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DETERMINATION OF DDT AND ITS METABOLITE IN MARULA PRODUCTS IN ESWATINI USING A MOLECULAR IMPRINTED POLYMER

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ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) is an effective organochlorine pesticide which is used in Indoor Residual Spraying (IRS) in Eswatini yet it is toxic, susceptible to long range environmental transport and bio-accumulate in fatty tissues. The main objective was to quantify the amount of DDT and its metabolites (DDE and DDD) in Marula brew and kernels. The samples were pre-extracted with acetonitrile, extracted using a periodic mesoporousorganosilica molecular imprinted polymer (PMO-MIP) with acetone in cyclohexane and then analyzed with GC/ECD. DDT was detectedin 88% of the samples with the highest concentrations at 0.903 ppm in brew and 148.686 mg/kg from the kernels. DDD was detected in 86% of the Marula brew samples with the highest concentration at 0.483 ppm and 68.219 mg/kg from the kernels. However, DDE was found in lower concentrations of 0.138 ppm and 30.132 mg/kg from the brew and kernels respectively. The study concluded that people and animals are at risk of DDT residual accumulation as 98% of the samples collected had the analytes detected at level above the WHO safety limit of 0.05 ppm.

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INTRODUCTION

Dichlorodiphenyltrichloroethane (DDT) is a best known organochlorine pesticide, which has been used extensively as an insect pest control in agriculture because of its broad-spectrum activity, long-lasting control, inexpensive to manufacture and effectiveness (Rathore and Nollet, 2012). However, DDT caused environmental impacts on animals which are what lead to it being banned in most countries under the Stockholm Convention on POPs in 2001 (Stein, 2013) because it is very persistent in the environment resulting to long range environmental transport and bio-accumulation in fatty tissues (Longnecker *et al*., 2001). DDT restricted use in vector control in Eswatini has led to reduction of malaria mortality (Tatem *et al*, 2010). A periodic mesoporous organosilica is an inorganic organic hybrid composition with ordered porous structures which can be molecularly imprinted to be able to bind specific chemical species. These molecular imprinted polymers are different in that; there exists a large number of organic functional groups homogeneously distributed throughout the pore wall of PMOs (Goto& Inagaki, 2002), increased hydrothermal stability, higher mechanical stability, variable hydrophilic-hydrophobic micro-environments, and myriad pore surface compositions that fine-tune interactions with guest molecules (Wang, 2011). These factors increase the extraction effieciency of the polymer toward DDT and its metabolites from environmental samples. The Marula fruits are used to make Marula brew (Mariod, 2012) while the Marula kernels are used as part of relish and kids just enjoy them as a snack, animals also enjoy the oil rich highly nutritious kernels (Xaba, 2011). Consequently, such activities increase the risk of DDT and its metabolites accumulation in organisms yet it is toxic.

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MATERIALS AND METHODS

Samples collection: Samples of Marula brew and kernels were collected from homesteads in the Lowveld and part of the Middleveld which are the Malaria endemic areas during the Marula season between January to April 2017 while the control samples were collected from Ntondozi and Hosea where there is no use of DDT. Marula brew samples were collected into clean sampling bottles and retained under refrigeration at 4 °C until extraction.

A B

Figure 1. Photographs showing; A samples of Marula brew and B samples of Marula kernels

A B

Figure 2. Photographs showing; A acetonitrile upper layer added into vials with the PMO-MIP and B elution of the analytes from the PMO-MIP using a syringe with the glass fiber microfilter

