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EFFECT OF ACTIVATED CHARCOAL AND RICE HUSK ASH ADDITION ON THE RESIDUES OF DINICONAZOLE AND THEIR EFFECT ON SOME BLOOD COMPONENTS OF CATFISH *(CLARIAS LAZERA)*

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ABSTRACT: The effect of activated charcoal and rice husk ash addition on the residues of diniconazole in water and catfish *clarias lazera* and their effects on some blood components was investigated. Summarized results show the following indications:

The initial amounts (after two hrs) of diniconazole residues in water alone and in water containing catfish were 2.85 and 0.46 ppm. These amounts were decreased by time till reached 0.71 and 0.070 ppm after 144 hrs post treatment indicating 75.08 and 84.78% dislodges of the initial amount, respectively. The initial amount of diniconazole residues in muscles, liver and kidney as determined after 2 hrs of application were 5.09, 12.47, 8.4 ppm, respectively. These amounts were increased gradually by time elapsed till reached 18.42 in muscles after 48 hrs while reached 19.50 and 14.43 ppm in liver and kidney after 24 and 48 hrs of treatment, respectively. After that the amount of residues were decreased and reached 3.96, 1.60 and 0.42 ppm in muscles, liver and kidney, respectively after 144 hrs of exposure period. Addition of activated charcoal and rice husk ash to water contaminated with diniconazole reduced clearly levels of residues in water and in fish muscles, liver and kidny. Total protein in catfish blood serum were reduced while glucose and GPT levels were increased compared to those in untreated fish. Thes effects were alleviated by activated charcoal and rice husk ash addition.

Key words: Diniconazole, residues , water , fish ,adsorben

INTRODUCTION

Pollution of aquatic environment by pesticides cause changes in the metabolic activities and alter physiological state, thereby change the biochemical constituents of aquatic organism. It is important to examine the toxic effect of pesticides on fish, as they constitute an important link in food chain and their contamination by pesticides imbalances the equatic ecosystem. Fish also form an important part of human food (Begum and vijayaraghavan, 1996).

The determination of the distribution, persistence and activity of pesticides in aquatic environments represents an important basic effort leading to an understanding of the true impact of pesticides on that ecosystem (Shalaby, 1997).

Diniconazole has been used as a systemic fungicide with protective and curative action. After 96 hours, LC_{50} values being 1.58, 6.4 and 4 mg/l. for rainbowtrout, Japanese killfish and carb, respectively. Diniconazole was rapidly metabolized in rat by hydroxylation of the tert-butyl methyl groups. Within 7 day, 52-87% were excrete in the feces and 13-46% in the urine (Tomlin, 1997).

With regard to the addition of sorbents as a detoxification strategy for xenobiotics, many investigators showed that adding adsorbent materials like humic substances, activated charcoal, cement kiln dust, inert materials such as natural clay and rice husk ash are known to reduce xenobiotics in water and in tissues of aquatic animals (Kargin, 1996; Uchida *et al.*, 1997; Romeh, 1999; Shalaby and Ayyat, 1999; Tao *et al.*, 2000; Romeh and Aioub 2001; Nagarnaik *et al.*, 2002; and Romeh and Abdel-Ghany, 2003).

The present study has been undertaken to explore the effect of sublethal concentration of the fungicide diniconazole on certain biochemical response i.e., levels of total protein, glucose and GPT in blood serum and the consequent role of activated charcoal and rice husk ash on these biochemical parameters. Also, to determine the residues of diniconazole in water and in different organs of catfish. The role of adsorbents in reducing diniconazole residues in water and fish was also investigated.

MATERIALS AND METHODS

1. Test Organism

The nile catfish *Clarias lazera* (45-50 gram each) were collected from Bahr-Mowase, Sharkia

governorate. The fish were maintained for 14 days in previously aerated tap water in large glass aquaria 160 liters capacity (5 fish / 25 L) for adaptation and clearance of pollutants from fish continous air flow and feeding on warms and shrimps during acclimatization.

