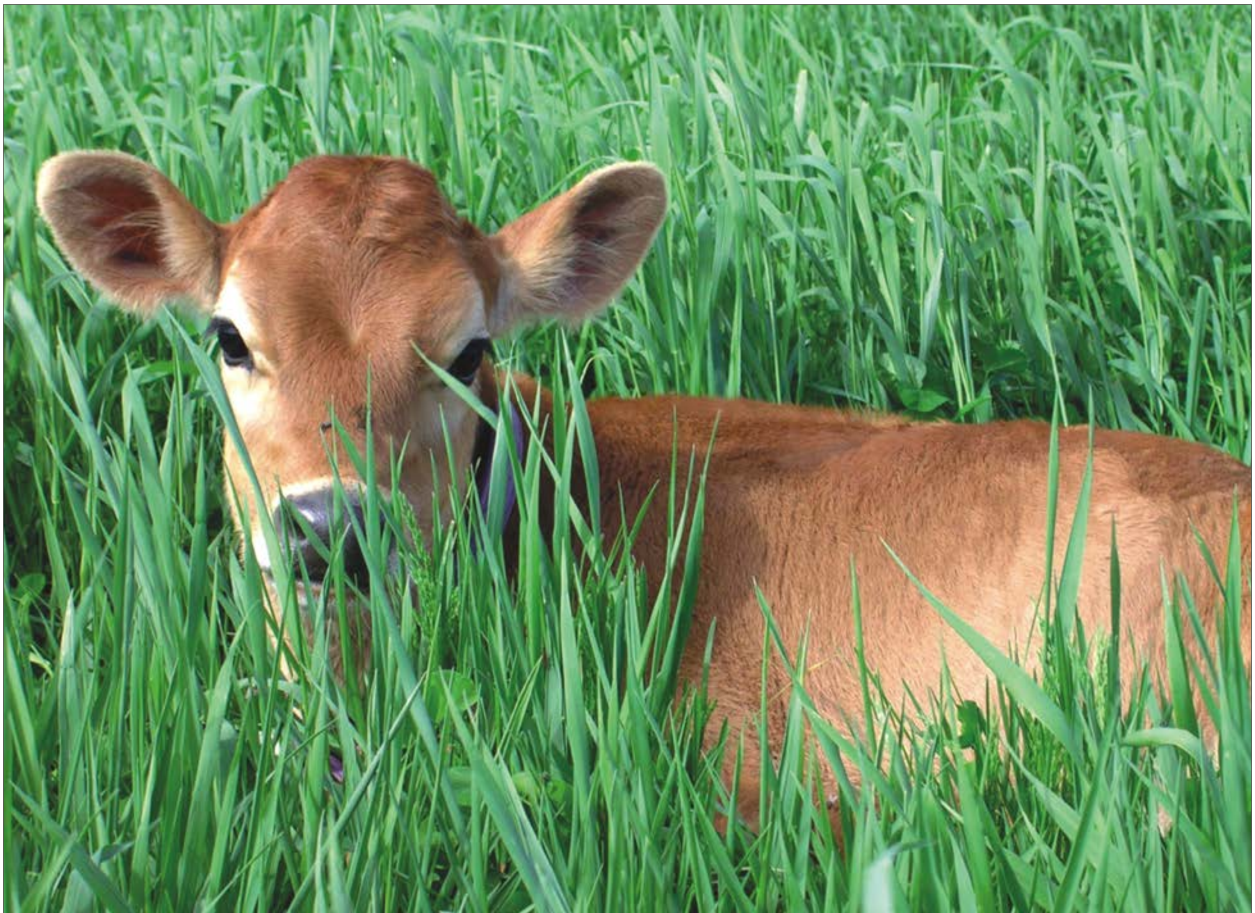

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Behavior of Certain Lactic Acid Bacteria in the Presence of Pesticides Residues

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ABSTRACT

Pesticides can pose risks and technological problems of dairy products if used improperly or frequently. The present work aims to investigate the effect of two fungicides (Anadol and Tasolen), herbicides (Round up and Saturn) and insecticides (Lannate and Reldan) commonly used in Egypt on certain lactic acid bacteria (*Streptococcus salivarius* subsp. *thermophilus* H, *Lactobacillus acidophilus* (Type 145), *Lactobacillus casei* subsp. *casei, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Bifidobacterium* spp. 420) and their impact on acid, acetaldehyde and diacetyl production by yogurt and ABT cultures. The concentrations of pesticides (1-10 ppm) were added to selective media immediately before inoculation with tested bacteria, incubated then colonies of bacteria were counted. The Pesticides were added to inoculated skim milk with tested bacteria then incubated, acidity development was determined. Yoghurt and ABT yoghurt were made in the presence of previous pesticides concentrations, acid, acetaldehyde and diacetyl production were estimated. The two insecticides caused the greatest harmful effect on acid production of most strains and acid, acetaldehyde and diacetyl production in yoghurt and ABT yoghurt followed by herbicides then fungicides, the effect of 2 ppm concentration on acetaldehyde, diacetyl and acid production was negligible reverse (8 and 10 ppm) concentrations. Nevertheless, *L. acidophilus* was more resistant to Anadol. While, *Bifidobacterium* spp. 420 was the most sensitive for Anadol and Tasolen. However, *L. bulgaricus* was the most resistant to Tasolen. *St. theromophilus* was the most sensitive for herbicides unlike *L. acidophilus*. *L. bulgaricus* surpassed other bacteria in tolerance insecticides, on the contrary, *Bifidobacteria* spp. exhibited excessive sensitive toward insecticides.