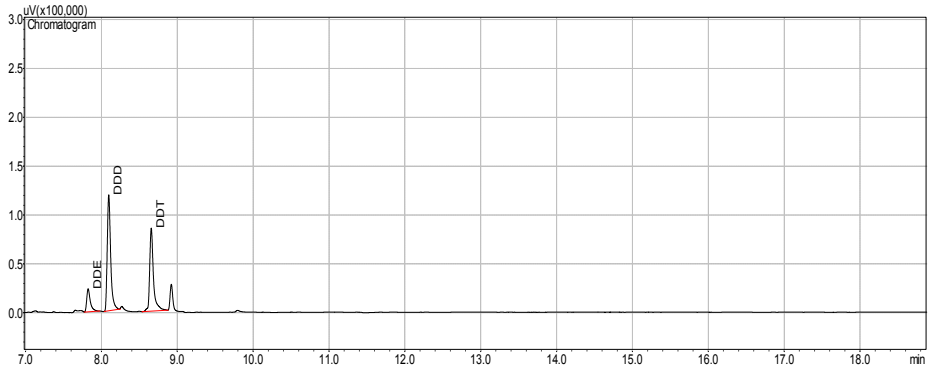

Figure 3. A chromatogram of Marula brew sample with DDT, DDD and DDE peaks

Marula kernels were collected into poly-ethene bags and ground into paste before extraction.

Sample pre-extraction: The Marula brew and kernels samples were pre-extracted with acetonitrile GC grade and un-buffered QuEChERS salt packet as described in (Ranganathan *et al*., 2017).

Sample extraction of analytesusing a MIP: Masses of 0.09 g of the PMO-MIP, 0.24 g of anhydrous magnesium sulfate and 0.06 g of sodium chloride were measured into a vial onto which 2 mL of the upper acetonitrile layer from the sample pre-treatment was added.

The mixture was thereafter shaken for 12 hours in a shaker and syringes with a glass fiber micro-filter were used to separate the PMO-MIP, salts and the acetonitrile. A volume of 3 mL cyclohexane:acetone (7:3) was used to elute DDT/DDD/DDE from the PMO-MIP and extracts were placed into GC vials for analysis (adapted Nothando *et al*., 2019).

Analytical Performance: Analytical performance evaluation was done through construction of calibration curves, quality control charts, detection limits, recoveries and error analysis. This was done to ensure that measurements were within statistically acceptable range.

RESULTS AND DISCUSSION

Marula brew results: A total of 56 Marula brew samples collected were analysed and 88% of those samples had DDT, detected. The mean concentration for DDT was 0.336 ppm, standard deviation of 0.171 ppm and RSD was 0.529. The highest concentration was 0.903 ppm from Newthulwane and the lowest concentration was 0.005 ppm from Mliba Figure 5, Table 2 and Figure 6.

DDD was detected in 86% saμmples with amean concentration of 0.136 ppm, standard deviation of 0.068 ppm and RSD of 0.504. The lowest concentration was 0.006 ppm from Hlane and the highest concentration of 0.483 ppm from Newthulwane which was also high in DDT levels. DDE had lower concentration in most of the 80% samples it was detected in from the following districts; Timphisini, Lomahasha, Lubulini, Ndzingeni, Nkilongo, Ntfonjeni, Hlane