2. Chemical Used

A. Diniconazole (Sumi-8 2% WP). (E)-(RS)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl) pent = 1-en-3-01. A formulated sample of diniconazole 2% WP. Under the trade name of Sumi-8 was supplied by central Agric, Pesticide laboratory, Agriculture Res. Center, Dokki, Giza, Egypt.

B. Adsorbent materials

1. Activated charcoal (4-14 mesh).
2. Rice husk ash (burned in open air) was heated at 450°C. for 4 hours in a muffle furnance in a steel container and control supply air rice husk ash with highly active SIO_2 content was obtained. The SIO_2 of the ash is mostly amorphous. The major components are silica 94.47%, calcium oxide 1.14%, alumina 2.03%, ferric oxide 0.4%, magnesium oxide 0.89%, and potassium oxide 1.52% (Abd El-wahed, 1990).

3. Experimental Design

Fish were starved for two days before initiation of experiments. One tenth of LC_{50} value (3 mg / L) of diniconazole fungicide was chosen as a sublethal concentration in which the fish survived for 13 days without physical symptoms and mortality.

For the study the effects of diniconazole on different blood serum biochemical parameters, residues of compound in different organs of fish (i.e. muscles, liver and kidney), water, and the effectiveness of adsorbents in diniconazole removal were studied. Fish individuals were divided into four groups of 45 fish each, except the second group contained 54. Three replicates were used for each group. Group one was maintained as control in free laboratory water (without diniconazole). The groups from 2nd to 4th were exposed to commercial formulation of diniconazole (3.0 mg / L). In addition, the groups from 3rd to 4th were treated separately with activated charcoal and rice husk ash, respectively, at the rate of 100 mg / L. (Ademoroti, 1980). The above experiment was repeated without fish. Representative samples of fish (3 fish from each replicate) were taken after 2, 24, 48, 72 and 144 hours of treatment for biochemical and residual analysis.

At the same periods, sample of water from each replicate of two experiments were taken for the determination of diniconazole residues. After that, the remaining contaminated diniconazole fish from the second group, were then transferred to clean fresh water for 168 hrs to investigate diniconazole elimination from the tissues. Three fish from each replicate were for biochemical and residual analysis.

4. Residue Dynamics and Biochemical Studies

After each test period, three replicates from each treatment were randomly selected to collect the blood samples. The blood samples were taken by cutting the tail of the fish. The exuding blood was collected into a clean centrifuge tubes and left at room temperature for clotting. After complete retration of the clot the blood samples were centrifuged at 3000 r.p.m for 15 minutes to separate the serum. Serum sample were kept in freezer for biochemical analysis. At the obovementioned periods, water and fish tissue samples (liver, kidney and muscles) were taken for residues determination.

A. Determination of fungicide residues

Fish tissue samples were extracted with methylene chloride according to Tindle (1972). The obtained dried extracts were subject to clean up procedures suggested by Gunter (1963).

Also, water samples were extracted and cleaned up according to Leppert *et al.* (1983). The residues of diniconazol.e were determined using HPLC with the following conditions: Dual delivery solvent system pump 40, UV. detector 166, integrator spectra physics 4270, attenuation 16 , chart speed 1.0 cm./ 1 min, stainless steel column (10 / 250nm) packed with C18 , flow rate 0.7 ml / min., wavelength 278 nm, mobile phase acetonitrile / water 70 /30 and retention time 2.0 min. Recovery percentages of fortified samples were 92.7 , 85.3 , 87.0 and 86.5 for the water , muscles, kidney and liver , respectively.

The rate of degradation was obtained form the following equation:

Rate of degradation (K) = 2.303 x slope

Medium degradation time 50% (DT_{50}) was obtained from the

following equation: DT_{50} = 0.693/k (Gomaa and Belal, 1975).

B. Biochemical studies

The activity of the blood serum enzyme transaminase Glutamic pyruvic transaminase (GPT) was determined using the method, of Reitman and Frankel, (1957). The levels of total serum protein and glucose were determined according to biuret method as described by Henry (1964) and Tinder (1969), respectively.