Key words: Pesticides, lactic acid bacteria, bacterial growth, acidity development, acetaldehyde and diacetyl

INTRODUCTION

In the last few years, the contamination of milk is considered as one of the main dangerous aspects. Milk can be contaminated by residues of organochlorine (OC) and organophosphorous (OP) pesticides (Fontcuberta *et al.*, 2008), through a variety of sources. The major source of (OC) residues is from fodder and soil, while for (OP) residues is mainly associated with ingestion (through licking) of insecticide used for controlling of parasites on animals (Snelson and Tuinstra, 1979; Waliszewski *et al.*, 1997). Pesticides are used for controlling weed, plant pests and diseases. As a result, their residues are transferred to milk (Ismail *et al.*, 1987). Pesticides have played an important role in the dramatic increases in agricultural productivity which has been achieved in the developed world over the last few decades. Production and use of organochlorine pesticides has been declining in recent years. This suggests that residue problems will decrease but the persistence of these materials in the environment means that some residues will be encountered for many years (Fries *et al.*, 1969). OC pesticides were widely used worldwide until restrictions were introduced in the late seventies both in Europe and the USA, initially for DDT (Fontcuberta *et al.*, 2008). Some of these pesticides are still widely used by farmers because of their effectiveness and their broad spectrum activity (Amoah *et al.*, 2006) and also, are being extensively used in tropical countries in malaria control programs and against livestock ectoparasites and agricultural pests (Curtis, 1994). Pesticide exposure independently or in synergism with modifiable risk factors, is recognized as an important environmental risk factor associated with hemopoetic cancers, cancers of the prostate, pancreas, liver and other body systems (Jaga and Dharmani, 2005). No entirely safe pesticides have been developed yet. It behooves us to emphasize that milk and dairy products can be contaminated by pesticides by improper handling and by feeding the animals on contaminated feeds. The misuse of any of such pesticides in dairy farms and for dairy industries may endanger the health of the consumer (Mitchell *et al.*, 1986). Besides presence of pesticides may lead to some technological problems. Many researchers studied growth rate and behavior of some bacterial starters in different of media containing insecticides. On the contrary, little information is available on the effect of fungicides, herbicides and insecticides on pure cultures of bacterial used in food and dairy industries. The present work was conducted to study the effect of two fungicides, herbicides and insecticides commonly used in Egypt, on the bacterial growth and lactic acid, acetaldehyde and diacetyl production of certain important lactic acid bacteria which are used in dairy industry.

MATERIALS AND METHODS

Bacterial strains: Lyophilized culture of *Streptococcus salivarius* subsp. *thermophilus* H, *Lactobacillus acidophilus* (type 145) and *Bifidobacterium* spp. 420 were obtained from Laboratorium wiesby, (2007) Niebull, Germany. *Lactobacillus delbrueckii* subsp. *burglarious*, *Lactobacillus casei* subsp. *casei*, yoghurt and ABT cultures were obtained from Chr. Hansen's Lab., Denmark (2008).

Milk and skimmed milk: Fresh buffaloes' milk were obtained from El-Serow Station for Animal Production Research and spray dried skim milk powder, low heat, of France origin was used during this study.

Pesticides: Six pesticides commonly used in Egypt were obtained from local market. Two fungicides, Anadol 80% WP {manganese ethylenebis (dithiocarbamate) polymeric complex with zinc salt} and Tazolen 72% WP {methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate and manganese ethylenebis (dithiocarbamate) polymeric complex with zinc salt}, two herbicides, Round up 48% WSC {N-(phosphonomethyle) glycine, isopropylammonium} and Saturn 50% (5-4 chlorobenzyl diethylthiocarbamate) and two insecticides, Lannate 90% SP {5-methyl N-(methyl carbamoyloxy) thioacetimidate} and Reldan 50% EC {0-0 diethyl 0-(3, 5, 6 trichloro-2-pyridyl)-phosphorothioate.

Preparation of solution pesticides: A stock solution for each pesticide in 5% (v/v) ethanol was prepared. Samples (media or milk) were artificially contaminated by adding sufficient stock solution, distilled water and by serial concentrations were used to give desired concentrations.

Effect of pesticides residues on growth certain lactic acid bacteria: This was determined by plate count according to Elliker *et al.* (1956). Separately pesticides were added to selective media immediately before inoculation with bacteria under study at concentrations 0.0, 1, 2, 3, 4, 5, 7 and 10 ppm.

Effect of pesticides residues on the acid production by certain lactic acid bacteria
a-Single strains of lactic acid tested bacteria: Reconstituted skimmed milk powder (11%TS) was filled in 100 mL lots into 250 mL conical flasks and autoclaved at 121°C/15 min. The pesticides (0.0, 2, 4, 6, 8 and 10 ppm) were added under sterile conditions to each flask. High activity cultures of bacteria under study were separately inculcated at level 2% into the flasks containing pesticides to give initial appropriate counts. The All flasks were incubated at 37-40°C with the exception of those *Bifidobacterium* sp. was incubated anaerobically. During this period the titratable acidity was determined as shown in Table 4-8.

In traditional and ABT yoghurt: Fresh buffaloes' milk (standardized 3%fat) was divided into thirty two 200 mL aliquots. Two served as a control, five 200 mL aliquots were contaminated with Anadol at levels 2, 4, 6, 8 and 10 ppm, this procedure has been pursued in the other pesticides (Tasolen, Round up, Saturn, Lannate and Reldan), all 32 (200-aliquots) were heated to 63°C for 30 min. and subsequently cooled to 40°C followed by addition 2% of traditional (zabady) culture. All treatments were incubated at 40°C. The same work was done in ABT yoghurt. Titratable acidity was determined as shown in Table 9 and 10, acetaldehyde and diacetyl were determined on the sixth day of cold storage.

