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INSECTICIDE RESIDUES IN MILK AND INFLUENCE OF HEAT TREATMENTS AND BACTERIAL FERMENTATION AS SAFEGUARD AGAINST THESE POLLUTANTS

(With 3 Tables)

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متبقيات المبيدات الحشريه فى اللبن وتأثير المعاملات الحراريه والتخمر البكتيرى ضد هذة الملوثات

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تم جمع ٦٨ عينة لبن من مزارع الجاموس فى محافظتى الأسكندرية والبحيرة وذلك خلال شهور الصيف أثناء إستخـــدام المبيدات الحشريه فى الأغراض الزراعيه والبيطريه ، وقد تم تحليل عينات اللبن بإستخدام جهاز الكروماتوجرافيا الغاز السائل لتقدير مدى تواجد متبقيات بعض المركبات الهيدروكلورينيه والفوسفوريه فى اللبن. وقد أظهرت النتائج تواجد متبقيات المبيدات الهيدروكلورينيه فى عينات اللبن بنسبة ٦, ٦٨٪ و ٤١ ,٧٩٪ و ١٨ ,٩١٪ و ٢٤ ,٨٨٪ و ٩٤ ,٥٢٪ وذلك لمركبات الـ د.د.ت ' د.د.إى ، اللندان و الدلدرين وذلك بمتوسطات ٠٢٣ ,٠ ± ٠٩٣ ,٠ و ٠١٨ ,٠ ± ١٠١ ,٠ ٠٨٩ ,٠ . ± ١٣٨ ,٠ و ٠١٣ ,٠ ± ٠٤٢ ,٠ . و ٠٣٠ ,٠ ± ٠٩٥ ,٠ جزء فى المليون على التوالى ، ومن ناحية أخرى تم تقدير متبقيات المبيدات الفوسفوريه فى عينات اللبن وقد دلت النتائج على أن عينات اللبن ملوثه بالديازينون والملاثيون والدورسبان بنسبة ٨٢ ,٣٣٪ و ٢٥٪ و ١٢ ,١٩٪ وبمتوسطات ٩ ,٢٧ ±٢٨ ,١٢٠ و ٠٣٩ , ±٠ ١١٠ ,٠ و ٠١٩ ,٠ ± ٠٥٩ ,٠ جزء فى المليون على التوالى بينما أشارت النتائج الى عدم تلوث اللبن بمركبات الألدرين واللانت والدايمثويت والباراثيون. وقد قيمت النتائج طبقاً للمعايير الدوليه التى أقرتها منظمة الصحه العالميه ومنظمة الأغذيه والزراعه الدوليه. وقد دلت النتائج على تلوث بعض عينات اللبن بالديازينون يزيد عن الحد المسموح به مما يشكل خطورة على صحة الإ نسان المستهلك لهذا اللبن. وقد وجد أن لكل من البسترة والغليان تأثير معنوى فى انخفاض قيمة متبقيات المبيدات الحشريه فى اللبن ، وقد أدت عملية البسترة الى إختزال كل من

باربار د.د.ت و باربار د.د.إي والديازينون والملاثيون والدورسبان بالنسب الآتيه على التوالى ٢٩, ٢٩ ، ٥٤, ٧٠ ، ٩٤, ٥١ ، ٦٨, ٤٤ ، ١١, ٢١ . بينما أدت عملية الغليان الى إختزال هذه المركبات بالنسب الآتيه على التوالى : ٢٨, ٧١ ، ٣٥, ٧٠ ، ١, ٧٨ ، ١١, ٥٦ ، ٣٢ ، ٥٥. على العكس لوحظ ثبات كل من الدلدرين واللندان عند معاملة اللبن حراريا بالبسترة والغليان , بينما وجد زيادة نسبة الـ بار بار د.د.د. ومن الناحيه الأخرى وجد أن تصنيع اللبن الى زبادى له تأثير معنوى فى تكسير متبقيات المبيدات الحشريه فى اللبن فكان اختزال كل من الـ د.د.ت واللندان والديازينون والدورسبان بالنسب الآتيه على التوالى ٨٣, ٦٥ ، ١, ٥٦ ، ٢٧, ٥١ ، ٣٧, ٤٧ ، ٢٢, ٦١ بينما كانت ٢, ٨ ٪ لمركب الدلدرين. وقد نوقشت الأهميه الصحيه لمتبقيات المبيدات الحشريه فى اللبن والاحتياطات الواجب إتخاذها لتقليل أو التخلص من تلوث الألبان بهذة المبيدات وحماية المستهلك ضد هذا التأثير الخطير لهذة الملوثات.

