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EFFECT OF THERMAL PROCESSING ON THE LEVEL OF MALACHITE GREEN RESIDUES IN *OREOCHROMIS NILOTICUS* WITH SPECIAL REFERENCES TO ITS PUBLIC HEALTH SIGNIFICANCE

(With 6 Tables and 2 Figures)

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تأثير بعض المعاملات الحرارية علي مستوي بقايا الملاكيت الأخضر في سمك البلطي مع الإشارة إلي تأثيره علي الصحة العامة

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يعتبر الملاكيت الأخضر من المواد الممنوع استخدامها دوليا في المزارع السمكية كعلاج أو كمطهر لما له من تأثيرات سلبية علي صحة المستهلك مسببا له أمراضاً سرطانية، لذلك تم جمع ١٥٠ عينة من أسماك البلطي أثناء صيف ٢٠١١ من مزارع مختلفة سبق تسجيل حالات مرضية بها؛ واستخدامها لبعض المطهرات، وذلك للوقوف علي مدي صلاحية أسماك البلطي للاستهلاك الآدمي عن طريق قياس مستوي الملاكيت الأخضر في أنسجة سمك البلطي وكذلك دراسة تأثير بعض المعاملات الحرارية علي نسبة وجودة في أنسجة تلك الأسماك. وبقياس مستوي الملاكيت الأخضر في العينات موضع الدراسة بواسطة جهاز الكروماتوجرافي عالي الكفاءة (HPLC) لم يستدل عليه في ٨١.٣٣% (١٢٢) من إجمالي العينات (١٥٠عينة)، ووجد أن متوسط مستوى الملاكيت الأخضر في عضلات البلطي النيلي كان ٢.٢٠ ± ٠.٥٠ جزء من البليون للعينات الطازجة. وبدراسة تأثير المعالجة الحرارية، كانت نسبة الانخفاض في مستوي الملاكيت الأخضر في أنسجة تلك الأسماك ٥٢.٥١ ٪ و٥٩.٩٨ ٪ للعينات المعالجة بالسلق وبالميكروويف علي التوالي. وبمقارنة مستوي الملاكيت الأخضر في عينات أسماك البلطي الطازجة والمعالجة بالسلق وبالميكروويف بالحد المسموح به طبقاً لمنظمة الأغذية والأدوية (FDA) وجد إن نسبة ٨١.٣٣ ٪ (١٢٢) من العينات الطازجة كان مطابقاً، علي الجانب الأخر وجد أن نسبة ١٨.٦٦% (٢٨) من العينات الطازجة والتي تم معالجتها

بالغليان والميكروويف قد تجاوزت الحد المسموح به طبقا لمنظمة الأغذية والأدوية (FDA)، وهذا يشير إلى أن الطهي بالسلق والميكروويف للأسماك يقلل نسبة وجود بقايا الملاكيت الأخضر ولكن لا تمنع وجوده نهائياً. وتم مناقشة العلاقة بين مستويات الملاكيت الأخضر في أنسجة الأسماك الطازجة والمعالجة حراريا (الغلي والميكروويف) علي الصحة العامة واقتراح التوصيات اللازمة.

SUMMARY

A total of 150 samples of *Oreochromis niloticus* were collected during summer season of 2011 from different intensive farms previously known be infected and treated by disinfectant at Kafr El-Sheikh, Governorate. The samples were assessed for the determination of malachite green residual levels in fresh, scalded (boiled) and microwave by HPLC. Malachite green residues not detected in 81.33% (122) of the examined *Oreochromis niloticus* samples. The mean concentrations of the malachite green residual level in the fresh *Oreochromis niloticus* muscle was 2.20 ± 0.50 ppb. On study of the effect of thermal processing, the reduction percentage in the levels of malachite green in the scalding (boiling) and microwave treated samples were 52.51% and 59.98% respectively. From the sum of the examined fresh *Oreochromis niloticus* samples 81.33% (122) were agree with the permissible limit established by FDA for malachite green, while 18.66% (28) of the fresh samples that treated by scalding (boiling) and microwave exceeded the permissible limit. This indicates that cooking by scalding (boiling) and microwave reduces the level of malachite green but not removed it from fish muscle. The relationship between the levels of malachite green in fresh, scalding and microwave treated samples as well as the public health significance of these residues were discussed.