RESULTS AND DISCUSSION

1. Residues of Diniconazole in Water and Catfish Tissues

The interaction between activated charcoal and rice husk ash addition to catfish *(Clarias lazera)* at 0.1 gm/L. and the residues of diniconazole at 0.1 of 96 h-LC_{50} in water and that on the distribution of such residues between the inner organs was investigated.

Data presented in Table 1 and Fig. 1 show that the initial amounts of diniconazole residues in water alone and in water containing catfish were 2.85 and 0.46 ppm as determined after two hours of treatments. These amounts were decreased by time elapsed till reached 0.71 and 0.07 ppm after 144 hrs of application indicating 75.08 and 84.78 % dislodges of the initial amounts, respectively.

The rate of degradation and medium degradation time (DT_{50}) value of diniconazole in fish surround water was 1.4×10^{-6} sec^{-1}. (DT_{50} = 52.8 hrs) while in water alone was 7.9×10^{-6} sec^{-1}. (DT_{50} = 81.6 hours). The study clearly show that the fish can speed up the diniconazole depletion from the water. This finding may be attributed to the metabolic detoxification process by the fish (Saxena *et al.*, 1989 and Singth *et al.*, 1996).

Addition of activated charcoal and rice husk ash in water alone and in water containing catfish resulted in removing higher amounts of the used fungicidal diniconazole as time elapsed from 2 hrs until the last period of detection (Table 1). Activated charcoal in water alone and in water containing the fish reduced diniconazole residues by 28.07-38.570% and 15.22-28.57% after 2-144 hrs of exposure periods, respectively. For rice husk ash, the values were 44.91 – 51.42% and 26.09 – 33.34% at the same periods.

Table 1: Persistance of diniconazole at 3.0 mg / L. in water with and without catfish *(Clarias lazera)* after treatment with activated charcoal and rice husk ash

Exposure periods (hours)	Water alone								Water containing catfish							
	Diniconazole		Diniconazole plus						Diniconazole		Diniconazole Plus					
			Activated charcoal			Rice husk ash					Activated charcoal			Rice husk ash		
	PPM	% loss	PPM	% loss	%elimination removal	PPM	% loss	%elimination removal	PPM	% loss	PPM	% loss	%elimination removal	PPM	% loss	%elimination removal
2	2.85	0.00	2.05	0.00	28.07	1.57	0.00	44.91	0.46	0.00	0.39	0.00	15.22	0.34	0.00	26.09
24	2.22	22.11	1.60	21.95	27.93	1.33	15.29	40.09	0.30	34.78	0.25	35.89	16.67	0.23	32.35	23.33
48	2.06	27.72	1.39	32.20	32.52	1.20	23.57	41.74	0.24	47.83	0.19	51.28	20.83	0.18	47.05	25.00
72	1.60	43.86	0.97	52.68	39.37	0.83	47.13	51.74	0.15	67.39	0.11	71.79	26.67	0.08	76.47	27.27
144	0.71	75.08	0.43	79.02	38.57	0.34	78.34	51.42	0.07	84.78	0.06	84.61	28.57	0.04	88.24	33.34
Rate of degradation	7.9×10^{-6} sec^{-1}.		6.6×10^{-6} sec^{-1}.			7.9×10^{-6} sec^{-1}.			1.4×10^{-6} sec^{-1}.		2.9×10^{-6} sec^{-1}.			1.3×10^{-6} sec^{-1}.		
DT_{50}	81.6		67.2			76.8			52.8		43.2			38.4		

DT_{50} = medium degradation time .