SUMMARY

Sixty eight buffaloe's milk samples were collected from the major production farms of Alexandria and Behera governorates over summer months during the period of pesticides application in agriculture and veterinary purposes. The samples were analysed for some organochlorine and organophosphorus insecticide residues using gas liquid chromatography. The obtained results revealed the presence of organochlorine insecticide residues in analysed milk samples at percentages of 86.76, 79.41, 91.18, 88.24 & 52.94% for p.p DDT, p.p DDD, p.p DDE, Lindane and Dieldrin with average values of 0.093±0.023, 0.101±0.018, 0.138±0.089, 0.042±0.013 and 0.095±0.0.030 p.p.m respectively. On the other hand determination of organophosphorus insecticide residues in investigated milk samples revealed that 33.82 , 9.25 & 19.12% of the samples were polluted with Diazinon, Malathion & Dursban residues with mean values of 120.8±27.9, 0.110±0.039 and 0.059±0.019, p.p.m respectively. However all analysed milk samples were free from detectable amounts of Aldrin, Lannate, Dimethoate and Parathion. The results were evaluated according to International Standards of WHO and FAO. The results indicated that presence of Diazinon residues above the permissible limit in some milk samples, may act as health hazard for man consuming such milk. It has been found that both pasteurization and boiling of milk were effective in minimizing the insecticide residues. The reduction percentages of p.p. DDT, p.p. DDE, Diazinon, Malathion and Dursban

residues were 29.79, 21.11, 70.54, 51.94 & 44.68% respectively in response to pasteurization. While values of 71.28, 70.35, 78.1, 56.11 & 55.32 respectively were resulted by boiling. In contrast, residues of p.p DDD were increased while Lindane and Dieldrin were relatively constant under used temperature. On the other hand the processing of milk into yoghurt has a significant role in degradation of the insecticide residues in milk. The reduction percent for each DDT, Lindane, Diazinon, Malathion and Dursban residues was 65.83, 56.1, 51.27, 47.37 & 61.22% respectively while it was 8.2% for Dieldrin residues. The public health importance of insecticide residues in milk and the suggestive measures for minimizing or eliminating the milk pollution and safeguard the consumers against the dangerous effect of these pollutants were discussed.

Key words: *Milk - Insecticide Residue - Heat - Bacterial Fermentation.*

INTRODUCTION

The pollution of milk and dairy products with insecticides are considered one of the main dangerous aspects of the last few years (F.D.A 1988). Nowadays there has been a steady increase in the use of insecticides in developing countries (Mowbray 1986 and Selim & Sebae, 1995). This might be resulted in an increased occurrence of these toxic organics in milk. The organic insecticides reach the milk through exposure of dairy animals to direct spraying with insecticides or via polluted food and water (I.D.F 1979). These pollutants are excreted into the milk by simple diffusion as milk is more acidic than plasma also considerable portion of milk is lipid which considered an important channel for excretion of fat soluble pesticides (Casarett and Doull 1980).

Residues of various insecticides and their metabolites has been reported in milk and milk products from various countries (Konar, 1983, Frank et al. 1985, El-Ghannam et al. 1987, Venant et al., 1991, Ahmed et al., 1992 and Kannan et al., 1992).

The effect of heat treatments on insecticide residues occurring in raw milk have been reported by (Leshchev et al., 1972 Gajduskova & Lat 1974, Molochnikov & Mochalov 1976, Skibniewska & Smoczynski 1985, Korolev 1987, Abdrabo et al., 1989 and Fekry & Bahout, 1992). However the influence of milk processing on the level of insecticide residues was

determined by Mendez et al., 1979, Serjeeva 1979, Sreenivasa et al, 1983, Abdel-Shaheed, 1984 and Ali et al., 1993.

The presence of insecticide residues in food create an actual hazard to public health. Many investigations were conducted to correlate between certain disease conditions of the nonetiological agents and the degree of environmental pollution with insecticide residues (Ingbedioh, 1991 and Heise, 1992).

The worldwide use of insecticides and their hygienic importance make it urgent to know as much as possible a more information about this subject. Therefore this work was carried out to study:

1- The presence of organochlorine and organophosphorus insecticide residues in buffaloe's milk of Alexandria and Behera governorate .

2- The influence of pasteurization and boiling on the level of insecticide residues in raw milk.

3- Effect of yoghurt processing on the content of insecticide residues in milk.

MATERIAL and METHODS

Sampling:

Sixty eight composite buffaloe's milk samples were collected in triplicate from major production farms of Alexandria and Behera governorate over a period of summer months of pesticides application in agriculture and veterinary purposes. The samples were collected in glass containers of one liter capacity and immediately transferred to the laboratory for analysis.