Key words: *Oreochromis niloticus, malachite green, thermal processing.*

INTRODUCTION

In the recent years, pollution of the environment is a topic problem of national and international concern. Aquatic ecosystems are being contaminated rapidly especially in the developing countries which provide a seldom efforts to evolve methods for pollution monitoring and control, in parallel with the rapid advancements in industry and technology. In aquaculture, parasitical, fungal and bacterial infections

concern a problem in fish industry, include increases in the incidence of infectious microorganisms (WHO, 1995; Howgate *et al.,* 2001; Huss *et al.,* 2003; Kesavan, 2010) and reduction in the productivity (volume and number) of fish farms (Subasinghe and Bernoth, 2000; Srivastava *et al.,* 2004; Kesavan, 2010). For that reason, uses of disinfectant and biocide in the aquaculture industry had been used extensively as aquaculture veterinary drug (Srivastava *et al.,* 2004; Andersen *et al.,* 2005). Due to the low cost, availability and efficacy of malachite green (MG) to treat parasitical infestation (protozoan ectoparasites, particularly *Ichthyophthirius multifiliis, Ichthyobodo necator*, *Trichodina* sp., *Trichodinella* sp., and *Chilodonella* sp., cestodes, trematodes and nematodes), fungal infections (*Saprolegnia* sp.) and bacterial infections (gill flavobacteriosis) in fish and fish eggs, malachite green have been using in aquaculture industries worldwide (Culp and Beland, 1996; Srivastava *et al.,* 2004; Halme *et al.,* 2007), also used as food coloring agent and food additive (Culp and Beland, 1996), although it is not approved for use in aquaculture by U. S. Food and Drug Administration "FDA" (Culp and Beland, 1996).

Malachite green (MG), also, called aniline green, basic green 4, diamond green B, or Victoria green B, is a toxic metallic looking crystal, easily dissolved in water as a blue-green solution and primarily used as a dyeing agent of textiles and as a biological stain for microscopic analysis of cell and tissue samples (Bergwerff and Scherpenisse, 2003; Srivastava *et al.,* 2004). Chemically, MG is a cationic triphenylmethane dye (N-methylated diaminotriphenylmethane), commonly known in a form called chromatic form in which it is a green dye and commercially available as the oxalate and hydrochloride salts (Plakas *et al.,* 1996; Cho *et al.,* 2003).

MG enters water cycles of aquaculture either through the excreted waste products of plant which used it in industry or the illegal use of it as a drug to treat diseased fish. The using of malachite green as a topical treatment by bath or flush methods without paying any attention to the fact that topically applied therapeutically might also be absorbed systemically and produce significant internal effects. On uptake by fish, it easily absorbed into the tissue and converted by body mechanisms into the carbinol form which spreads across cell membranes faster, then metabolized and rapidly reduced inside the cell into leucomalachite green (LMG) which is toxic, lipophilic, accumulated and stored in fatty tissue of the fish for longer period than MG (UNESCO,

2000; Cho *et al.,* 2003; Srivastava *et al.,* 2004). The progress of intoxication in fish is very rapid depending on the exposure time, temperature, concentration and the environmental condition (water hardness, pH, temperature and amount of dissolved oxygen in water). Typical, clinical symptoms include restlessness and uncoordinated movements, move near the surface of water and leap above the water surface for gasping air, then loss its balance and death. The pathological anatomical picture is characterized by greenish tinge of their skin and increased production of skin slime. The gills are edematous, with excessive amounts of mucous, discolored and damaged. Vessels of body cavity were dilated and muscle tissues and internal organs were often light green in color (Machova *et al.,* 2001). Also, necrosis in the liver (Srivastava *et al.,* 1998a), kidney and intestine (Srivastava *et al.,* 1998b) and degenerative changes in gonads (Srivastava *et al.,* 1998c), were recorded as toxicological effect of MG in fish. On the other hand lower weight gains, higher incidence of tumors in the abdomen, intestines and liver, overall anemia later in their life and higher mortality were recorded as a side effects on treated fish (Sudova *et al.,* 2007).