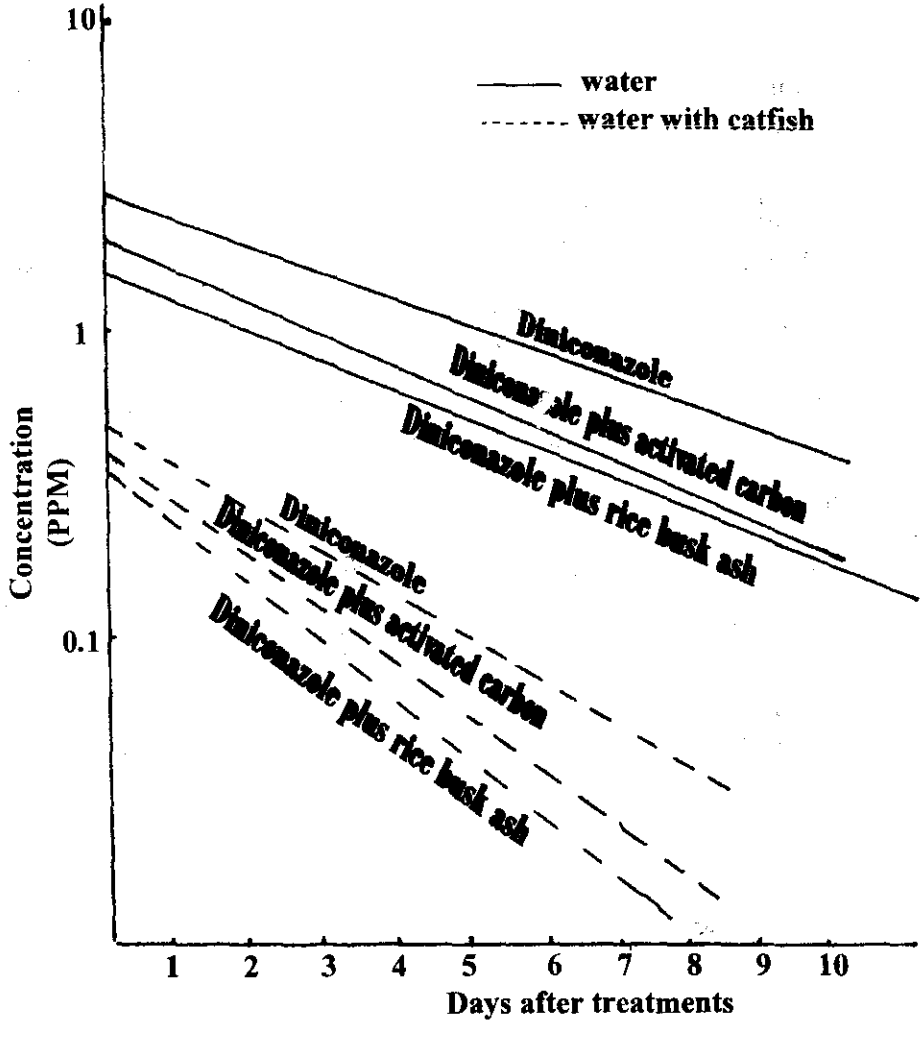

Fig. 1: Degradation of diniconazole in water with and without catfish

Rice husk ash exhibited high capacity in eliminating diniconazole residues in water than activated charcoal.

Data concerning the distribution of diniconazole residues in muscles, liver, and kidney of catfish after 2, 24, 48, 72 and 144 hrs from exposure to 0.1 of LC_{50} are presented in Table 2. The obtained results indicated the occurrence of approximate distribution of the same amount of fungicide in muscles, liver and kidney of exposed fish immediately after exposure (zero-time). The initial detected residues of diniconazole in muscles, liver and kidney were 5.09, 12.47 and 8.4 ppm respectively. These amounts were increased gradually by time lapse till reached 18.42 ppm in muscle after 48 hrs while reached 19.50 and 14.43 ppm in liver and kidney after 24 and 48 hrs respectively. The amount of residues were decreased and reached 3.96, 1.60, and 0.42 ppm in muscles, liver and kidney, respectively. Quite high residues were accumulated in liver followed descendingly with that in kidney and the lowest accumulated residues were determined in muscles. The accumulated residues in fish may be attributed to the penetration and binding residues in fish tissues, the inner organs and the exposure periods (Hayes, 1994). Diniconazole was rapidly metabolized in rat by hydroxylation of the tert-butyl methyl groups. Within 7 days, 52-87 % were excreted in the feces and 13-46 % in the urine (Tomlin, 1997). The amount of pesticide residues increased gradually in the tissues of fish to reach the maximum level, after which gradual decrease in residues took place, El-Sheamy *et al.* (1991), El-Kenawy (1995) and Afifi *et al.* (2002)

