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PROTECTIVE ROLE OF EFFECTIVE MICROORGANISMS AGAINST PESTICIDES RESIDUES TOXICITY ON TOMATO HAULMS AND THEIR EFFECTS ON PERFORMANCE OF DAIRY GOATS

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Abstract

Fifteen lactating Zaraibi does were assigned to study the effect of EM ability to prevent the probably toxicology effect of the residues of pesticides remained in tomato haulms (TH). Animals were fed ad libitum fresh TH (T1), TH silage (T2) and TH silage treated with EM (T3) and concentrate feed mixture (CFM). Treatment with EM (T3) was resulted in less concentrations of pesticides residues compared to T1 and T2. Higher milk yield, milk composition and 4% FCM were followed EM treatment; T1 had less milk fat content than T2 and T3. All pesticides concentrations residues in milk were higher in T1, but it had less degree in T2 and not detected for T3 Treatment TH with EM (T3) had higher concentrations of TVFA's, acetate, gas production, total count of cellulosic bacteria and less protozoa. T1 had higher concentrations of cholesterol, triglyceride, urea, creatinine, AST, ALT and less glucose, albumin and globulin than those for T2 and T3. So, biological (EM) treatment could be advisable to overcome the harmful effect of feeding TH exposure to pesticides.

Key words: *effective microorganisms (EM), pesticides residues, rumen metabolites, nutrient utilization, goats*

INTRODUCTION

Animal production in Egypt is mainly based on smallholder farms. Several by-products have potential value, especially for ruminants, due to their ability to digest fiber. So, use of local resources and crop byproducts as livestock feeds is become a necessary for profitable production. Presently, indoor use of pesticides for pest control is widespread in Egypt. Meantime, as these agrochemicals are used intensively and excessively in the production system; major problems are caused from the contamination of food by pesticide residues, and pollution of environmental ecosystems. Not only that, but it created many problems on human and animal health. Consequently, there has been a growing interest in nature farming and organic agriculture by consumers and environmentalists. However, no accurate information of the types and amounts of Egyptian household pesticide use, or numbers of contamination incidents is available (Hassan et al., 2010). The misuse and excessive use of chemical fertilizers and pesticides had resulted that Feed and fodder offered to animals are often contaminated with pesticide residues (Raikwar and Nag, 2003). However, they have usually found that such problems cannot be solved without using microbial methods and technologies in coordination with agricultural production (Parr and Hornick, 1992). Ways of degrading pesticide residues became to be fundamentally worthy of attention. Studies on microbial degradation of pesticide residues originated in 1940s, and the researchers were paid more attention on the degradation process and degradation mechanism of organic (Akbar and Sultan, 2016). Bacteria in nature could degrade the pesticide residues, with low cost and environmentally friendly and it would not cause secondary pollution, it converts organic macromolecules into small non-toxic molecules, thus avoiding the secondary pollution. Studies have shown that mineralization and co-metabolism were the main mechanisms for the further degradation of pesticides and their intermediate products (Ye et al., 2018). A number of bacteria that could degrade and convert pesticides have been isolated (Ramya et al., 2016).

EM contains selected species of microorganisms including predominant populations of lactic acid bacteria and yeasts, and smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms. All of these are mutually compatible with one another and can coexist in liquid culture. There are three types of microorganisms which are categorized into decomposing or degenerative, opportunistic or neutral and constructive or regenerative. EM belongs to the regenerative category whereby they can prevent decomposition in any type of substances and thus maintain the health of both living organisms and the environment (PSDC, 2009). Therefore, the EM has great potential in creating an environment most suitable for the existence, propagation, and prosperity of life (Higa and Parr, 1994). The objective of this experiment was to determinate the poisonous effects of pesticides residues in tomato haulm and whether EM is able to prevent the probably toxicological effects of pesticides on rumen degradability parameters in the rumen environment or not.