Fish containing MG and its major metabolic, leucomalachite green cause significant health hazard for humans who eat contaminated fish. They have mutagenic, carcinogenic and teratogenic effects based on its structural similarity to known carcinogens, for example cause bladder cancer and liver tumor in human (Culp *et al.,* 2002). It also has an adverse effect on immune system and reproductive system as well as its genotoxic and carcinogenic potential (Gouranchat, 2000). In rare case, methemoglobinemia in children was recorded (Spiller *et al.,* 2008). It place on annex IV list of the European Union Council Regulation 2377/90/EEC the drug has been banned in many nations outside the EU as well.

The monitoring of malachite green residue levels is essential to assure the safety and manage global health risks of fish. Thus the present study was carried out to evaluate the effect of scalding (boiling) and microwave treatment on the level of malachite green residues in *Oreochromis niloticus* and its public health significance.

MATERIALS and METHODS

1: Samples collection:

A total of 150 samples of fresh *Oreochromis niloticus* were collected during summer season of 2011 from different intensive farms previously known be infected and treated by disinfectant at Kafr El-Sheikh, Governorate. The samples were separately placed into sealed sterile plastic bag, thoroughly identified and delivered to the laboratory in a refrigerated container as soon as they catch to assess the levels of malachite green residues by high performance liquid chromatography.

2: Samples homogenate preparation:

Each individual fish sample was cleaned before preparation. Heads, scales, tails, fins, guts and inedible bones of fish sample were removed and discarded, then filleted to obtain all flesh. The muscle of each fish sample was separately passed rapidly through meat chopper 3 times with plate opening as small as practical (1.5-3 mm in diameter) mixed thoroughly after each grinding to obtain a uniform mass and finally began all determinations promptly. Each homogenate sample was divided into 3 equal parts, the 1st part (fresh) was directly conducted to assess the levels of malachite green residues by high performance liquid chromatography, while the 2nd and 3rd parts of each sample, that contain malachite green residues, were processed by scalding (boiling) and microwave respectively before the assessment of malachite green residues. The homogenates sample was transferred to a wide mouth glass stoppered. The analysis was carried out as soon as possible, if any delay, the sample was chilled to inhibit the decomposition (A.O.A.C., 2000).

3: Sample processing:

3:1: Scalding (Boiling):

The 2nd part of each individual sample was processed by boiling for 15 min. After cooling, the processed sample was subjected for the assessment of malachite green level by HPLC (Mitrowska *et al.,* 2007).

3:2: Microwave:

The 3rd part of each sample was treated in the microwave for 1 min. The treated sample was carried out to the technique recommended for the assessment of malachite green level by HPLC (Mitrowska *et al.,* 2007).

4: Assessment of malachite green residues levels:

The homogenate samples were conducted individually for determination of malachite green levels by high performance liquid chromatography (Agilent 1200 Series-Germany) according to FDA (2005).

4-1: Reagents:

All used chemicals were HPLC grade. Malachite green standards, hydroxylamine hydrochloride, ammonium acetate and *P*-Toluene sulfonic acid " *P*-TSA" obtained from Sigma-Aldrich (Sigma, St. Louis, MO, USA), while acetic acid, acetonitrile, methanol, methylene chloride and HPLC water obtained from Merck Inc. (Merck, Darmstadt, Germany).

4-2-1: Sample Extraction:

To 5 g homogenate sample of *Oreochromis niloticus* tissue either fresh or processed was added 1 ml hydroxylamine (in 100 ml volumetric flask, 25 g of hydroxylamine hydrochloride was added and diluted to the mark by deionized water "0.25 g/ml"), 2 ml 1 M toluene sulfonic acid (in 50 ml volumetric flask, 9.5 g of *P*-Toluene sulfonic acid " *P*-TSA" was added and diluted to the mark by deionized water), 2 ml of 0.1 M ammonium acetate buffer (7.7 g of ammonium acetate was dissolved in 1000 ml deionized water and adjusted to pH 4.5 by adding 8 ml acetic acid and 5 ml of 1 M *P*-Toluene sulfonic acid) and 40 ml acetonitrile. The mixture was homogenized for 2 min, then the supernatant was decanted and to the precipitate was added 20 ml acetonitrile. This was filtered and added to the supernatant. To the combined acetonitrile extracts, 35 ml water and 30 ml methylene chloride were added. The solution was shaken and the methylene chloride layer collected. A second extract of 20 ml methylene chloride was made and this layer added to the first layer and thoroughly mixed (FDA, 2005).