Addition of activated charcoal and rice husk ash to water contaminated with diniconazole reduced clearly the levels of residues in fish muscles, liver and kidney. The removed percentages of diniconazole by activated charcoal and rice husk ash in fish muscles were 49.70- 27.53% and 65.03- 34.34% after 2-144 hrs, respectively and reached 64.72– 25% and 71.93– 38.13% in liver compared with 61.90- 50% and 69.04- 52.38% in kidney at the same periods, respectively (Table 2). Also, data showed that when the polluted fish were transferred to clean fresh water for 168 hours, the residues of diniconazole were decreased and reached 0.078, 0.014 and 0.00 ppm in muscles, liver and kidney, respectively (Table 2).

Addition of adsorbent materials to water contaminated

Table 2: Effect of activated charcoal and rice husk ash addition on diniconazole residues in some organs of catfish *(Clarias lazera)* contaminated with 1/10 from their LC_{50} values

Exposure periods (days)	Muscles						Liver						Kidney					
	Diniconazole		Diniconazole Plus				Diniconazole	Diniconazole Plus					Diniconazole	Diniconazole Plus				
	PPM	% (increase)	Activated charcoal		Rice husk ash		PPM	% increase	Activated charcoal		Rice husk ash		PPM	% increase	Activated charcoal		Rice husk ash	
			PPM	%*E.R	PPM	%*E.R			PPM	%*E.R	PPM	%*E.R			PPM	%*E.R	PPM	%*E.R
2	5.09	(0.00)	2.56	49.70	1.78	65.03	12.47	(0.00)	4.40	64.72	3.50	71.93	8.4	(0.00)	3.2	61.90	2.6	69.04
24	8.29	(+62.87)	5.18	37.52	3.92	52.71	19.50	(56.38)	9.70	50.26	9.41	51.74	10.86	(28.57)	6.41	40. 65	5.24	51.48
48	18.42	(+261.89	13.86	24.76	11.54	37.35	7.87	(-36.89)	4.99	36.59	3.72	52.73	14.43	(71.78)	8.24	42.90	3.88	73.11
72	14.50	(+184.87)	9.70	33.10	9.19	36.62	3.19	(-74.42)	2.11	33.86	1.80	43.57	6.18	(-26.43)	2.16	65.05	1.44	76.69
144	3.96	(-77.79)	2.87	27.53	2.60	34.34	1.60	(-87.16)	1.20	25.0	0.99	38.13	0.42	(-95.0)	0.21	50.0	0.20	52.38
****168**	0.078	(-98.47)	-	-	-	-	0.014	(-99.92)	-	-	-	-	0.00	(-100)	-	-	-	-

* E.R. refere to percent elimination removal.

** The fish were transferred to fresh water for 168 hours after exposure to the water contaminated with diniconazole for 144 hours.

with diniconazole reduced clearly the levels of residues in water and fish by adsorption and binding properties of adsorbents. In this respect Carson and smith (1983) reported that pentonite feeding prevents T-2 toxicity by reducing intestinal adsorption and increasing feceasl excretion of the toxin, i.e. natural clays adsorb the toxic material and excrete it in feces. Shalaby and Ayat (1999) reported that the levels of profenofos and monocrotophos residues .were reduced clearly by tafla addition (5 %) to chicken diet. Romeh (1999) showed that addition of activated charcoal (0.01) to water contained 0.58 ppm of carbosulfan had higher magnitude effect on the removal of active carbosulfan residues. Romeh and Aioub (2001) reported that addition of peat-moss, activated carbon, cement kiln dust, rice straw ash and aluminum sulfate caused 76.96, 67.97, 66.29, 62.36 and 56.47% elimination of anilofos residues in water, while reached 79.39, 76.96, 78.18, 78.18 and 70.30% in fish muscles after 4 hours of treatment, respectively. Nagarnaik *et al.* (2002) indicated that rice husk carbon can be used as an adsorbent for the removal of As (III) from aqueous solution. Ghaudhary *et al.* (2003) found that the granular activated carbon adsorption system was effective in removing total organic carbon form the waste water.