Table 1. Concentration of DDT, DDD and DDE (ppm) from the Marula brew samples

Districts	Place	DDT	DDD	DDE
Timphisini	Timphisini (kaDlamini)	0.262	0.193	0.126
Ntfonjeni	Vusweni (kaMkhonta)	0.357	0.139	0.045
	Mahlabatsini (kaNdwandwe)	0.134	0.118	0.074
Mayiwane	Mkhuzweni (kaKunene)	0.484	0.142	0.030
Mhlangatane	Lohlalane (kaTsabedze)	0.256	0.077	0.008
	Nyakafo (Maganu)	0.158	0.101	0.049
Ndzingeni	Bulandzeni (kaMethula)	0.377	0.124	0.085
	Mgungundlovu (kaMnisi)	0.511	0.157	0.037
Madlangempisi	NkambenikaMaziya	0.542	0.131	0.019
	NkambenikaGumedze	0.390	0.106	0.025
Mkhweni	Mliba (kaManana)	0.005	nd	nd
Hlane	KhuphukakaSifundza	0.183	0.169	0.136
	KhuphukaHlane sample	0.424	0.055	nd
	MnjoliMphafeni	0.097	0.125	0.028
	HlanekalaNkumbula	0.027	0.006	0.006
	Hlane sample	nd	nd	nd
	Skhuphe (kaMtsetfwa)	0.381	0.114	0.054
Mhlume	Vuvulane (Maganu)	nd	nd	nd
Lomahasha	Lomahasha (kaHlophe)	0.183	0.166	0.108
	Shewula	0.163	0.114	0.035
	Maphiveni	0.235	0.245	0.138
Lugongolweni	Lugongolweni (kaMaziya)	0.124	0.094	0.079
	Sitsatsaweni (kaMnisi)	0.362	0.102	0.020
	Sitsatsaweni (kaJele)	0.444	0.106	0.027
	Mhlumeni (kaMatsenjwa)	nd	nd	nd
	Emacaceni (kaJele)	0.295	0.107	0.019
	Mambane (kaMatsenjwa)	0.441	0.143	0.043
Dvokodweni	Malindza (kaSifundza)	0.450	0.142	0.057
	Ebhodweni	0.567	0.152	0.026
	Sigcaweni	0.438	0.143	0.034
	Newthulwane (kaNkambule)	0.903	0.483	0.037
	Mdumezulu (kaMkhabela)	0.374	0.116	0.033
Mpolonjeni	Lukhula (Maganu)	nd	nd	nd
	KaLanga (kaHlophe)	0.333	0.087	0.002
	Mpolonjeni (kaMamba)	0.011	0.074	0.074
	Ngcina (kaMkhabela)	0.125	0.121	0.061
	KaDzangu (kaDlamini)	0.195	0.153	0.079
Nhlambeni	NhlambenikaVilakati	0.200	0.116	0.026
	Sidvokodvo (kaSimelane)	0.413	0.125	0.040
	Gudvwini (Maganu)	0.468	0.287	0.014
Siphofaneni	Eduze (Siphofaneni)	0.389	0.110	nd
	Eduze (kaJimba)	0.434	0.125	0.041
	Mganyaneni (Siphofaneni)	0.453	0.143	0.043
Sithobelweni	Gucuka (kaDlamini)	0.205	0.145	0.022
	Sithobelweni (kaDlamini)	nd	nd	nd
Nkilongo	Gamula (kaMyeni)	0.217	0.171	0.067
Lubulini	Ncwandweni (kaButhelezi)	0.417	0.185	0.095
	Lubulini (kaMngometulu)	0.438	0.132	0.056
Somntongo	Maplotini 1 (Lavumisa)	0.515	0.129	0.009
	Maplotini 2 (Lavumisa)	0.379	0.132	0.077
	Somtongo (kalaGina)	nd	nd	nd
Ntontozi	KhalangililekaKhumalo	0.448	0.118	0.015
	KhalangililekaNgwenya	0.401	0.114	0.029
	Velezizweni	0.404	0.069	nd
Kubuta	Kholwane (kaManana)	0.121	0.078	0.020
Hosea	Hosea	nd	nd	nd
	Mean	0.336	0.136	0.048
	Std dev.	0.171	0.068	0.034
	RSD	0.529	0.504	0.715

The districts with high levels of DDT were Dvokodweni, Mayiwane, Madlangempisi, Somntongo, Ndzingeni, Lubulini, Siphofaneni, Ntontozi, Nhlambeni and Lugongolweni which ranged from 0.333 to 0.546 ppm.

and Mpolonjeni. The highest concentration was 0.138 ppm from Maphiveni and the lowest concentration was 0.002 ppm from kaLanga. The mean concentration was 0.048 ppm with standard deviation of 0.034 and RSD of 0.715.