4-2-2: Clean-up procedure:

Liquid phase was evaporated to 5 ml then 20 ml deionized water was added. 25 ml of the aliquot was passed through the Agilent ZORBAX C18 (3 µm, 150 X 4.6 mm) which previously activated with 2 ml methanol for cleanup. After the solution had passed, the column was washed by 2 ml water and elute with 2 x 2 ml of acidified methanol. The methanol was dried under rotary evaporator and the residue was redissolved in 1 ml mobile phase before the injection in HPLC (FDA, 2005).

4-2-3: Preparation malachite green working standards:

Analytical grade standard for malachite green purchased from Sigma (St. Louis, MO, USA). Into separate 100 ml volumetric flask, 10.0 mg of reference standard malachite green (Corrected for MG purity and also for MG oxalate, which contains only 7.109 mg of MG for 10 mg of MG-oxalate) was added and diluted to the volume by methanol and thoroughly mixed to obtain a stock solution at a concentration of 100 µg/ml. For calibration curve standards, intermediate standards were prepared by pipette 1.0 ml of MG stock solution into a 100 ml volumetric flask, diluted to the volume with methanol and mixed to prepare MG1 working standard (MG1-ICV, 1.0 µg/ml). A second 1.0 µg/ml MG working standard from MG-ICV stock solution was prepared. For preparation of MG2 working standard (MG2, 0.1 µg/ml), 1.0 ml of MG1 was pipette into a 15 ml graduated glass centrifuge tube and diluted to 10 ml with methanol. These standards were diluted to 5 ml of acetonitrile-acetate buffer mixture (50:50 v/v) to make final solutions in the range of 0, 1, 2, 4, 10 and 20 ppb of Mg (FDA, 2005).

4-2-4: HPLC determination:

Malachite green level was determined in *Oreochromis niloticus* samples either fresh or processed by HPLC (Agilent 1200 Series, Germany) at wavelength 618 nm (4.0 nm bandwidth) with reference of 725 nm (8 nm bandwidth) using a tungsten lamp. The column used was the Agilent ZORBAX C18 (3 µm, 150 X 4.6 mm) and the mobile phase was composed of 2 phase (Mobile phase A was ammonium acetate buffer: acetonitrile, "1:1, v/v", Mobile phase B was acetonitrile and the elution profile was 95% and 5% for A and B respectively) which pumped with constant flow rate at 1 ml/min. 100 µl of the reconstituted sample were injected in the HPLC at 35°C to achieve the optimum resolution of malachite green. Several blanks (methanol only) and malachite green standard solution were injected also. The examined samples were done in triplicates. Calculations to get the level of malachite green in the examined samples were carried out automatically by Agilent ChemStation Software System (FDA, 2005).

5- Statistical methods

One-Way ANOA test was performed on the parameter studied to describe data using Statistical Package for Social Scientists (SPSS) for windows 16.0 (*SPSS Inc., Chicago, IL, and USA*). Correlations between the level of malachite green residues in the fresh *Oreochromis niloticus* samples and that processed by boiling and microwave were also done to examine whether there were significant differences in parameters

analyzed. P value was considered significant if less than 0.05 and 0.01 at 95% and 99% respectively (SPSS, 2007).

RESULTS

Table 1: Statistical analytical results of malachite green residues (expressed as ppb) recovered from fresh *Oreochromis niloticus* samples (n=150).

			Fresh *Oreochromis niloticus* samples
Samples	Total	No.	150
		%	100
	(ND)	No.	122
		%	81.33
	(D)	No.	28
		%	18.66
Statistic for malachite green levels		Min.	0.00
		Max.	35.45
		Mean	2.20
		S.E.	0.50

ppb = Part per billion. ND = Non detectable level (< 1 ppb). D = Detectable level. Min. = Minimum. Max. = Maximum. SE = Standard error.

Table 2: Statistical analytical results of the effects of thermal processing (scalding and microwave) on malachite green levels (expressed as ppb) recovered from *Oreochromis niloticus* samples (n=28).

