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Full Length Research Paper

Comparison among solarization kind of pailes and their impact in the control of *Cryptosporidium parvum* and *Giardia lamblia*

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Studies have determined that the manure contains pathogenic to human parasites such as *Cryptosporidium parvum* and *Giardia lamblia*. In this study we provide results from a study of nine treatments with four replicates tested under field conditions consisting of 36 piles of 2 meters long and 1.5m wide and 1.0m high. Three piles were left at 25cm below the surface, three at 50cm below the surface and the other three on the surface with 0%, 25% and 50% moisture respectively, at three replications. Temperatures and samples for identification of parasites, were collected from two different scanning depths (0-7.5 and 7.5-15 cm). In the manure piles and non-solarized (control), samples were collected according to NOM-044-SEMARNAT-2002. Solarized for manure sampling was at the end of the period of solarization to determine *Cryptosporidium parvum* and *Giardia lamblia*. For the manure non solarized (control), there was a homogeneous mixture of the various stables in North area of the Ejido Fresno. The experimental results indicate that achieving an increase in temperatures in solarized piles. The pile with 50% moisture with an average measure of 56.41, under these temperatures was eliminated *Cryptosporidium parvum* and *Giardia lamblia*. In the sample of manure without solarization was detected the presence of *Escherichia coli, F.enterobacteriaceae, Enterobacter* sp., *Bacille* sp., *Mucor* sp. and *F. Sanccharonmycetaceae*, *Cryptosporidium* sp. and *Giardia* sp.

Keywords*: Criptosporidim,* pathogen, manure.

INTRODUCTION

At the national level, livestock inventory of the 2002 indicated that there were nearly 30 million heads of cattle for meat and little more than two million head of cattle milk. Most of the production of cattle for meat occurs extensively in livestock dry lands, so there is not a confined production of manure. Only in the case of the bovine dairy, the estimated manure production goes from 3.8 million per year^{-1}. In the Comarca Lagunera in the State of Coahuila Mexico, cattle produce around 2.6 million kg (dry matter) per day (Cueto et al., 2005). In general, this farm manure is collected and accumulated in cells without any treatment to subsequently incorporate it into agricultural land, in order to add nutrients, improving the physical structure of the soil and facilitate better use of water for irrigation of crops (Salazar et al., 2010).

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The global frequency of various intestinal parasites is high in geographical areas where ecological conditions favor the presence of parasites, in addition to the socio-economic characteristics of population, such as poverty, ignorance and poor infrastructure; factors that shared by developing countries (Sánchez et al., 2000; Thompson, 2004). The pathogenic protozoa *Cryptosporidium parvum and Giardia lamblia* has shown its dangerous and negative impact on the health of thousands of people both in industrialized and in developing countries (Cifuentes et al., 2004; Solarte et al., 2006).

Today, agriculture has become another factor of contamination by enter pathogens, one of the main sources are the livestock excreta (Barwick, 2003; Kucik et al., 2004). Experiments in calves, suggest that these go to excrete up to 10 million oquistes of *Cryptosporidium parvum* during a period of 7 to 10 days, they may have contact with rivers, streams, or any source of water. (Videorecording, 2004; Meihardt et al., 1996).

Cryptosporidium parvum is a genus of parasitic protozoa of the Apicomplexa phylum that is associated with a disease called diarrheal Cryptosporidiosis in humans. Cryptosporidiosis is typically an acute disease of short duration, but the infection can be serious and persistent in children and immune compromised patients such as those infected with the HIV Virus (AIDS). The parasite is transmitted in the environment through Hardy cysts (oocysts) that, once ingested, they exist in the small intestine and lead to the infection of intestinal epithelial tissue. (Garcia, 1991)

In the host, *Cryptosporidium parvum* invades cells of the digestive tract, developed in the edge brush of intestinal epithelial cells (Forney et al., 1996) and in the respiratory tract (Atlas, 1991). It may also affect the cecum, colon, gall bladder and the kidneys of a wide variety of hosts (Georgi and Georgi, 1994; Romero, 2007). It is thus that they are susceptible to calves, piglets, foals, mice, goats, fish, birds, snakes, and even man (Atías, 1991; Harp and Harley, 1991; Quevedo et al., 1990; Guerrant, 1997).Cryptosporium Oocyst parvum is very resistant to weather conditions, and can remain viable from two to six months at 4 ° C in the atmosphere and it is resistant to most disinfectants used in the laboratory (Atias, 1991; Forney, 1996). The oquist can be found even in drinking water because they resist the chlorination of water, and it could survive in filtered water. They are also very sensitive to desiccation and freezing (Gorman, 1987; Keusch et al., 1995).

Statistics carried out by the National System of Epidemiological Surveillance of Mexico, shows that diseases with prevalence in the country are: the intestinal amebiasis, Giardiasis, enterovirus and other intestinal protozoan infections (Flore, 2000; Flowers, 2006). Enteropathogens cause mainly gastroenteritis and, 50% of the cases are due to the consumption of contaminated water for both human feces for animals (Solarte et al., 2006). The prevalence varies the Country being studied or even in different regions of a Country (Atias, 1991). In the United States of America it has been determined that Cryptosporidium sp and the Giardia lamblia is one of the causes for more frequent diarrheal cases, mainly because it resists the chlorination of drinking water, since this bacteria is small and difficult to filter, and is present in many animals (Guerrant, 1997; Suárez et al., 1997). In Peru, there have been studies to know the prevalence of Cryptosporidium in humans, determining a 14% of positive samples from a total of 28,165 samples of diarrheal patients; the samples were processed by the method of Ziehl Neelsen (Ramos, 2000).