Bacterial count: Bifidobacteria were enumerated according to Dave and Shah (1996) using modified MRS agar supplemented with 0.05% L-cystein and 0.3% lithium chloride. The plates anaerobically incubated at 37°C for 48 h.

Lctobacilli: *L. acidophilus* was enumerated according to Gilliland and Walker (1990) using modified MRS agar supplement with 0.2% Oxagal. The plates incubated at 37°C for 48 h. *L. bulgaricus* and *L. casei* counts were determined using MRS agar according to De Man *et al.* (1960) and the plates incubated at 40°C for 48 h.

***St. theromophilus*:** *St. theromophilus* count was determined using M17 agar (Terzaghi and Sandine, 1975). The plates incubated at 37°C for 48 h.

Chemical analysis
Titratable acidity: Titratable acidity expressed as lactic acid (%) was determined according to Ling (1963).

Determination of acetaldehyde: Acetaldehyde was estimated as given by Lees and Jago (1969) using Conway micro diffusion-semicarbazide method. One milliliter of micromole semicarbazide solution was pipetted in inner wall of Conway micro diffusion cell. Three grams of the sample were rapidly pipetted the outer and the cell was covered and placed in an incubator at 30°C for 90 min. The solution in the inner wall transferred to 10 mL volumetric flask and made up to volume. The absorption was measured at 224 nm using type CE 595 Double Beam Digital U.V.

Spectro-photometer. The concentration of acetaldehyde was calculated from standard curve of acetaldehyde solution ranging from 1 μmol to 20 μmol/100 mL mixed with raw milk and treated as samples.

Determination of diacetyl: Diacety was estimated as described by Westerfeld (1945) with some modification: five grams of samples were mixed with 15% trichloroacetic acid solution to 50 mL; the solution was filtered then through Whatman No. 42 filter paper. The filtrate was readjusted to the same pH as before, then 1 mL filtrate was mixed with 4 mL distilled water, 1 mL of 0.5% creatine and 1 mL 5% alpha-naphthol solution (in 2.5 N-NaOH) after 10 min at room temperature, the optical density was measured at 540 nm after 10 min at room temperature. The optical density was measured at 540 nm after 10 min using Double Beam Digital U.V. Spectro photometer type (E595). The diacetycontent was estimated from a standard calibrated curve for solution containing known concentration of pure diacetyl.

RESULTS AND DISCUSSION

Effect of pesticides on bacterial growth: Initially, growth all studied lactic acid bacteria was inhibited by pesticides. The inhibition increased with increasing concentration of pesticides. Table 1-3 explain that all studied strains were strongly affected by the presence of fungicides, herbicides and insecticides pesticides. From Table 1 it can be observed that *L. acidophilus* was more resistant to Anadol followed *L. casei, St. theromophilus, L. bulgaricus* then *Bifidobacterium* spp. which was the most inhibition of Anadol and Tasolen. However, *L. bulgaricus* was more resistant than other cultures in the existence Tasolen. Generally, the last fungicide was more inhibition of tested lactic acid bacteria than Anadol. Dhanalakshmi *et al.* (1997) reported that *L. acidophilus*

Table 1: Count of lactic acid bacteria in the present fungicides

	Count of lactic acid bacteria				
Concentration of fungicides	B. sp. $\times 10^6$	L.a $\times 10^5$	L.b $\times 10^7$	L. c $\times 10^6$	St. th $\times 10^7$
Anadol					
0.0	21.0	22.3	23.1	19.0	16.3
1	20.0	21.4	22.5	18.4	16.0
2	19.2	20.8	21.7	17.6	15.5
3	17.1	19.3	20.5	16.4	14.6
4	15.7	18.1	18.8	15.2	13.6
5	11.0	14.0	15.0	11.1	9.9
7	8.1	11.5	12.3	8.1	7.5
10	3.0	10.0	11.2	7.0	5.9
Tasolen					
0.0	21.0	22.3	23.1	19.0	16.3
1	20.0	21.2	22.1	18.5	16.1
2	18.3	20.4	21.2	17.2	15.1
3	17.1	19.0	20.0	15.9	14.0
4	15.0	18.0	18.8	14.6	13.0
5	10.5	13.6	14.5	9.9	8.9
7	7.2	10.9	12.0	7.9	6.8
10	2.3	9.3	10.0	5.8	4.4

B. sp.: *Bifidobacterium* sp.; L. a: *Lactobacillus acidophilus*; L. b: *Lactobacillus delbrueckii* subsp. *burglarious*; L. c: *Lactobacillus casei* subsp. *casei*; St. th: *Streptococcus salivarius* subsp. *thermophilus*. These results the average of three replicates

Table 2: Count of lactic acid bacteria in the present herbicides

	Count of lactic acid bacteria				
Concentration of herbicides	B. sp.×10^6	L .a×10^5	L .b×10^7	L .c×10^6	St .th×10^7
Round up					
0.0	21.1	22.2	23.0	19.3	16.3
1	20.2	21.6	22.7	18.7	16.0
2	19.0	21.1	21.2	17.5	15.2
3	16.7	17.8	18.7	15.0	14.5
4	15.3	16.3	17.7	13.2	12.8
5	10.9	13.9	15.3	10.7	8.0
7	7.8	10.5	12.0	8.3	5.6
10	5.5	8.5	6.8	4.9	3.7
Saturn					
0.0	21.1	22.2	23.0	19.4	16.2
1	20.1	22.0	22.7	19.0	15.7
2	18.9	20.4	21.5	17.8	15.0
3	17.0	18.2	18.7	15.5	13.5
4	14.5	16.3	18.1	14.1	8.8
5	10.6	14.3	15.9	11.1	6.5
7	8.1	10.9	12.5	8.6	5.0
10	5.8	8.9	7 .3	5.0	4.1

