-4-amino	8.33	16.67	33.33	66.67	100	100	100
2-Nitro-4-amino	8.33	16.67	25.00	58.33	70.83	100	100
2-amino-5-nitro	25.00	33.33	41.67	66.67	100	100	100

Table 3: The type of fungitoxicity of phenylisothiocyanate derivatives (47 days old).

Derivatives	Conc. ppm	Dilution percentage 10%	20%	30%	50%	Type of Toxicity
3-nitro-4-amino	25	N	N	N	N	
	50	N	N	N	N	Fungitoxic
	100	N	N	N	N	
2-nitro-4-amino	50	G	G	G	G	
	100	G	G	N	N	Fungistatic
2-amino-5-nitro	25	G	G	G	N	
	50	G	G	G	N	
	100	G	G	N	N	Fungistatic

* Dilution percentages of the medium containing non germed spores.
N = No germination observed. G = Spore germination observed

العلاقه بين التركيب الكيماوى والنشاط الأبادى لمشتقات الأريل ايزوثيوسيانات الجديده ٣ـ النشاط الابادى الفطرى والبكتيرى لمشتقات الفينيل ايزوثيوسيانات

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لقد تم دراسة العلاقه بين النشاط الأبادى والتركيب الكيماوى لمشتقات الفينيل ايزوثيوسيانات ضد فطريات ريزوكتونيا سولانى وفيوزاريوم اوكسيسبورم وبوترتيس شيوروميا • وكذلك البكتريا بزيد وموناس سولاناسيرم وأجروبكتيريم تومينانسيس• بالاضافه الى ذلك فانه تم تقدير نشاط هذه المركبات وقدرتها على تثبيط نشاط انزيمات بكتين ميثيل استيريز (من الفطر) والد يهيد روجينيز (من البكتريا) وكذلك الانزيم للاد ينوزين تراى فوسفاتيز (من الفطر) • لقد لوحظ أن المشتقات المختبره لها خصائص أباديه مختلفه تعتمد على التركيب الكيماوى للمركب ،حيث وجد ان المشتق ٣ـ نيترو ـ ٤ـ أمينو كان فعال جدا كمبيد أو كماده سامه تقرب أو تفوق فى بعض الأحيان المبيدات المستخدمه فعلا • ويتبعه فى هذا النشاط المشتق ٢ـ أمينو ـ ٥ـ نيترو فى حين كان المشتق ٢ـ نيترو ـ ٤ـ أمينو أقلهم نشاطا وفاعليه ومن ناحيه أخرى فان قدرة هذه المشتقات على تثبيط نشاط الأنزيمات كان يتدنى مع التأثير على الكائن بصفه عامه وكان أقل الانزيمات تأثرا بالمركبات هو اد ينوزين تراى فوسفاتيز •