Table 2. Concentrations of DDT, DDD and DDE (mg/kg) in Marula kernels

Districts	Place		DDT	DDD	DDE
Timphisini	Timphisini (kaDlamini)		9.215	2.039	4.579
Ntfonjeni	Vusweni (kaMkhonta)		9.143	2.469	1.845
	Mahlabatsini (kaNdwandwe)		8.909	5.870	2.348
Mayiwane	Mkhuzweni (kaKunene)		13.903	1.879	5.925
Mhlangatane	Lohlalane (kaTsabedze)		133.792	43.406	27.007
	Mpofu		8.585	0.569	1.042
Ndzingeni	Bulandzeni (kaMethula)		11.907	2.882	3.775
	Mgungundlovu (kaMnisi)		15.562	5.987	9.564
Madlangempisi	Nkambeni (kaMaziya)		146.673	66.895	25.215
	Nkambeni (kaGumedze)		11.843	2.321	1.612
	NkambenikaMdluli		113.325	51.619	19.315
Mkhiweni	Mliba		74.328	32.265	14.077
Hlane	KhuphukakaSifundza		102.012	41.979	22.132
	KhuphukakaMagagula		109.671	42.120	25.474
	MnjoliMphafeni		128.925	45.340	22.653
	MphalakaMagwaziphalishi		129.145	58.822	23.532
	Mpumalanga malaria campsite		148.468	65.289	30.132
	HlanekalaNkumbula		127.469	51.762	25.474
	SkhuphekaMtsetfwa		124.967	55.433	11.686
Lomahasha	LomahashakaHlophe		119.381	42.636	24.180
	Maphiveni		108.103	45.442	14.177
Lugongolweni	LugongolwenikaMaziya		116.241	43.776	22.548
	SitsatsawenikaMnisi		81.661	33.323	15.884
	SitsatsawenikaJele		110.058	45.334	19.920
	MhlumenikaMatsenjwa		84.298	34.136	16.660
	EmacacenikaJele		135.379	43.955	8.774
	MambanekaMatsenjwa		119.123	46.161	19.822
Dvokodweni	MalindzakaSifundza		98.779	43.582	18.369
	Ebhodweni		123.089	51.985	25.966
	Sigcaweni		107.665	47.357	21.837
	NewthulwanekaNkambule		129.291	65.279	21.647
	MdumezulukaMkhabela		108.751	43.545	24.007
Mpolonjeni	KaLangakaHlophe		106.743	47.864	11.894
	MpolonjenikaMamba		10.031	1.856	5.618
	NgcinakaMkhabela		15.808	4.320	13.626
	KaDzangukaDlamini		6.538	0.988	1.186
Nhlambeni	NhlambenikaNdwandwe		7.573	1.383	2.279
	NhlambenikaVilakati		12.402	2.853	11.607
	SidvokodvokaSimelane		8.259	1.677	1.855
Siphofaneni	Eduze		120.464	60.415	25.559
	Mganyaneni		126.051	58.180	13.503
	Madlenya		141.157	62.196	26.126
Sithobelweni	GucukakaDlamini		104.129	45.234	15.033
	SithobelwenikaDlamini		117.751	52.832	23.361
Nkilongo	GamulakaMyeni		131.136	53.950	22.826
	GamulakaButhelezi		113.116	50.526	16.704
Lubulini	NcwandwenikaButhelezi		90.811	38.078	14.471
	LubulinikaMngometulu		140.354	61.475	27.561
Somntongo	Maplotini 1 - Lavumisa		145.708	68.219	26.516
	Maplotini 2 - Lavumisa		118.322	46.721	22.887
	SomntongokalaGina		116.558	61.210	20.471
Ntontozi	KhalangililekaKhumalo		126.031	57.162	23.013
	KhalangililekaNgwenya		125.318	53.031	23.583
	Velezizweni		84.535	36.621	14.540
Kubuta	KholwanekaManana		10.377	2.732	3.661
Nkwene	BuselenikaNyembe		9.827	4.342	4.945
Matsanjeni South	Hluti		101.985	45.907	17.910
		Mean	87.029	36.583	16.073
		Std dev.	50.504	22.772	8.570
		RSD	0.580	0.622	0.533