B. sp.: *Bifidobacterium* sp.; L. a: *Lactobacillus acidophilus*; L. b: *Lactobacillus delbrueckii* subsp. *Burglarious*; L. c: *Lactobacillus casei* subsp.*casei*; St. th: *Streptococcus salivarius* subsp. *thermophilus*. These results the average of three replicates

Table 3: Count of lactic acid bacteria in the present of insecticides

Concentration of insecticides		Count of lactic acid bacteria				
		B. spp. ×10^6	L.a ×10^6	L.b ×10^7	L.c ×10^6	St.th ×10^7
Lannate	0	21.0	22.2	24.3	19.1	16.3
	1	20.0	21.3	22.3	18.0	16.1
	2	18.8	19.6	20.8	15.9	15.0
	3	16.1	17.5	19.3	14.1	14.1
	4	14.7	16.3	17.6	13.0	12.5
	5	10.2	13.2	14.5	9.2	7.1
	7	7.0	10.0	11.1	6.7	4.9
	10	--	8.1	9.2	4.8	3.0
Reldan	0	21.1	22.1	23.3	19.3	16.2
	1	20.8	21.5	22.4	18.5	16.2
	2	18.6	20.0	21.2	16.8	15.0
	3	16.3	18.0	18.9	15.1	14.4
	4	14.8	16.7	17.5	13.8	13.1
	5	10.3	14.0	15.2	11.3	9.3
	7	7.2	11.1	12.3	8.5	6.3
	10	--	9.1	10.3	3.2	3.9

B. spp: *Bifidobacterium* spp.; L.a: *Lactobacillus acidophilus*; L.b: *Lactobacillus delbrueckii* subsp. *Burglarious*; L.c: *Lactobacillus casei* subsp. *casei*; St.th: *Streptococcus salivarius* subsp. *Thermophilus*; -: not detected. These results the average of three replicates

was found the most sensitive to Lindane unlike our results concerning *L. acidophilus*. Results of Ismail *et al*. (1987) may explain our results; they mentioned that no morphological changes in bacterial cell of *L. acidophilus* and *L. casei* were observed post incubation with Aldicarb LC50 for 90 min.

All tested lactic acid bacteria were more sensitivity herbicides than fungicides with the exception *Bifidobacterium* sp. On the other hand, round up was more destructive to all tested strains than Saturn. However, *St. theromophilus* was the most sensitive to herbicides followed by *L. casei, Bifidobacterium* spp., *L. bulgaricus* then *L. acidophilus* which recorded the highest resistance among the tested bacteria (Table 2). The obtained results agreed with Zidan *et al.* (1990) mentioned that *St. theromophilus* had the highest sensitivity, i.e., the lowest growth in the presence of Fenvalerate and DDT.

According to data in Table 3, insecticides were the most inhibition lactic acid bacteria compared to herbicides or fungicides. Lactobacilli genera showed more resistant than other bacteria. Nevertheless *L. bulgaricus* surpassed other bacteria in tolerance insecticides; on the contrary, *Bifidobacterium* sp. exhibited excessive sensitive toward insecticides. Present results correspond with Zidan *et al.* (1990) attested to that *L. bulgaricus* appeared to be more tolerant to the organochlorine insecticides (DDT). Moreover, *L. helveticus was* the most resistant strain to the pyrethroid Fenvalerate. In that way Cabras *et al.* (1994) affirmed that *L. plantarum* resisted effectiveness Dichlofluanid.

Effect of pesticides on acidity development by single strain of lactic acid bacteria: Variation either in type or concentration of pesticides affected the lactic acid production by most strains studied. Inhibition effect of lactic acid production by lactic acid bacteria appeared at more than 2 ppm concentration of Lindane and Endrin (Abdou *et al.,* 1983). Therefore, 2, 4, 6, 8 and 10 ppm concentrations of pesticides were used in our study. Generally, as the incubation period progressed, the Titratable Acidity (TA) increased in all samples. Noteworthy, insecticides were the most obstruction on lactic acid production by all organisms studied followed by herbicides then fungicides. With regard to acidity development by *Bifidobacterium* sp. in sterilized skim milk containing different amount of pesticides, data presented in Table 4 represent that the effect of 2 ppm of all pesticides didn't clearly affect on Acidity Development (AD) compared with control except Lannet. Concentrations of all pesticides higher than 2 ppm influenced gradually on (TA) by *Bifidobacterium* sp. (AD) was moderate In the presence of 4 and 6 ppm concentrations of fungicides but 8 and 10 ppm concentrations were High Impact. The same trend appeared in other treatments except 6, 8 and 10 ppm violently slowed (AD) especially in the presence of (Lannet and Reldan). Finally, (A.D.) by *Bifidobacterium* spp. was extremely slow compared to other strains. These results might be due to the highly sensitivity of *Bifidobacterium* spp. toward presence of tested pesticides.