		Fresh samples	Processed samples	
			Scalding	Microwave
Samples	No.	28	28	28
	%	100	100	100
Statistic for malachite green levels	Min.	2.37	1.14	0.95
	Max.	35.45	17.02	14.18
	Mean	11.77	5.59	4.71
	S.E.	1.81	0.87	0.73

ppb = Part per billion. Min. = Minimum. Max. = Maximum. SE = Standard error.

Table 3: Frequency distribution of malachite green levels (expressed as ppb) under the effect of thermal processing (scalding and microwave) for *Oreochromis niloticus* samples (n=28).

Levels range (ppb)	Fresh samples		Processed samples			
			Scalding		Microwave	
	No.	%	No.	%	No.	%
> 0.0 - < 4.0	6	21.43	16	57.14	17	60.71
> 4.0 - < 8.0	10	35.71	4	14.29	4	14.29
> 8.0 - < 12.0	1	3.57	5	17.86	5	17.86
> 12.0 - < 16.0	3	10.71	2	7.14	2	7.14
> 16.0 - < 20.0	1	3.57	1	3.57	0.00	0.00
> 20.0 - < 24.0	3	10.71	0.00	0.00	0.00	0.00
> 24.0 - < 28.0	2	7.14	0.00	0.00	0.00	0.00
> 28.0 - < 32.0	0.00	0.00	0.00	0.00	0.00	0.00
> 32.0 - < 36.0	2	7.14	0.00	0.00	0.00	0.00
Total	28	100	28	100	28	100

ppb = Part per billion.

Table 4: Reduction effect of thermal processing (scalding and microwave) on malachite green levels (expressed as ppb) recovered from *Oreochromis niloticus* samples (n=28).

		Fresh samples	Processed samples	
			Scalding	Microwave
Samples	No.	28	28	28
	%	100	100	100
Mean		11.77	5.59	4.71
Reduction %		--------	52.51	59.98

Table 5: Correlation between the different mean values of malachite green residues recovered from fresh and processed *Oreochromis niloticus* samples (n=28).

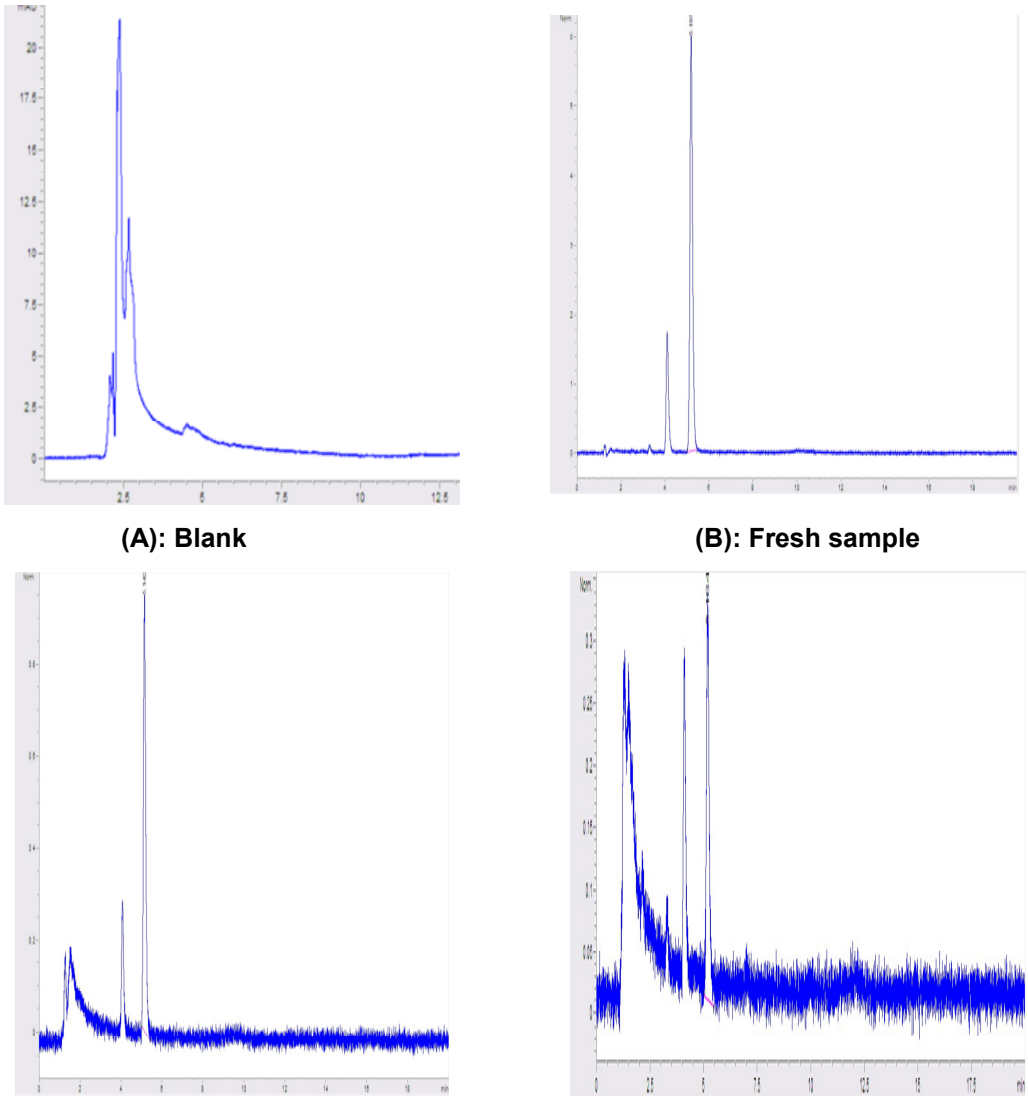
		Fresh	Processed samples	
			Scalding	Microwave
Fresh		----------	0.003 (**)	0.001 (**)
Processed samples	Scalding	0.003 (**)	----------	0.881
	Microwave	0.001 (**)	0.881	----------