2. Biochemical Changes

Data presented in Table 3 show that the concentration of diniconazole at 3.0 ppm produced dysfunctions in several biochemical processes in *Clarias lazera*. The levels of total protein in blood serum of catfish were reduced while glucose and GPT levels were increased in blood serum by diniconazole residues compared to un-treated fish. This general trend was the case after all exposure periods of experiments.

Addition of activated chrcoal and rice husk ash lowered dinicaonazole residues in water to which fish exposed and accordingly tended to alleviate the reduction of total protein 12.25-118.18, and 32.30-152.07 respectively (Table 3). On the other hand, adsorbent materials addition , as above mentioned, tended to alleviate the elevation of blood serum glucose 74.74- 69.59 and 73.77-58.43, respectively. Moreover, addition of activeated charcoal and rice husk ash lowered comparatively GPT activity levels. The respective percentages of reduction in GPT were 18.57-60.16, and 12.57-52.19 respectively.

Table 3: Levels of some catfish blood serum components (total protein, glucose and GPT) as affected by the interaction between diniconazole and activated charcoal and rice husk ash.

Exposure periods (hours)	Serum total protein (g/100ml)				Blood serum glucose (g/100ml)						GPT (U/L)					
		Diniconazole plus				Diniconazole plus						Diniconazole plus				
	Control Mean ± S.D	Diniconzole Mean ± S.D	% change	Activated charcoal Mean± S. D	% change	Rice husk ash Mean± S.D	% change	Control Mean ± S.D	Dinicanzole Mean ± S.D	% change	Activated charcoal Mean ± S.D	% change	Rice husk ash Mean ± S.D	% change	Control Mean ± S.D	Diniconzole Mean ± S.D	% change	Activated charcoal Mean ± S.D	% change	Rice husk ash Mean ± S.D	% change
2	3.57± 0.5	3.59±0.09	(+0.56)	4.03±0.2	(+12.25)	3.40 ± 0.1	(-5.29)	52.12± 0.13	204.88± 3.4	(+293.09)	51.78 ± 0.9	(-74.74)	53.74 ± 2.00	(-73.77)	4.8 ± 0.3	14.0 ± 0.4	(+191.067)	11.4± 0.2	(-18.57)	12.24 ± 0.09	(-12.57)
24	3.75± 0.4	3.51±0.09	(+6.4)	4.06±0.3	(+15.06)	3.28 ± 0.19	(-6.55)	67.10± 0.3	246.90± 2.6	(+267.90)	62.57 ± 1.3	(-74.65)	67.55 ± 3.2	(-72.56)	8.0 ± 0.4	29.0 ± 0.08	(+262.5)	20.8± 0.1	(-28.28)	24.0 ± 0.4	(-17.24)
48	3.89± 0.03	2.63± 0.09	(32.39)	4.02±0.2	(+52.85)	3.48 ± 0.4	(+32.30)	45.60± 0.5	215.30± 2.5	(+372.15)	63.51 ± 3.4	(-70.50)	55.04± 1.5	(-74.4)	8.0 ± 0.2	19.5 ± 0.06	(+143.75)	16.0± 0.08	(-17.95)	35.2 ± 0.3	(-80.51)
72	3.92± 0.08	1.17± 0.3	(-70.15)	2.90±0.09	(147.86)	3.50 ± 0.08	(+199.15)	70.01± 0.4	191.53± 4.11	(+173.58)	45.92 ± 4.5	(-76.02)	55.37 ± 2.5	(-71.09)	6.4 ± 0.3	25.4 ± 0.9	(+296.88)	14.4± 0.3	(-43.31)	12.8 ± 0.20	(-49.61)
144	3.80± 0.08	1.21± 0.06	(68.16)	2.64±0.04	(118.18)	3.05± 0.03	(+152.07)	65.15± 0.06	137.13± (2.08)	(+110.48)	41.69 ± 2.00	(-69.59)	57.00 ± 3.00	(-58.43)	5.2 ± 0.1	20.08 ± 0.2	(+286.15)	8.0± 0.2	(-60.16)	9.6 ± 0.3	(-52.19)
*168	3.67± 0.09	3.26± 0.05	(13.30)	--		--		59.57± 0.4	44.63± 1.9	(-25.08)	--				5.0 ± 0.09	5.8 ± 0.2	(+16)	--		--	