In this regard, must not forget it is well Known, technological problems have arisen with using the therapeutic bacteria (*Bifidobacterium* spp, *L. acidophilus* and *L. casei*) in preparing fermented milks, particularly, the relatively long time needed for obtaining a satisfactory yoghurt coagulum (Mahmoud, 1999). Obviously from Table 5 both insecticides were more effect on (AD) by *L. acidophilus* than fungicides or herbicides. The effect of inhibition extremely appeared at 8 and 10 ppm concentrations of fungicides or herbicides. However, in the presence of (Lannet and Reldan) *L. acidophilus* was very slow on lactic acid production after 4 ppm also. The effect of both fungicides on (A.D.) by *L. acidophilus* was alike as did both herbicides, but Reldan had a less effect than Lannet in the same way, these findings are in agreement with those found by Zidan *et al.* (1990) who reported that presence of Fenvalerate, Malathion and DDT redused acid production by *L. helveticus* by 17, 9 and 20% at the end of incubation period.

Table 4: Acidity development by *Bifidobacterium* spp. in sterilized skimmed milk containing pesticides

			Concentration of fungicides (ppm)						Concentration of herbicides (ppm)				
Time (h)	Fungicides	Control	2	4	6	8	10	Herbicides	2	4	6	8	10
0	Anadol	0.17	0.18	0.17	0.17	0.18	0.18	Round up	0.18	0.17	0.17	0.18	0.18
4		0.24	0.20	0.19	0.19	0.19	0.18		0.20	0.19	0.19	0.18	0.18
8		0.42	0.39	0.28	0.25	0.20	0.19		0.36	0.26	0.22	0.2	0.19
12		0.58	0.45	0.43	0.38	0.25	0.23		0.54	0.41	0.35	0.22	0.20
16		0.65	0.60	0.53	0.50	0.37	0.32		0.63	0.57	0.50	0.35	0.28
20		-	-	0.63	0.58	0.48	0.43		-	0.60	0.60	0.44	0.40
24		-	-	-	-	0.56	0.48		-	-	0.64	0.55	0.44
0	Tasolen	0.17	0.17	0.18	0.18	0.18	0.17	Saturn	0.18	0.18	0.18	0.18	0.18
4		0.24	0.20	0.18	0.19	0.19	0.18		0.22	0.20	0.20	0.19	0.19
8		0.42	0.38	0.26	0.22	0.2	0.19		0.38	0.24	0.23	0.21	0.20
12		0.55	0.45	0.40	0.30	0.23	0.20		0.56	0.37	0.37	0.32	0.23
16		0.65	0.57	0.55	0.50	0.33	0.26		0.65	0.53	0.50	0.37	0.35
20		-	-	0.59	0.55	0.42	0.40		-	0.62	0.61	0.48	0.44
24		-	-	-	-	0.52	0.46		-	-	0.64	0.59	0.47

		Concentration of insecticides (ppm)				
Time (h)	Insecticides	2	4	6	8	10
0	Lannet	0.18	0.18	0.17	0.18	0.18
4		0.20	0.19	0.19	0.19	0.18
8		0.33	0.23	0.20	0.20	0.19
12		0.50	0.38	0.35	0.20	0.20
16		0.55	0.51	0.42	0.30	0.28
20		-	0.56	0.44	0.40	0.30
24		-	-	0.49	0.44	0.37
0	Reldan	0.18	0.18	0.18	0.18	0.18
4		0.22	0.20	0.20	0.19	0.18
8		0.35	0.24	0.22	0.20	0.19
12		0.53	0.41	0.37	0.21	0.22
16		0.59	0.53	0.44	0.32	0.30
20		-	0.58	0.47	0.43	0.34
24		-	-	0.51	0.46	0.40

These results the average of three replicates. -: Did not estimate

The results presented in Table 6 clearly indicate that *L. bulgaricus* behaved the same way *L. acidophilus* in the presence of both fungicides, but Round up and Lannet were more delay on lactic acid production than Reldan and Saturn. Interesting observation that differences in (TA) were little at the end of fermentation period, As well, these results indicate that the all tested pesticides had a slight effect in less than 4ppm concentration, which confirms previous results. Though Fenvalerate increased acid production by *L. bulgaricus* (17%) particularly after 72 h incubation and uptill the end of the incubation period (120 h) on the contrary acid production by *L. bulgaricus* reduced in the presence of DDT and Malathion by between 12 to 44% (Zidan *et al.*, 1990).

From preceding results became plain that *L. casei* was mild resistant for all tested pesticides, materialized that from results recorded in Table 7 whereas, the effect of the two fungicides on lactic acid production were similar such as the effect of the two herbicides and both of insecticides

Table 5: Acidity development by *L .acidophilus* in sterilized skimmed milk containing pesticides

			Concentration of fungicides (ppm)						Concentration of herbicides (ppm)				
Time (h)	Fungicides	Control	2	4	6	8	10	Herbicides	2	4	6	8	10
0	Anadol	0.18	0.18	0.18	0.18	0.18	0.18	Round up	0.18	0.18	0.18	0.18	0.18
4		0.36	0.30	0.30	0.30	0.25	0.22		0.30	0.30	0.28	0.22	0.22
8		0.55	0.51	0.44	0.39	0.35	0.31		0.52	0.41	0.33	0.30	0.30
12		0.68	0.64	0.57	0.49	0.41	0.35		0.58	0.53	0.45	0.37	0.35
14	-	-	-	-	0.48	0.41	-		-	-	-	0.44	0.41
0	Tasolen	0.17	0.17	0.18	0.18	0.18	0.17	Saturn	0.18	0.18	0.18	0.18	0.18
4		0.33	0.30	0.30	0.28	0.25	0.20		0.32	0.30	0.30	0.24	0.20
8		0.50	0.47	0.41	0.36	0.32	0.28		0.48	0.41	0.35	0.32	0.33
12		0.60	0.58	0.5	0.45	0.38	0.32		0.60	0.53	0.46	0.4	0.39
14		-	-	-	-	0.45	0.41		-	-	-	0.45	0.42