MATERIALS AND METHODS

Three tomato haulms with capacity of 3 tons (1 ton each) were used to be fed as fresh, silage and silage treated with EM. The Tomato haulms were collected from Noburia area, Egypt; after harvesting, chopped (1 to 3 cm in length). The silage was prepared by filling successive layers of the chopped materials and heavily trod ten before adding the next layer; molasses was added at the rate of 3% at the ensiling time and EM was add (1 liter/ton silage).

Fifteen lactating Zaraibi does (post weaning) in the 2nd and 3rd season of lactation, aging 2.5-3.5 years with 38.50 ±1.37 Kg in average body weights were randomly divided into three equal groups, (5 does each) for an experimental period of 60 days in randomized complete block design . Animals were offered roughage *ad libitum* twice a day at 8.00 and 16.00 plus CFM was fed to supply the CP requirements according to NRC (2007), while, tomato haulm were allowed to be fed ad libitum in each group, the actual amount of tomato haulm fed was recorded. The CFM consisted of 36% yellow corn, 30% wheat bran, 12% soybean meal (44% CP), 13% cottonseeds meal, 5% molasses, 2% limestone, 1.5% common salt and 0.5% vitamins minerals premix (Table 1). Milk yield was individually recorded on two successive days, milk samples were collected twice daily for 4 times in the 60 days through the collection period from all goats according to Galyean (1989). Milk samples (about 0.5% of total milk produced) were taken biweekly from doses of all groups during lactation. Milk samples were chemically analyzed for total solid (TS), protein, fat and ash according to AOAC (2006), while lactose was calculated by difference.

Three experimental rations were composed of concentrate feed mixture (CFM) plus fresh tomato haulm (F) as control ration, CFM + tomato haulm silage (S) and CFM + tomato haulm silage treated with (EM).

Table 1. Chemical analysis and cell wall constituents of the concentrate feed mixture (CFM) and tomato haulm fed to dairy goats (on DM basis)

Item	CFM	Tomato haulm		
		Fresh	Silage	Silage treated with EM
DM	89.14	28.73	29.38	28.91
OM	94.26	92.15	92.03	91.98
CP	15.62	7.71	7.53	7.69
CF	6.31	37.88	33.14	29.87
EE	3.07	1.69	1.65	1.61
NFE	69.26	44.87	49.71	52.81
Ash	5.74	7.85	7.97	8.02
NDF	27.34	64.89	61.19	57.83
ADF	19.33	46.24	43.77	40.74
ADL	4.02	12.21	11.01	10.49
Hemicellulose	8.01	18.65	17.42	17.09
Cellulose	15.31	34.03	32.76	30.25

Three Zaraibi does were used for rumen fermentation and in situ trials. On the last day of the experiment, ruminal fluid samples (200 mL) were collected at 0 and 4 h post-feeding (morning feeding) from each goat. Ruminal fluid samples were collected via a stomach tube connected with a vacuum pump; pH and temperature were recorded using a portable pH and temperature meter (HANNA instrument HI 8424 microcomputer, Singapore). Ruminal fluid samples were then strained through three layers of cheesecloth. The strained fluid samples (10 mL) were collected for measurement of the number of total viable bacteria as well as cellulolytic, amylolytic and proteolytic bacteria by the roll-tube technique (Hungate, 1969). The remaining strained fluid samples were then immediately mixed with 5 mL of 2 M H_2SO_4 to stop microbial activity. Ruminal fluid samples were then centrifuged at 3000 cycle for 10 min

and the supernatant (100 mL) was taken and divided into two portions. The first portion was kept in a plastic bottle where 5 mL of 1 M H_2SO_4 was added and frozen (20ºC) for later NH3-N and VFA analyses. The second portion was kept in a plastic bottle, immediately fixed with 10% formalin solution (1: 9 v/v, ruminal fluid: 10% formalin) (Galyean, 1989) and stored at 4ºC for later measurement of the ruminal microbial populations. Ruminal fluid was analyzed for NH_3-N by using the hypochlorite–phenol procedure (Beecher and Whitten 1970) and VFA by using HPLC, according to the method of Samuel et al. (1997). The total direct counts of bacteria, protozoa (holotrichs and entodiniomorphs) and fungal zoospores were measured using the methods of Galyean (1989) based on the use of a haemocyto meter (Boeco, Hamburg, Germany).