(**) = Highly significant correlation at < 0.01 by using one way ANOVA test.

Table 6: Comparison between malachite green levels (expressed as ppb) recovered from fresh and processed *Oreochromis niloticus* samples with the permissible limit recommended by FDA (0 ppb).

		Fresh		Processed samples			
				Scalding		Microwave	
		No.	%	No.	%	No.	%
Samples	Acceptable	122	81.33	0.0	0.0	0.0	0.0
	Not Acceptable	28	18.66	28	100	28	100
	Total Number	150	100	28	100	28	100

ppb = Part per billion. FDA = Food and Drug Administration.



(A): Blank **(B): Fresh sample**

(C): Scalding **(D): Microwave treated sample**

Fig. 1: Malachite green chromatograms for analysed fresh and processed (Scalding and microwave) *Oreochromis niloticus* samples (group III) and blank (A): Blank. (B): Extracted fresh *Oreochromis niloticus* samples containing malachite green. (C): Extracted scalded *Oreochromis niloticus* samples containing malachite green. (D): Extracted microwave treated *Oreochromis niloticus* samples containing malachite green.

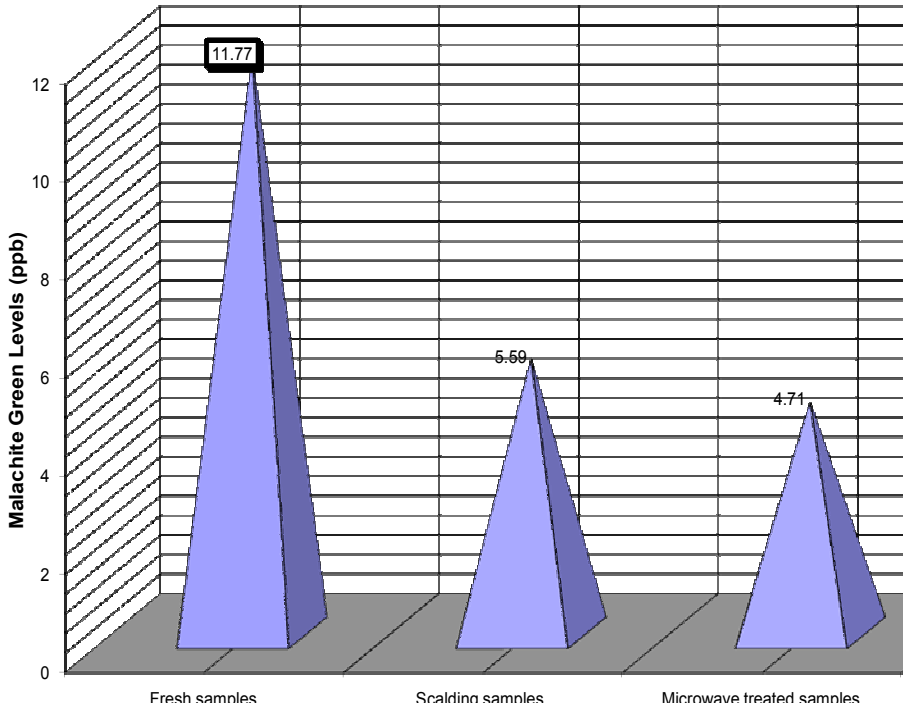


Fig. 2: Reduction effect of thermal processing (scalding and microwave) on malachite green levels (expressed as ppb) recovered from *Oreochromis niloticus* samples (n=28).

DISCUSSION

The assessment of malachite green residual levels in the examined *Oreochromis niloticus* samples were showed in Table 1 and Fig. 1. Malachite green not detected in the examined fresh samples with an incidence of 81.33% (122) but detected in 18.66% (28) of the fresh samples. This result was less than the results recorded by Rasmussen (2007); Schuetze *et al.* (2008) where they recorded an incidence of 55.55% and 83.31% in fresh farmed fish respectively. The mean values of malachite green levels in the examined *Oreochromis niloticus* samples (n=150) was 2.20 ± 0.50 ppb. The obtained result was higher than the results recorded by Allen *et al.* (1994); Plakas *et al.* (1996);