		Concentration of insecticides (ppm)				
Time (h)	Insecticides	2	4	6	8	10
0	Lnnet	0.18	0.18	0.18	0.18	0.18
4		0.26	0.25	0.22	0.2	0.20
8		0.45	0.38	0.30	0.24	0.22
12		0.55	0.50	0.38	0.34	0.31
14		-	-	0.49	0.42	0.40
0	Reldan	0.18	0.18	0.18	0.18	0.18
4		0.28	0.26	0.24	0.22	0.20
8		0.45	0.38	0.33	0.28	0.26
12		0.58	0.54	0.40	0.38	0.34
14		-	-	0.53	0.45	0.41

These results the average of three replicates; -: Did not estimated

Table 6: Acidity development by *L. bulgaricus* in sterilized skimmed milk containing pesticides

			Concentration of fungicides (ppm)						Concentration of herbicides (ppm)				
Time (h)	Fungicides	Control	2	4	6	8	10	Herbicides	2	4	6	8	10
0	Anadol	0.19	0.19	0.18	0.19	0.19	0.19	Round up	0.19	0.19	0.19	0.19	0.19
2		0.51	0.47	0.35	0.28	0.25	0.22		0.51	0.41	0.33	0.24	0.20
4		0.71	0.62	0.58	0.49	0.41	0.33		0.68	0.60	0.50	0.37	0.30
6		-	-	-	0.58	0.52	0.48		-	-	0.55	0.47	0.44
0	Tasolen	0.19	0.19	0.19	0.19	0.19	0.19	Saturn	0.19	0.19	0.19	0.19	0.19
2		0.53	0.45	0.32	0.25	0.23	0.20		0.52	0.42	0.35	0.29	0.24
4		0.72	0.60	0.55	0.44	0.38	0.31		0.72	0.63	0.57	0.40	0.35
6		-	-	-	0.54	0.50	0.48		-	-	-	0.50	0.48

		Concentration of insecticides (ppm)				
Time (h)	Insecticides	2	4	6	8	10
0	Lannet	0.19	0.19	0.19	0.19	0.19
2		0.37	0.28	0.22	0.22	0.2
4		0.61	0.51	0.37	0.31	0.33
6		-	-	0.48	0.42	0.38
0	Reldan	0.19	0.19	0.19	0.19	0.19
2		0.4	0.33	0.25	0.23	0.21
4		0.63	0.53	0.42	0.36	0.35
6		-	-	0.51	0.46	0.41

These results the average of three replicates; -: Did not estimated

Table 7: Acidity development by *L. casei* in sterilized skimmed milk containing pesticides

			Concentration of fungicides (ppm)						Concentration of herbicides (ppm)				
Time (h)	Fungicides	Control	2	4	6	8	10	Herbicides	2	4	6	8	10
0	Anadol	0.18	0.18	0.18	0.18	0.18	0.18	Round up	0.18	0.18	0.18	0.18	0.18
2		0.28	0.26	0.25	0.20	0.20	0.19		0.24	0.22	0.20	0.20	0.19
4		0.42	0.38	0.32	0.28	0.22	0.20		0.34	0.30	0.25	0.22	0.22
6		0.63	0.58	0.50	0.43	0.38	0.33		0.58	0.53	48.0	0.40	0.35
7		-	-	-	-	0.48	0.44		-	-	-	0.48	0.42
0	Tasolen	0.18	0.18	0.18	0.18	0.18	0.18	Saturn	0.18	0.18	0.17	0.18	0.18
2		0.29	0.27	0.25	0.20	0.20	0.19		0.25	0.24	0.21	0.20	0.19
4		0.41	0.40	0.34	0.28	0.23	0.21		0.36	0.33	0.26	0.24	0.22
6		0.61	0.59	0.50	0.43	0.39	0.34		0.60	0.53	0.50	0.43	0.36
7		-	-	-	-	0.49	0.44		-	-	-	0.48	0.43

		Concentration of insecticides (ppm)				
Time (h)	Insecticides	2	4	6	8	10
0	Lannet	0.18	0.18	0.18	0.18	0.18
2		0.23	0.22	0.2	0.2	0.19
4		0.33	0.28	0.23	0.21	0.2
6		0.56	0.51	0.43	0.33	0.28
7		-	-	-	0.41	0.36
0	Reldan	0.18	0.18	0.18	0.18	0.17
2		0.24	0.22	0.21	0.21	0.20
4		0.35	0.28	0.24	0.23	0.28
6		0.56	0.55	0.46	0.35	0.32
7		-	-	-	0.44	0.39

These results the average of three replicates; -: Did not estimated

approximately. Another noteworthy observation that (AD) by *L. casei* was affected by 8 and 10 ppm concentrations of all pesticides more pronounced than others. These findings conflicted with Kim and Harmon (1968) who confirmed that growth and fermentative ability of *L. casei* not affected by Methoxychlor in the milk.

In the case of *St. thermophiles*, Table 8 indicated that the samples without pesticides (control) had 0.72% (TA) after 5 h of incubation, this value gradually decreased with increasing of fungicides concentrations, this strain produced adequate quantity of lactic acid after 5 h except in the presence of 10 ppm of fungicides, this harmonious with bacterial growth experiment. On the completely contrary in the presence of herbicides whereas it was the most sensitive in the bacterial growth experiment, herbicides clearly delayed acid production by *St. thermophiles*. Presence of insecticides delayed acid production by that strain though it was more capability in acid production than other studied strains at high concentrations. Worth mentioning (AD) by *St. thermophiles* slightly affected by Tasolen, Round up and Lannet more than others. These results may be due to the different in the growth media (Synthetic growth medium M17 or milk). The results agreed with (Zidan *et al.*, 1990) they reported that Fenvalerate, DDT and Malathion decreased acid production by between 12 to 31% by *St. lactis* and *St. thermophiles*, also our results compatible with Ismet *et al.* (2004) who announced that the spores of the bacteria are sensitive to chemical pesticides.