Blood samples were collected twice (the first one was taken before the beginning of the experiment and the other one at the end of the experimental period), from all goats. Blood samples were obtained from the jugular vein of the goats in the morning before access to feed and water. Serum was obtained by centrifugation of blood and was stored at –20ºC until used for analysis. Glucose concentration was determined by the method of Trinder (1969). Serum cholesterol was determined using the colorimetric method of Stein (1986). Serum total protein (TP) was measured as described by the Biuret method according to Henry et al. (1974). Albumin (A) concentration was determined according to Doumas et al. (1977). Kidney function was evaluated by measuring blood urea using the colorimetric methods of Henry and Todd (1974) using commercial kits. Creatinine was measured using the colorimetric method according to Faulkner and King (1976). Liver function was assessed by measuring the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by the method of Reitman and Frankel (1957).

Pesticides in feed and milk samples

Solvents and other reagents used (acetone, benzene, ethyl acetate, methylene chloride, n-hexane, florisil 60-100 mesh (pre-treated as in the method of Kadenczki et al. (1992); sodium hydroxide, stannous chloride, carbon disulfide, cupric acetate monohydrate, hydrochloric acid, ethanol, diethanol amine were analytical reagent grade. The analytical standards of the tested pesticides were kindly provided by Department of Environmental Studies, Institute of Graduate Studies and Research, University of Alexandria, Egypt. The selected analytical standards are: (a) - Chlorinated hydrocarbon insecticides: HCB, lindane, p,p'-DDD, p-p' DDE and p-p' DDT. (b)- Halogenated pyrothroids: Cypermethrin, lambda-cyhalothrin. (c)- Organophosphorus insecticides: Dimethoate, malathion.

A simple multi-residue method according to Kadenczki et al. (1992) was applied to extract several pesticides (chlorinated hydrocarbon, halogenated pyrothriod insecticides and organophosphate) from tomato haulms and milk. The principle of this method is based on having a homogeneous sample pulp adsorbed on the surface of activated florisil to obtain a free-flowing powder, which is extracted in a glass column with methylene chloride-acetone (9:1, v/v). The gas chromatograph (GC) used was HP-5890 Series II. Polychlorinated biphenyls (PCBs) was determined by gas chromatograph according to Willett and Hess (1975).

Statistical Analysis

Means were calculated for all variables by goats within period. Data were analyzed using the MIXED procedure of SAS (SAS, 2000). Differences were tested using the PDIFF option in SAS (SAS, 2000) using protected ($P<0.10$) LSD test. Differences were declared significant at a $P<0.05$; and trends were discussed at a $P<0.15$, unless stated otherwise.

RESULTS AND DISCUSSIONS

The concentration of pesticides residues of the tomato haulms are presented in Table 2.

Table 2. Concentration (mg/kg) of pesticides residues of tomato haulm

Items	Tomato haulm		
	Fresh	Silage	Silage treated with EM
Deltamathrin	0.92	0.58	0.21
Aldrin, Dieldrin	1.11	0.69	0.25
Malathion	0.78	0.35	0.11
Cypermethrin	0.96	0.44	0.07
Permethin	0.83	0.40	0.16
HCB	0.33	0.17	0.04
Lindine	0.28	0.11	0.02
PP DDE	0.17	0.07	0.01

The tomato haulms silage treated with EM showed lower values of pesticides residue compared with the fresh and silage form.

Brajesh and Allan (2006) reported that the biochemistry of organophosphorus compound degradation by most of the bacteria seems to be identical, in which a structurally similar enzyme (organophosphate hydrolase or phospho-triesterase) catalyzes the first step of the degradation.

Catherine et al. (2002) found that organo-phosphorus hydrolase is a bacterial enzyme that has been shown to degrade a wide range of neurotoxic organophosphate nerve agents. However, the effectiveness of degradation varies dramatically, ranging from highly efficient with paraoxon to relatively slow with methyl parathion. Plants have evolved interactions and association with micoorganisms that can accelerate breakdown or transformation of certain pollutants in the plant root zones to products that no longer pose environmental hazards (Brimecombe et al., 2001). Sharaf et al. (2006) reported that understanding pesticide metabolism in plants and microorganisms is necessary for pesticide development, for safe and efficient use, as well as for developing pesticide bioremediation strategies for contaminated soil and water.