Schuetze *et al.* (2008) and less than the results recoded by Mitrowska and Posyniak (2005). The differences between the obtained results and the results of other studies may be attributed to the difference in the time, concentration, methods of application of MG and its purity and the varying concentration of residual impurities (Sudova *et al.,* 2007). Fat content, weigh and growth rate of fish affect on the persistence of malachite green in fish tissues (Bauer *et al.,* 1988; Belitz and Grosch, 1999). Also malachite green toxicity heavily influenced by the condition and quality of water such as hardness, pH, temperature and amount of dissolved oxygen whereas their efficacy increase as temperature increased and pH decreased (Cho *et al.,* 2003). The presence of Ca^{2+} ions compete MG for negative charged humic substances (HS) results in reduction in the efficacy of MG (Meinelt *et al.,* 2001). The levels of pollution either natural of industrial effluents increase the levels of contamination by MG (FAO 1992; Olowosegun *et al.,* 2005; Obasohan, 2009).

On studying, the effect of cooking on the malachite green residual levels in the examined *Oreochromis niloticus* samples showed in Tables 2-4 and Fig. 1-2. The mean values of malachite green levels in fresh, scalding (boiling) and microwave treated samples were 11.77 ± 1.81, 5.59 ± 0.87 and 4.71 ± 0.73 ppb respectively. The frequency distribution of the malachite green detected residues in the examined fresh *Oreochromis niloticus* samples (n=28) ranged from > 0.0 to <28.0 ppb and from > 32.0 to <36.0 ppb with an incidence of 92.86% (26) and 7.14% (2) respectively. After processing of the fresh samples (n=28), the frequency distribution of the malachite green detected residues in the processed *Oreochromis niloticus* samples ranged from > 0.0 to <20.0 ppb and from > 0.0 to <16.0 ppb with an incidence of 100% (28) for each of scalding (boiling) and microwave treated samples respectively. These results agree with the results recoded by Long *et al.* (2008); Schuetze *et al.* (2008). The wide range of the obtained results may be regards to the uneven distribution of the used or contaminated MG in the aquaculture media, stopped the flow of water, less adequate aeration and defect in the cleaning after treatment (Citek *et al.,* 1997; Mitrowska and Posyniak, 2004).