Analytical methods:

The levels of insecticide residues were determined according to the procedure described by A.O.A.C. (1990) for organochlorine insecticides and the method of Toyoda et al. (1990) for organophosphorus, insecticides using Hewlett packard 5890 G.L.C. equipped with electron capture detector (Ni^{63}) and flame photometric detector.

Effect of heat treatments on the levels of insecticide residues in milk:

1- Pasteurization :

Five positive milk samples were heated to 62.8 °C for 1/2 hour then cooled immediately to 10 °C (laboratory pasteurization).

2- Boiling :

Five milk samples contained various types of present insecticide residues were heated with stirring to boiling point for five minutes then cooled.

Effect of yoghurt processing on the levels of insecticide residues in milk:

Five positive milk samples were heated to 85 °C in a water bath for 20 minutes, cooled to 40 to 42 °C, inoculated with 2% yoghurt starter culture (1:1). Streptococcus thermophilous and Lactobacillus bulgaricus at 40 °C for 3-4 hours until it was coagulated and then refrigerated over night. Yoghurt samples were then analysed for insecticide residues.

Statistical analysis:

Data achieved were statistically analysed according to Sendecor and Cochran (1980).

RESULTS

The obtained data are recorded in Tables 1, 2 & 3 .

DISCUSSION

1 - Occurrence of insecticide residues in milk:

1.1- Organochlorine:

The residual properties and fate of the chemical which make them effective for long periods, have been a cause of considerable importance in milk pollution. In the present study, it is clear from Table (1) that the organochlorine insecticide residues were detected in analysed milk samples at percentages of 86.76 , 79.41 , 91.18 , 88.24 and 52.94 % for p.p. DDT, p.p. DDD, p.p. DDE, Lindane and Dieldrin respectively. The concentration of p.p. DDT were ranged from 0.048 to 0.199 with a mean value of 0.093±0.023 p.p.m and p.p. DDD varied from 0.036 to 0.209 with an average value of 0.101±0.018 p.p.m. Also the concentrations of p.p. DDE were ranged from 0.011 to 0.288 with a mean value of 0.138±0.089 p.p.m. Moreover the average value of Lindane was 0.042±0.013 p.p.m and its quantities lie between 0.014 to 0.062 p.p.m but the mean value of Dieldrin was 0.095±0.030 p.p.m and its residues varied from 0.06 to 0.168 p.p.m.

It is obvious from above mentioned results a comparatively high incidence of organochlorine insecticide residues in milk samples but their

average levels are below the acceptable maximum residues levels of F.A.O & W.H.O (1972). These limits are 1.25 p.p.m for total DDT and its isomers, 0.20 p.p.m for Lindane and 0.15 p.p.m for Dieldrin including Aldrin. These results are nearly similar to those reported by Bluthgen et al. (1977), Enb, (1987), Abdrabo et al. (1989) and Ali et al. (1993). In contrast concentrations of some organochlorine compounds in milk in other countries and other localities in Egypt were found to be above the maximum residues limits set forth by the F.A.O and W.H.O as recorded by Kannan et al. (1992) and Amr et al. (1996).

These results indicated that Aldrin residues was not detected in all examined milk samples which may be explained by its continuous degradation into Dieldrin within living tissues through different metabolic processes (Dogheim et al., 1989).

1.2- Organophosphorus:

The analytical results listed in Table (1) revealed that Diazinon residues could be detected in 33.82% of examined milk samples ranging from 11.39 to 590.20 ug/L with a mean value of 120.8±27.9 ug/L. Such level is considered comparatively high and might be due to large scale application of this insecticide for the control of external parasites in livestock practice during this period of the year. These findings are nearly similar with those recorded by Baldl et al. (1979). On the other hand, higher concentrations of Diazinon residues in milk samples were recorded by experimental study of Kholif et al. (1994). However the Food and Agriculture Organization and World Health Organization (1972) stated that the acceptable daily intake for Diazinon residues in milk has been set at a maximum of 2 ug/Kg body weight. According, the average level of Diazinon in analysed milk samples still below the acceptable limit except seven samples (30.43 %) which exceed this limit.

Estimation of Malathion residues in milk samples revealed that 9.25% of the samples were contaminated with Malathion and its residues were varied from 0.051 to 0.660 ug/L with a mean value of 0.110 ug/L. These values are considered slightly higher than those reported by Abd-Alla et al. (1991).