* Fish were placed in a clean fresh water for 168 hours after exposure to dinicoazole for 144 hours.

Our results are in agreement with many investigators. Graham (1955), Coughlin and Ezra (1968), Puri *et al.* (1976), Ademoroti (1986), Uchida *et al.* (1997) and Ghaudhary *et al.* (2003) reported that the adsorptive capacity of carbon to organic pollutants depends on their physical properties (i.e. surface hydrophobicity, acidic group and surface oxygen groups on activated charcoal). Romeh and Abd El-Ghany (2003) reported that rice husk ash fired at 450°C (amorphous) has high reactivity toward lead and cyanophos pesticide. The maximum specific adsorption (Ym) rates were 109.89 and 909.09 mg/gm for lead and cyanophos respectively.

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تأثير إضافة الفحم النشط وناتج حرق سرس الأرز على متبقيات مبيد الداينيكونازول وتأثيراتهما على بعض مكونات الدم في القراميط

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تم دراسة تأثير إضافة الفحم النشط وناتج حرق سرس الأرز على متبقيات مبيد الداينيكونازول في الماء وسمك القراميط وتأثيرهما على بعض مكونات الدم وأوضحت النتائج ما يلى:

كانت المتبقيات الأولية لمبيد الداينيكونازول بعد ٢ ساعة من المعاملة في الماء وحده وفي الماء المحتوى على السمك ٢,٨٥ ، ٠,٤٦ جزء من المليون. تناقصت هذه الكميات بمرور الوقت حتى وصلت ٠,٧١ ، ٠,٠٧ جزء في المليون بعد ١٤٤ ساعة من العاملة وهذه الكميات تمثل نسب فقد مقدراها ٧٥,٠٨ ، ٨٤,٨٧ % من الكمية الأولية.

ازدادت الكميات الأولية لمتبقى الداينيكونازول في العضلات والكبد والكلية إلي ٥,٠٩، ١٢,٤٧ ، ٨,٤ ، جزء في المليون على التوالى بعد ساعتين من المعاملة ثم تزايدت تدريجياً مع تقدم الوقت حتى وصلت ١٨,٤٢ جزء في المليون في العضلات بعد ٤٨ ساعة بينما وصلت إلي ١٩,٥٥ ، ١٤,٤٣ جزء في المليون فى الكبد والكلية بعد ٢٤ و ٤٨ ساعة من المعاملة على التوالى ثم تناقصت المتبيقات بعد ذلك حتى وصلت ٣,٩٦ ، ١,٦٠، ٠,٤٢ جزء في المليون في العضلات والكبد والكلية بعد ١٤٤ ساعة من المعاملة على التوالى. أدى إضافة الفحم النشط وناتج حرق سرس الأرز إلي المياه الملوثة بالداينيكونازول إلي إختزال متبقياته في الماء وفي كلا من العضلات والكبد والكلى لسمك القراميط. إنخفض مستوى البروتين الكلى في سيرم دم القراميط بينما زاد مستوى الجلوكوز، GPT وذلك بالمقارنة بنظيرتها فى غير المعامل. تحسنت هذه التأثيرات بإضافة الفحم النشط وناتج حرق سرس الأرز.