Table 4 showed a reduction in the levels of malachite green after treatment of *Oreochromis niloticus* samples. The reduction percentage in the residual levels of malachite green after treatment of *Oreochromis niloticus* samples by scalding (boiling) and microwave were 52.51 %

and 59.98 % respectively. These results approximately agree with the results recorded by Mitrowska *et al.* (2007) who recorded a reduction percentage of 54±3% and 61±2 % for boiling and microwave treated samples. The lesser variation between the reduction effect of scalding (boiling) and microwave may be regards to malachite green is stable at 100°C and the internal temperature of the meat by cooking (boiling and microwave) did not rise above 100°C and not more than 15 minutes where the highest achieved temperature were 96.4 °C and 99.6°C during boiling and microwave treatment respectively (Mitrowska *et al.,* 2007). More reduction or elimination of malachite green from fish needs more temperature and more time than the normal cooking conditions. So the cooking by high temperature was not a guarantee for full and complete breakdown of the malachite green residues which may be occur in *Oreochromis niloticus*.

One-Way ANOVA test was used to compare the means values of malachite green in the examined *Oreochromis niloticus* fresh and processed samples (Table 5). A highly significant relationship between the mean values of malachite green residues of fresh and each of scalded (boiling) and microwave treated samples were recorded and a non significant relationship between the mean values of MG residues of boiling and microwave treated samples. This indicates that the cooking either by scalding (boiling) or microwave reduce the levels of malachite green but not eliminates it from fish muscle. Also, there is lesser difference in the reduction of MG either by scalding (boiling) or microwave treatment.

In comparison of the mean values of malachite green in the examined *Oreochromis niloticus* samples with the permissible limit established by FDA (2007b) showed in Table (6). It is evident that malachite green was not detected in 81.33% (122) of the fresh samples and this results were agree with the limit recommended by FDA (2007b) for malachite green residues in fish. On the other hand, malachite green were detected in examined samples before treatment (fresh) and after treatment either by scalding (boiling) or microwave with an incidence of 18.66% (28) for each of them. This levels were exceeded the permissible limits of malachite green residues in fish established by FDA (2007b). From the public health significant, the fish which exceed the permissible limits of malachite green according to FDA (2007b) consider unfit for human consumption and cause significant health hazard for humans who eat contaminated fish (Culp *et al.,* 2002).

CONCLUSION

In spite that malachite green (MG) has been banned in several countries, as a results of the risks it poses to the consumers, it is still being used in many parts of the world due to its success in the treatment of some aquaculture diseases and lack of a proper alternative. Thus, in conclusion, application of good manufacture practices and Hazards Analysis Critical Control System (HACCP) in tilapia farms must be done for improving the malachite green residual problem in fish muscles. Such control include good management program to overcome the outbreaks in the aquaculture by microorganisms, adequate supply of good-quality water with minimum contamination by organic substances and good-quality feeds should be applied to keep fish in the best health condition possible and increase their resistance to infections. Strictly prevention the malachite green to enter the food chain either through, stop the uses of malachite green as veterinary drug in aquaculture or prevention and treated the waste waters of factories or industries and agricultural chemicals containing malachite green to come into the aquatic environment. The treatment of wastewaters with biosorbents which able to decolorizes, biosorb or biodegrade MG such as microorganisms (*Citrobacter* sp. and *Pithophora* sp.) and microalgae (*Cosmarium* sp.) enhanced the removal of this dye. Comprehensive system regulations based on the practice for handling and disposal of chemicals and wastes should be established to ensure the safety removal. On the other hand, any company or establishment found to discharge wastes that are dangerous to fish should be made to pay compensation to the nearby fish farms. Accurate monitoring of chemical residue levels in food and agriculture products is essential to assure the safety of the food supply and manage global health risks. So, it is necessary for established an early warning system by using the biochemical markers of oxidative stress in response to the malachite green exposure before marketing. Also, tilapia containing malachite green residues (either treated or contaminated) must be held for the recommended withdrawal time before they can be marketed for ensuring their safety for human consumption due to MG persist in the aquatic environment for a long time. A proper alternative and approved drugs for malachite green should be introduced in aquaculture treatment such as the active ingredient Bronopol, stable chlorine dioxide, hydrogen peroxide and humic acid. A non-conventional sorbents such as sugarcane dust, sawdust bottom ash fly ash de-oiled soya, maize cob and peat should be used to remove excess malachite green left in aquaculture medium.

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