Data concerning milk yield and its composition of lactating goats fed the experimental rations are presented in Table 3.

Table 3. Milk yields and milk composition for lactating goats fed the experimental rations

Items	Tomato haulm				
	Fresh	Silage	Silage treated with EM	SEM	*P Value*
Milk yields (g/d)	563.25[c]	899.33[b]	955.18[a]	0.31	0.042
4% FCM (g)	479.55[c]	817.08[b]	880.67[a]	0.47	0.019
Fat, (g/d)	16.95[c]	30.49[b]	33.24[a]	0.39	0.001
Protein, (g/d)	15.49[c]	28.42[b]	31.43[a]	0.51	0.004
Milk composition (%):					
Total solids	13.07[b]	13.81[a]	13.85[a]	0.15	0.032
Solids not fat	10.06[b]	10.65[a]	10.56[a]	0.14	0.027
Fat	3.01[b]	3.39[a]	3.48[a]	0.11	0.005
Protein	2.75[c]	3.16[b]	3.29[a]	0.07	0.016
Lactose	6.35	6.68	6.43	0.31	0.744
Ash	0.96[a]	0.81[b]	0.84[b]	0.05	0.004

[a,,b, and c] *Means within rows with different superscripts are significantly different (P<0.05).*

The milk yield and fat corrected milk (FCM) were significantly increased (P<0.05) for goats fed tomato haulm silage treated with EM compared with the other experimental groups (tomato haulm silage and tomato haulm fresh). Milk fat and protein yield were also significantly increased (P<0.05).

Concerning milk composition and milk produced from animals fed tomato haulm silage and tomato haulm silage treated with EM had significantly (P<0.05) higher contents of fat, protein, total solids (TS) and solids not fat (SNF) compared with the tomato haulm fresh group. While milk lactose was not significantly differed (P>0.05) among experimental groups.

The concentration of pesticides residues (µg/kg on fat basis) in goat's milk are presented in (Table 4). The pesticides residue in the milk of goats fed tomato haulm silage and tomato haulm silage treated with EM showed low values of pesticides residues compared with tomato haulm fresh.

Table 4. Concentrations of pesticides residues (µg/kg on fat basis) of the milk goat's samples

Items	Tomato haulm		
	Fresh	Silage	Silage treated with EM
Deltamathrin	0.18	0.04	ND
Aldrin, Dieldrin	0.21	0.09	0.02
Malathion	0.11	0.01	ND
Cypermethrin	0.20	0.05	ND
Permethin	0.13	0.01	ND
HCB	0.09	ND	ND
Lindine	0.09	ND	ND
PP DDE	0.05	ND	ND

ND, not detected.

DebMandal et al. (2008) reported that microbes (fungi, bacteria, and other microorganisms) could degrade or breakdown the pesticides whereas they used them as food source. Quintero et al. (2008) reported that white-rot fungi species have demonstrated a high capacity to degrade organic pollutants such as the insecticide lindane (y-HCH). Also, pesticides (malathion, delatmethrin, cypermethrin, and permethrin, etc.) were used in some dairy farms to protect the animals from house flies and ticks. Some farmers were facing pesticide resistance problem in vector control which is also reflected by Singh et al. (2014) in the region of the present study. Additionally, in some farms those pesticide containers were kept in animal feed storage sites which may result into accidental spillage on feed. Moreover, while spraying pesticides on crops adjacent to dairy farms, the drift and volatilization of these pesticides may result in their deposition on the non-target sites which may include feed, fodder and water.