Dursban could be detected in 19.12% of milk samples with a mean concentration value of 0.059±0.019 ug/L. These results are considered a good agreement with those obtained by El-Ghannam et al. (1987) who found that Dursban residues in milk after spraying stayed for beyond the toxic level

for human according to WHO (1973) which reported that the level of 0.03 mg/Kg body weight per day appear to be the minimal threshold response level in human to Dursban. Application of 0.014 mg/Kg body weight per day orally for one month is a level causing no harmful effect.

In connection with organophosphorus insecticide residues in milk Table(1) indicated that all analysed milk samples were free from detectable amounts of Parathion, Dimethoate and Lannate. In contrast a large number of reports including of Froslie et al. (1982), Stijve (1984), Camoni et al. (1990), and Abd-Alla (1991). Those indicated deposition of these residues and other pollutants in milk.

In this investigation the incidence of detected organochlorine residues in milk is comparatively high in comparison with organophosphorus insecticide residues. These results could be explained on the basis of organophosphorus insecticides have an advantage over organochlorine insecticides that they are rapidly decomposed by physicochemical processes in the environment as well as by enzymatic processes in the animal body which prevents the building up of significant residues in milk (Downey 1971). Moreover, most of these compounds are water soluble, therefore, the animals secrete most of them in urine and faeces (I.D.F. 1979). Consequently, a little or no residues would be secreted in milk. On the other hand, organochlorine are very stable compounds resulted in extensive environmental pollution. These compounds have a high fat / water partition coefficient (Abbott et al. 1986). Consequently these materials accumulated in adipose tissue of milking animals hence high residues would be secreated in milk.

2 - Effect of common heat treatments on the levels of natural insecticide residues in milk.

2.1- Organochlorine:

Table (2) revealed that reduction percentage of each p.p DDT and p.p DDE residues as result of pasteurization was 29.79 and 21.11%, respectively, while it was 71.28 & 70.35% due to boiling. On the other hand p.p DDD residues was increased at percentages of 26% and 64.8% due to pasteurization and boiling respectively. These results are in accordance with that mentioned by Abdrabo et al. (1989), and Amr et al. (1996). Moreover the increasing level of p.p DDD may be attributed to decomposing of p.p DDT & p.p DDE during thermal treatment into p.p DDD. The conversion of

p.p DDT by thermal treatment into p.p DDD has reported by many authors (Hiroko et al. 1971, Dick et al. 1978 and Heschen et al. 1978). It is evident from the data illustrated in Table (1) that Lindane and Dieldrin residues were thermostable against boiling and pasteurization. Similar finding was determined by Abdrabo et al. (1989).

2.2- Organophosphorus:

Table (2) showed that each of boiling and pasteurization had a significant effect in degradation of Diazinon, Malathion and Dursban residues where the reduction percentages as result of pasteurization were 70.54, 51.94 and 44.68% respectively. While values were 78.1, 56.11 and 55.32% due to boiling. These results are nearly similar to these reported by Leshchev et al. (1972). Also Fekry & Bahout (1992) recorded that each of boiling and pasteurization was effective in reducing Diazinon content in milk. In addition, Korolev (1987) investigated the efficiency of heating the milk to 40. 100 or 126 °C in water bath for 15 or 30 minutes for decontamination of milk from Methatim, Cyclophos, Sebacyl, Sulphidophos & Cgodrin as organophosphorus insecticides. He concluded that thermal treatment at 100 & 126 °C were effective in destruction of all examined organophosphorus insecticide residues at percentages of 93.3 % & 100 %, respectively, with exception of Sebacyl which showed only 34-40 % destruction.

3- Effect of yoghurt processing on the levels of natural insecticides residues in milk:

3.1- Organochlorine:

It is evident from the data illustrated in Table (3) that processing of milk into yoghurt reduced the content of organochlorine insecticides residues at percentages of 65.83%, 56.1% and 8.2% for DDT, Lindane and Dieldrin, respectively. These results are nearly similar to Ali et al. (1993). The slight reduction in Dieldrin residues may be attributed to unability of lactic acid bacteria to destruct this compound.

3.2- Organophosphorus:

Yoghurt processing reduced the insecticide residues content of milk by 51.27%, 47.37% & 61.22% for Diazinon, Malathion and Dursban, respectively. These findings are partially similar to those recorded by Gajduskova & Lat (1974).

The reduction in insecticide residues during yoghurt processing may be attributed to milk protein adsorption, bacterial cell incorporation and or to microbial degradation (Magdoub et al. 1989).

Generally the results obtained about the efficiency of lactic acid bacteria in the degradation of insecticides in milk are coincided with those reported by Serjeeva (1979) who indicated that polluted milk with insecticides should be preferably processed into yoghurt or white pickled cheese. However, a contradictory opinion was given by Mendez et al. (1979) who indicated no treatment ensure complete removal of insecticide residues.