Marula Kernels Results: A total of 57 samples of the Marula kernels were analyzed for DDT, DDD and DDE and all three analytes were detected in all samples (100%).For DDT, the concentration range was 6.538 mg/kg from kaDzangu to 148.468 mg/kg from Mpumalanga Malaria campsite as the highest level. The mean concentration was 87.029 mg/kg, standard deviation of 50.504 mg/kg and RSD of 0.580. Siphofaneni was the district with the highest mean concentration of 129.2 mg/kg. Analysis for DDD showed that 72% of the samples were above the WHO limit with the mean concentration at 36.583 mg/kg, standard deviation of 22.772 mg/kg and RSD at 0.622. The concentrations ranged from 0.569 mg/kg to 68.219 mg/kg, the lowest concentration being from Mpofu and the highest from Maplotini. Analysis for DDE indicated that 77% of the samples had concentrations exceeding the WHO safety limit. The mean concentration was 16.073 mg/kg, standard deviation was 8.570mg/kg and RSD was 0.533. Sample from Mpofu had the lowest concentration of 1.042 mg/kg while sample from Mpumalanga Malaria campsite had the highest concentration of 30.132 mg/kg.

Analytical performance results: Calibration curves were constructed using the concentrations and the peak areas of the standards where R^2 were 0.9973, 0.9958 and 0.9966 for DDT,

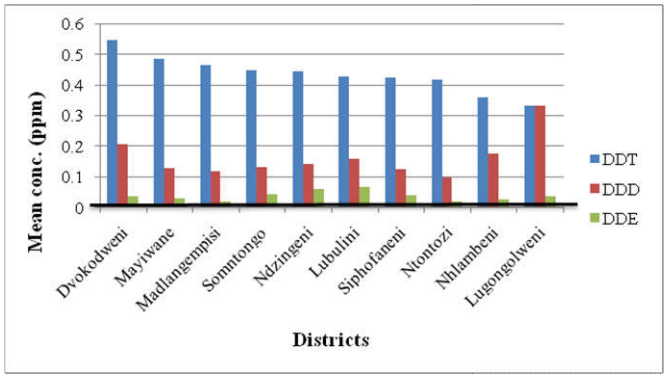

Figure 4. Districts with high concentrations of DDT and their concentrations of DDD and DDE from the Marula brew in relation to the WHO limit (0.05 ppm) shown using the black line

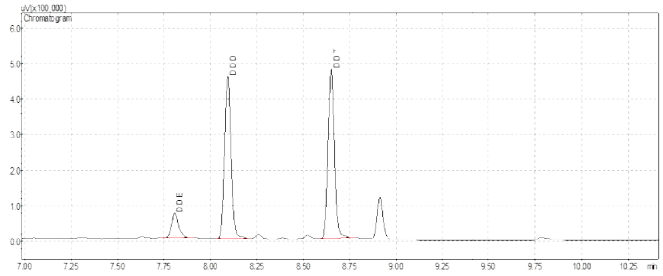

Figure 5. A Chromatogram showing a Marula kernel sample with DDT, DDD and DDE peaks

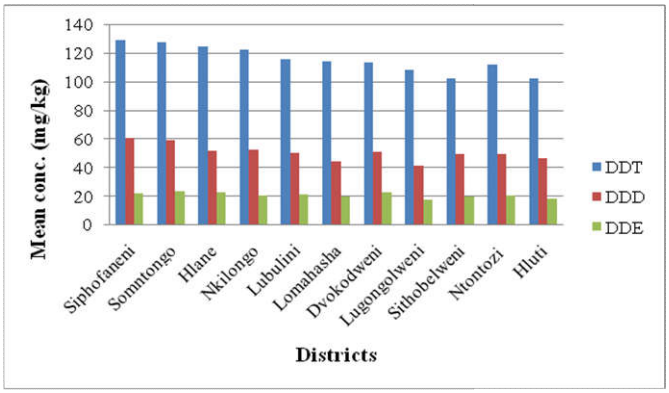

Figure 6. Districts with high concentrations of DDT and their concentrations of DDD and DDE