Data of ruminal pH, NH_3-N and VFA's concentrations are shown in Table 5. The ruminal pH was significantly (P<0.05) higher in both silage groups and increased linearly with silage treated with EM than in fresh tomato haulm group, but there were no significant differences among the both silage groups. Ruminal NH_3-N concentration was significantly (P<0.05) higher in both silage groups and increased linearly with silage treated with EM than in fresh tomato haulm group, but there were no significant differences among both silage groups. Total ruminal VFA concentrations and molar proportions of ruminal acetate were significantly (P<0.05) higher in silage treated with EM group than in fresh tomato haulm group. There was no difference among treatments for molar proportions of propionate and butyrate.

Table 5. Rumen liquor parameters of lactating goats fed the experimental diets

Items	Tomato haulm				
	Fresh	Silage	Silage treated with EM	SEM	*P Value*
pH	6.14[b]	6.53[a]	6.58[a]	0.06	0.038
NH_3. N(mg/$dL_)$	11.83[b]	13.66[a]	13.14[a]	0.52	0.021
TVFA's ((mM)	9.47[c]	11.38[b]	12.42[a]	0.19	0.029
Acetate (mol/100 mol)	61.75[c]	66.48[b]	68.77[a]	0.89	0.011
Propionate (mol/100 mol)	21.97	22.37	22.41	0.53	0.684
Butyrate (mol/100 mol)	8.52	8.66	8.68	0.27	0.773
Acetate: propionate ratio	2.81	2.97	3.07	0.23	0.693
Gas production volume at 24h, ml	28.8[c]	35.2[b]	37.1[a]	0.41	0.001
CO_2, %	46.82[a]	44.37[b]	43.29[c]	0.25	0.029
CH_4, %	28.79[a]	24.33[b]	22.89[c]	0.16	0.001

[a,,b, and c] *Means within rows with different superscripts are significantly different (P<0.05).*

It is interesting to note that the ruminal pH of fresh tomato haulm group was below 6.2, whereas both silage groups were in the normal range (6.53–6.58) and stable. Firkins (1996) stated that the pH range for optimal ruminal microbial digestion is 6.5–7.0. The low ruminal pH of the fresh tomato haulm group may have led to lower NDF digestibility and reduced bacterial populations compared with both silage groups (Table 5).

The higher NH_3-N concentrations of both silage groups may be reflected in greater numbers of rumen microbes. The greater number of rumen microbes in the both silage groups may be explained by the fact that pesticides in fresh tomato haulm group may be detoxified by the EM and pesticides may be adsorbed by the inorganic components, resulting in the removal of the suppression on microbial growth and activity. These microbes in turn ferment more feed, and generate more VFA's and a greater supply of microbial N for both silage groups. This also allows both silage groups to consume more feed as it is disappearing from the rumen faster. The experimental diet contained urea which is used as N source for microbial protein synthesis in the rumen, and as more feed was consumed by both silage groups, this lead to a higher ruminal NH_3-N concentration. The present results show that the EM supplementation increased the NH_3-N concentration, with NH_3 being the main N source for growth and protein synthesis by ruminal bacteria to achieve maximum fermentation. These results are in agreement with those of Dänicke et al. (2005) who reported an elevation in rumen NH_3 concentration and a reduction in duodenal flow of microbial protein in cows fed deoxynivalenol.

Table 6. Effect of the EM supplementation on rumen microorganism population

Items	Tomato haulm				
	Fresh	Silage	Silage treated with EM	SEM	*P Value*
		Rumen microbes			
Bacteria ($\times 10^{12}$ cells/mL)	6.7[c]	8.9[b]	11.4[a]	0.69	0.001
Protozoa ($\times 10^{5}$ cells/mL)	4.3[a]	3.1[b]	2.6[c]	0.25	0.015
Fungal zoospore ($\times 10^{7}$ cells/mL)	3.2[c]	4.8[b]	5.9[a]	0.16	0.004
		Viable bacteria (CFU/mL)			
Total ($\times 10^{9}$)	3.6[c]	7.7[b]	9.5[a]	0.47	0.027
Cellulolytic ($\times 10^{9}$)	2.1[c]	5.9[b]	7.2[a]	0.38	0.013

[a,,b, and c] *Means within rows with different superscripts are significantly different (P<0.05).*