In the light of the results obtained from the present study, it could be concluded that pollution of milk samples with various insecticide residues in different concentrations act as kind of human health hazard. The most dangerous problem of milk pollution with insecticides, is that it causes changes in milk component configuration, which give chance to be teratogenic substances to human consumption and cause a generation of unexpected diseases. Furthermore, some insecticides are known to accumulate in foetal tissues indicating placental transfer of these chemicals. Among the potential hazards effects of insecticides are carcinogenesis, renal failure, liver cirrhosis and optic nerve manifestation of special concern (Dixon, 1980 and Amr, 1992). Hence from hygienic health point of view, milk containing insecticide residues should be directed to industrial manufacturing as some of these compounds affected by boiling and pasteurization as well as by high acidity produced during processing of fermented milk.

From the toxicological point of view, milk is unable to escape from insecticide pollutants once dairy animals exposed to these compounds. Some of these chemicals are too much dangerous that usual method of removal from milk dose not affect their residues. For this we advice regulations and control on using of these insecticides in order to minimize or eliminate the hazard resulting from missusing of these compounds. We hope to restrict pollution of food and water supplied to dairy animals. Subsequently we recommend that if it is necessary to use a specific insecticide for veterinary or agricultural purposes, complete information must be available so that the risk to milk quality from residues can be directly evaluated. Also the condition under which residues will disappear from milk and the efficiency of milk processing as well as the permissible limit of insecticide residues must be known before its proposal.

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Tahble (1) : Residues levels of organochlorine and organophosphorus insecticides in buffaloes milk (p.p.m)

Type of insecticides	No. of Samples	No. of Positive	%	Range of concentration Minimum	Maximum	Mean +Standar error	Permissible limit of daily intake limit, ug/60 Kg B.W	FAO/WHO Limit in milk p.p.m	No. of positive samples exceeded the permissible	%
Organochlorine:										
p.p DDT	68	59	86.76	0.048	0.199	0.093+0.023	--			
p.p DDD	68	54	79.41	0.036	0.209	0.101+0.018	1200.0	1.25		
p.p DDE	68	62	91.18	0.011	0.208	0.130+0.089	--			
Lindane	68	60	88.24	0.014	0.062	0.042+0.013	600.0	0.20		
Dieldrin	68	36	52.94	0.06	0.168	0.095+0.038	6.0	0.15		
Aldrin	68	N.D								
Organophosphorus:										
Diazinon	68	23	33.82	11.39	590.208	120.8+27.9	2.0		7.0	30.43
Malathion	68	17	9.25	0.051	0.660	0.110+0.039				
Dursban	68	13	19.12	0.012	0.658	0.059+0.019	30.0			
Lannate	68	N.D								
Dimethoate	68	N.D								
Parathion	68	N.D								

Table (2) Effect of common heat treatments on the levels of insecticide residues in buffaloes milk (pp.m)

Type of treatments / Type insecticides	Raw milk Mean + S.E	Mean + S.E	Pastaurization Degradation %	increasing %	Mean + S.E	Boiling Degradation %	Increasing %
Organochlorine:							
p.p DDT	0.188+0.037	0.132+0.026	29.79		0.054+0.011	71.28	
p.p DDE	0.199+0.053	0.157+0.044	21.11		0.059+0.009	70.35	
p.p DDD	0.250+0.066	0.315+0.078		26%	0.412+0.098		64.8
Lindane	0.028+0.009	0.027+0.007			0.027+0.008		
Dieldrin	0.092+0.024	0.091+0.023			0.090+0.026		
Organophosphorus:							
Diazinon	455.300+15.91	134.138+11.21	70.54		99.850+9.66	78.1	
Malathion	0.360+0.089	0.173+0.053	51.94		0.158+0.086	56.11	
Dursban	0.047+0.012	0.026+0.008	44.68		0.021+0.007	55.32	

Table (3): Effect of yoghurt processing on insecticide residues level in boffaloe s milk

Type Insecicides	Raw milk Mean +S.E	Yoghurt Mean +S.E	Loss	Degradation %
Organochlorine:				
Total DDT	0.998+0.109	0.341+0.092	0.657	65.83
Lindane	0.041+0.013	0.018+0.005	0.023	56.1
Dieldrin	0.085+0.028	0.078+0.019	0.007	8.2
Organophosphorus				
Diazinon	511.20 +16.90	249.09 +21.60	262.11	51.27
Malathion	0.38 + 0.091	0.20 + 0.051	0.18	47.37
Dursban	0.049+ 0.014	0.019+ 0.06	0.030	61.22