DDD and DDE respectively which indicated that the linear regression method can be used to quantify the amounts of DDT from environmental samples. Detection limits of the GC were determined to be 0.176 µg/L, 0.236 µg/L and 0.168 µg/L for DDT, DDD and DDE respectively.Quality control charts were also drawn and the samples concentrations obtained from the GC/ECD were within the statistically acceptable ranges. The PMO-MIP used in the extraction process was able to recover 106%, 85%and 99% of DDT, DDD and DDE respectively from the Marula brew samples. The total errors for DDT, DDD and DDE were 0.0282, 0.0375 and 0.0077 ppm respectively and measurement errors due to day to day variation were the most contributing error as it showed high values.

DISCUSSION

To this day only two studies have been on DDT and its metabolites in Eswatini and both studies indicated DDT residues in environmental samples.

Similarly, 88% of the Marula brew samples had DDT detected with the highest concentration of 0.903 ppm. DDD was also detected in 86% of the samples with the highest concentration of 0.483ppm. DDE had lower concentration in most of the 48% samples it was detected with the highest concentration of 0.138 ppm. 98% of these samples exceeded the WHO safety limit in water of 0.05 ppm. Also the Marula kernels analysis had DDT detected in all samples and at levels above the WHO safety limit. This showed that people are at risk of ingesting DDT through drinking the Marula brew and through eating the kernels as they are used for domestic purposes. In a study done by Okonkwo and his colleagues in 1999, milk samples from lactacting mothers in Siphofaneni showed accumulation of p,p-DDT, o,p-DDT and p,p-DDE in 83.5%, 76.7% and 47.6% of the samples respectively. This study gives an insight on how these women had residues of DDT in breastmilk, DDT is liphophilic therefore accumulates in the fatty tissues.

Drinking the Marula brew and eating the kernels could be one way of exposure to DDT residues accumulation in humans. In a study by Gumedze (2012) soil samples from Mpumalanga Malaria campsite had concentration of DDT at 0.087 mg/kgand 67% of the samples collected had DDT concentrations above the WHO safety limit. The same was observed in this study as high levels of DDT were detected in the Marula nuts collected at the Mpumalanga campsite. This was because the nut were left to dry on the ground therefore DDT accumulated in the oily kernels. Surrounding areas such as Mnjoli, Mphafeni and Mphala also had DDT levels detected at high concentrations. The same study indicated that cow's milk samples collected also had traces of DDT detected at a mean of 50 ppb which could be another route of human exposure. Samples collected from Ntontozi also showed high levels of DDT yet IRS is not done in that area. This shows that DDT is now found in areas it has never been used in Eswatini because of its persistence and susceptibility to long range environmental transport. Gumedze (2012) also reported DDT levels to be at 0.991 ppm in sediments collected along rivers, which is a direct indication of long range environmental transport. This also threatens the aquatic organisms which lead directly to DDT bio-accumulation and bio-magnification in humans.

Conclusion

This study discovered that the Swati Nation and international tourists who enjoy the Marula brew are at risk of ingesting DDT and its metabolites as 98% of the samples collected had the analytes detected at levels above the WHO safety limit. The same was observed from the Marula kernels which pose threat not only to the older generation but also the children who enjoy the kernels as snack. Animals that feed on the kernels are also susceptible to DDT and its metabolites exposure which directly leads to its accumulation in humans. It is recommended that a system of monitoring pesticide residues should be included as a key component to generate data for policy making and curtail the use of pesticidesin Eswatini.

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