Table 8: Acidity development by *St. thermophiles* in sterilized skimmed milk containing pesticides

			Concentration of fungicides (ppm)						Concentration of herbicides (ppm)				
Time (h)	Fungicides	Control	2	4	6	8	10	Herbicides	2	4	6	8	10
0	Anadol	0.18	0.18	0.18	0.18	0.18	0.18	Round up	0.18	0.18	0.18	0.18	0.18
2		0.36	0.33	0.30	0.28	0.26	0.24		0.35	0.32	0.30	0.24	0.22
4		0.58	0.55	0.51	0.48	0.41	0.33		0.55	0.46	0.44	0.38	0.30
5		0.72	0.69	0.64	0.57	0.52	0.46		0.69	0.65	0.56	0.45	0.42
6		-	-	-	-		-	Saturn	-	-	-	0.52	0.43
0	Tasolen	0.17	0.17	0.18	0.18	0.18	0.17		0.18	0.18	0.17	0.18	0.18
2		0.36	0.31	0.29	0.25	0.23	0.21		0.35	0.34	0.33	0.26	0.22
4		0.57	0.53	0.48	0.46	38.0	0.30		0.57	0.51	0.48	0.38	0.31
5		0.72	0.68	0.61	0.53	0.47	0.44		0.70	0.67	0.60	0.53	0.44
6		-	-	-	-	-	-		-	-	-	0.56	0.45

		Concentration of insecticides (ppm)				
Time (h)	Insecticides	2	4	6	8	10
0	Lannet	0.18	0.18	0.18	0.18	0.18
2		0.32	0.30	0.28	0.22	0.20
4		0.48	0.42	0.38	0.33	0.28
5		0.63	0.60	0.52	0.42	0.39
6		-	-	-	0.52	0.46
0	Reldan	0.18	0.18	0.18	0.18	0.17
2		0.33	0.31	0.30	0.23	0.20
4		0.49	0.44	0.40	0.35	0.31
5		0.65	0.62	0.55	0.46	0.41
6		-	-	-	0.54	0.48

These results the average of three replicates; -: Did not estimated

Behavior yoghurt and ABT culture in the presence of pesticides: In the terminal section of this study, yoghurt and ABT yoghurt were made in the presence of (0.0, 2, 4, 6, 8 and 10 ppm) concentrations of pesticides (AD) and flavor compounds (acetaldehyde and diacetyle) were determined. From Table 9 progressive increase in (AD) occurred with time of incubation in all samples. Also, it is interesting to note that insecticides (Lannet and Reldan) delayed the acid production by yoghurt culture more than herbicides and fungicides, quaint that (TA) was higher in the presence of herbicide (Saturn) than other pesticides contrary to Lannet in the end of incubation period. Presence 2 ppm of all pesticides didn't retard acid production except Lannet, Presence 8 and 10 ppm of all pesticides had a violent effect. These findings correspond with Abdou *et al.* (1983), they attested to Lindane retarded (AD) at each stage. Lindane and Endrin caused little effect until their concentration reached 0.5 ppm.

Regarding ABT culture, control treatment reached 0.65% (TA) after 6 h Table 10, this quantity gradually decreased with incubation time or increasing pesticides concentration in the presence all studied pesticides but after 8ppm caused a sharp decreased after 7 h. Anther noteworthy observation, the variations between two fungicides, herbicides and insecticides were negligible. Furthermore ABT culture affected by presence tested pesticides more than yoghurt culture.

From The results of experiments of acidity development by the single strain and these findings in the current point appeared that pasteurization had not apparent impact on pesticides destroyed, these results agreed with Dhanalakshmi *et al.* (1997).

Table 9: Acidity development by yoghurt culture in milk (standardized 3%fat) in the presence of pesticides

			Concentration of fungicides (ppm)						Concentration of herbicides (ppm)				
Time (h)	Fungicides	Control	2	4	6	8	10	Herbicides	2	4	6	8	10
0	Anadol	0.17	0.17	0.17	0.17	0.17	0.17	Round up	0.17	0.17	0.17	0.17	0.17
1		0.22	0.2	0.18	0.18	0.18	0.17		0.2	0.2	0.19	0.18	0.18
2		0.31	0.29	0.26	0.25	0.22	0.19		0.28	0.25	0.21	0.21	0.2
3		0.53	0.48	0.45	0.4	0.38	0.33		0.46	0.42	0.38	0.34	0.29
4		0.71	0.66	0.52	0.47	0.42	0.38		0.65	0.51	0.44	0.39	0.35
0	Tasolen	0.17	0.17	0.17	0.17	0.17	0.17	Saturn	0.17	0.17	0.17	0.17	0.17
1		0.22	0.19	0.18	0.18	0.18	0.18		0.2	0.18	0.19	0.18	0.18
2		0.31	0.27	0.24	0.22	0.2	0.19		0.29	0.24	0.22	0.23	0.2
3		0.53	0.45	0.43	0.38	0.34	0.27		0.46	0.42	0.4	0.36	0.31
4		0.71	0.62	0.48	0.44	0.39	0.34		0.69	0.53	0.46	0.42	0.39