Ruminal bacterial counts and fungal-zoospore counts were significantly higher with both silage groups and increased linearly with silage treated with EM, whereas protozoa counts were lower,

and decreased linearly with the silage treated with EM than in fresh tomato haulm group. Total viable-bacterial and counts cellulolytic bacterial counts were significantly higher in both silage groups and increased linearly with silage treated with EM than in fresh tomato haulm group. It is interesting to note that the populations of total bacteria, total fungal zoospores, total viable bacteria and cellulolytic bacteria increased, while protozoa population decreased with both silage groups and increased linearly with silage treated with EM. These results show that the EM played an important role in changing ruminal microbial populations. A possible explanation for the greater numbers of rumen microbes in both silage groups and their subsequent effects have already been discussed previously. Lower populations of ruminal bacteria in fresh tomato haulm groups in the current study were possibly associated with a fall in ruminal pH below 6.2 (Rode 2008).

Table 7. Blood serum parameters for lactating goats fed the experimental rations

Items	Tomato haulm				
	Fresh	Silage	Silage treated with EM	SEM	*P Value*
Glucose (mg/dl)	65.95^{b}	68.55^{a}	68.95^{a}	0.63	0.036
Cholesterol (mg/dl)	78.88^{a}	74.66^{b}	73.64^{b}	0.93	0.021
Triglyceride(mg /dl)	64.47^{a}	60.27^{b}	59.72^{b}	0.88	0.016
Total Protein (g /dl)	5.92	6.89^{a}	6.97^{a}	1.46	0.006
Albumin (g /dl)	3.06^{b}	3.47^{a}	3.44^{a}	0.07	0.001
Globulin (g/dl)	2.86^{b}	3.42^{a}	3.53^{a}	0.11	0.001
Urea (mg/dl)	43.85^{a}	38.66^{b}	38.83^{b}	0.32	0.004
Creatinine (mg/dl)	1.42^{a}	0.89^{b}	0.91^{b}	0.04	0.001
AST (U/L)	33.64^{a}	29.58^{b}	29.48^{b}	0.15	0.006
ALT (U/L)	22.84^{a}	19.46^{b}	18.66^{b}	0.89	0.09

$^{a, and\ b}$ Means within rows with different superscripts are significantly different (P<0.05).

Data of blood serum parameters for lactating goats are presented in Table 7. Data showed that silage and silage treated with EM rations caused a significant (P<0.05) increase in glucose, total protein, albumin and globulin levels than ration containing fresh silage. While fresh ration had significant (P<0.05) increase in cholesterol, triglyceride, urea, creatinine, AST and ALT than silage and silage treated with EM rations. The changes in carbohydrate metabolism induced by pesticides can be correlated with the effects of these chemicals on the activities of hepatic enzyme system which are intimately involved in glucose production, storage and metabolism and/or correlated with the endocrine activity of the pancreas (insulin activity). Pesticides exposure could cause hyperglycemia which could be a result of glycogenolysis in muscle and liver causing a significant increase in blood glucose level. This disturbance in carbohydrate metabolism may be responsible for the toxic action of pesticides (Ferrando and Andreu–Moliner, 1991).

The reduction of serum proteins, particularly albumin, in animals fed fresh TH contaminated with pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver (Rivarola and Blegno, 1991).The increased in blood urea and creatinine concentrations revealed in the present study should be due to pesticides effect. Elevated blood urea is known to be correlated with an increase in protein catabolism in mammalian body or it could be resulted from more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production (Rodwell, 1979).The increased of serum AST and ALT activity indicated liver damage and disruption of normal liver function (Shakoori et al., 1994). The increment of the activities of AST and ALT in plasma are mainly due to the leakage of these enzymes from the hepatic cytosol into the blood stream (Navarro et al., 1993), which gives an indication on the hepatotoxic effect of lindane which leads to the liver damage.

CONCLUSIONS

It could be concluded that biological treatment with fungi or bacteria (silage) could be advisable in order to overcome the harmful effect of TH exposure to pesticides. However, more studies are needed in this respect.

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