		Concentration of insecticides (ppm)				
Time (h)	Insecticides	2	4	6	8	10
0	Lannet	0.17	0.17	0.17	0.17	0.17
1		0.19	0.19	0.19	0.18	0.18
2		0.25	0.23	0.22	0.2	0.19
3		0.38	0.35	0.33	0.28	0.25
4		0.56	0.44	0.4	0.37	0.3
0	Reldan	0.17	0.17	0.17	0.17	0.17
1		0.2	0.21	0.2	0.18	0.18
2		0.23	0.24	0.24	0.22	0.2
3		0.41	0.38	0.36	0.3	0.27
4		0.59	0.46	0.43	0.41	0.37

Table 10: Acidity development by ABT yoghurt culture in milk (standardized 3%fat) in the presence of pesticides

			Concentration of fungicides (ppm)						Concentration of herbicides (ppm)				
Time (h)	Fungicides	Control	2	4	6	8	10	Herbicides	2	4	6	8	10
0	Anadol	0.17	0.17	0.17	0.17	0.17	0.17	Round up	0.17	0.17	0.17	0.17	0.17
2		0.22	0.21	0.18	0.18	0.18	0.18		0.2	0.20	0.19	0.18	0.18
4		0.37	0.33	0.28	0.25	0.22	0.19		0.28	0.25	0.23	0.20	0.20
6		0.65	0.49	0.42	0.38	0.36	0.33		0.44	0.41	0.41	0.30	0.26
7		-	0.65	0.55	0.48	0.42	0.38		0.58	0.50	0.45	0.39	0.3
0	Tasolen	0.17	0.17	0.17	0.17	0.17	0.17	Saturn	0.17	0.17	0.17	0.17	0.17
2		0.22	0.21	0.18	0.18	0.18	0.18		0.20	0.18	0.19	0.18	0.18
4		0.33	0.30	0.24	0.23	0.20	0.19		0.29	0.24	0.24	0.22	0.20
6		0.64	0.45	0.41	0.36	0.34	0.29		0.46	0.42	0.43	0.32	0.27
7		-	0.62	0.51	0.46	0.4	0.37		0.58	0.51	0.46	0.41	0.32

		Concentration of insecticides (ppm)				
Time (h)	Insecticides	2	4	6	8	10
0	Lannet	0.17	0.17	0.17	0.17	0.18
2		0.20	0.20	0.19	0.18	0.18
4		0.28	0.25	0.22	0.19	0.19
6		0.43	0.40	0.33	0.25	0.22
7		0.55	0.50	0.41	0.33	0.25

Table 10: Continued

		Concentration of insecticides (ppm)				
Time (h)	Insecticides	2	4	6	8	10
0	Reldan	0.17	0.17	0.17	0.17	0.18
2		0.21	0.20	0.19	0.18	0.18
4		0.3	0.26	0.23	0.19	0.21
6		0.45	0.42	0.35	0.27	0.25
7		0.55	0.52	0.43	0.35	0.27

Table 11: Values of acetaldehyde and diacetyl by yoghurt culture in the presence of pesticides on the 6th day from cold storing at 6-8°C

	Concentration of pesticides											
	Control		2 ppm		4 ppm		6 ppm		8 ppm		10 ppm	
Pesticides	Ace	Dia	Ace	Dia	Ace	Dia	Ace	Dia	Ace	Dia	Ace	Dia
Fungicides												
Anadol	290	108	270	101	248	91	208	79	168	62	112	41
Tasolen	290	109	262	95	240	85	196	66	160	58	105	38
Herbicides												
Round up	285	109	260	99	240	85	201	70	160	51	102	36
Saturn	288	108	263	102	243	87	205	73	162	55	104	36
Insecticides												
Lannate	290	108	250	85	232	80	170	52	120	40	80	30
Reldan	289	110	255	90	235	82	175	55	128	45	85	32

Table 12: Values of acetaldehyde and diacetyl by ABT culture in the presence of pesticides on the 6th day from cold storing at 6-8°C

	Concentration of pesticides											
	Control		2 ppm		4 ppm		6 ppm		8 ppm		10 ppm	
Pesticides	Ace	Dia	Ace	Dia	Ace	Dia	Ace	Dia	Ace	Dia	Ace	Dia
Fungicides												
Anadol	210	105	175	100	155	85	120	70	80	55	33	25
Tasolen	210	105	170	95	150	80	115	65	76	50	30	20
Herbicides												
Round up	208	103	170	85	150	80	110	60	72	50	35	25
Saturn	210	105	175	85	150	83	115	62	72	52	35	25
Insecticides												
Lannate	210	105	155	70	140	72	100	55	65	50	30	15
Reldan	205	105	160	75	133	74	103	57	65	52	35	17

Acetaldehyde and diacetyl are considered as important constituents of the flavor of fermented milks. Data presented in Table 11 indicated that the highest acetaldehyde and diacetyl on the sixth day were attained with yoghurt free pesticides (control) samples and decreased with increasing of pesticides. The 2 ppm of all pesticides studied slightly delayed the flavor compounds production, the high concentrations 8 and 10 ppm decreased sharply of acetaldehyde and diacetyl. Worth mentioning, Lannet was the most effect in disable the acetaldehyde and diacetyl production followed by Reldan, Round up, Saturn, Tasolen then Anadol.

As for the Acetaldehyde and diacetyl production by ABT culture, it took the same direction with some differences Table 12, the studied pesticides strongly affected on flavor compounds production more than they did in the yoghurt culture, the difference between the two fungicides, herbicides or insecticides was very slightly, However, presence of insecticides delayed acetaldehyde and diacetyl production by ABT followed herbicides then fungicides which had the lowest effect in that way. These observations might be due to harmful effect of pesticides on the cells metabolism.

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