In developed countries is one of the most high causes acute diarrhea, persistent, that is given by interpersonal infection, ingestion of contaminated food, lack of sanitation and ignorance of hygiene standards; Although also occurs in epidemic form by swallowing water or contaminated products (Quevedo et al., 1990; Atías, 1991; Yoshiyama, 2000).

Giardiasis is an infection caused by a flagellate Protozoan Hexamitidae, Giardia (G) family of sp, is characteristic of cosmopolitan, identified by Loewenkoeck in their own droppings in 1681, however the first description was held in 1859 by Lambl (Acha and Szyfres, 1989; Atías, 1991). It is classified in the subphylum Mastigophora (Flagllata), class Zoomastigophorea. Order Diplomonads, Giardia gender and according to their host in Giardia Lamblia (Intestinalis, intestinalis, enteric) in humans, the rabbit G.duodenalis, g. bovis from cattle, g. caprae of sheep and goats and g. canis dog (Acha and Szyfres, 1989). Giardia SP. It is found mainly in the small intestine of its host and its life cycle differs from others in terms of the formation of resistant cysts (Georgi and Georgi, 1994), there are always flagellate forms or vegetative plays automatically for participation binary, nuclear division often takes place inside the cyst, while the cell division occurs only after the dissolution of the cyst wall, inside the new host (Mehlhorn y Piekarsiki, 1993)

The representatives of the Diplomonadina, as it is the case of Giardia lamblia live in the intestine of the host and in the light of the intestine feed by phagocytosis of the intestinal contents, stored carbohydrates that take the glycogen, in the presence of oxygen actively breathing through what is called aerobic aero tolerant. Defecation transmission is carried out through the cysts and are eliminated in the stool; the walls of the cysts contain filamentous stabilizing elements and are separated from the surface of the parasite through a process of exocytosis (Mehlhorn et al., 1993; Geogri and Geogry, 1994)

There have been studies on the effect of ultra violet disinfection of drinking water by determining the total

elimination of the ineffectiveness of Cryptosporidium parvum with exposure to UV light for 150 minutes or more (Lorenzo et al., 1993), has also shown that exposure to high temperatures (> 55 ºC) for short periods of time (5, 10 and 15 stems) are sufficient to destroy the oocytes of Cryptosporidium sp. in water and milk (Harp et al., 1996). The giardia cyst is Hardy in drinking water, likewise cysts kept its feasibility in water to 8 ° C for more than two months to 21 ° C for a month and 37 ° C about four days Atías (1991).

In United States is considered to be the intestinal parasite most common and cause more frequent of traveler's diarrhea, being also very common in Great Britain and Canada, where outbreaks of water-borne in children's institutions, groups of gay and transmission have been reported in person. In Latin America is estimated at 15% and affects 200 million people worldwide. It ranks first in etiology of enteroparasites in Argentina. Cuba is the fourth country of medical importance (Gamboa et al., 1998).

On the other hand Peru determine a prevalence of 30.6 per cent of positive samples of Giardia Lamblia in a total of 912 samples of patients of the Institute of Tropical Medicine in Lima (Tantalean; 1993). Giardiasis is considered the infection caused by parasites more common that affects humans in the world (Kicik, 2004).

The fecal matter of animal and human origin contains a variety of pathogens to humans. Manure and biosolids treated properly are fertilizers effective and safe, so it is necessary that producers apply good agricultural practices to handle these natural fertilizers, in order to minimize the dangerous microbiological (Salazar et al., 2010).

Transformation of organic waste into fertilizer treatments can be divided into two groups, treatments passive and active treatments.

Passive treatments are based on the maintenance of organic waste under natural conditions. Compost piles are not removed and the free oxygen present in them is used rapidly, giving rise to anaerobic conditions that delayed the process of transformation in fertilizer. However, environmental factors such as temperature, moisture and radiation UV, when acting with sufficient time, inhibit the growth of pathogenic organisms and eventually destroy them (FDA, 1998; Salazar et al., 2009).

The biggest obstacle faced by this method is that it takes too much time to reduce significantly the number of pathogens in organic matter and it is difficult to determine the time required for this process takes place. The amount of time it takes depends on the climate of the region, season of the year; as well as the origin, the type of manure and organic matter used. Because of these variables, the passive transformation is not recommended (FDA, 1998).

Active treatments are those in which organic batteries are treated in conditions that accelerate the transformation process of the waste into fertilizer. Active treatment to convert organic matter into fertilizer is the most used by farmers.

In active treatments, organic batteries are often removed instand another kind of acceleration in order to maintain conditions of oxygen (aerobic) within the stack. Controlled temperature, moisture levels and added supplements needed to maintain optimal moisture and an adequate rate of carbon/nitrogen to complete the process of transformation in payment. This process has completed, when the battery is no longer hot. Under appropriate conditions, the high temperatures generated during the fermentation process destroy most of pathogens in a relatively short time period Salazar et al. (2010), Mexico, use plastics in agriculture, to capture solar energy and to raise the temperature of the ground with the aim of eliminating pathogens that affect the root system of the plant, which I call solar heating and process which is now known as solarization.

Several terms have been used to describe this method: solar heating, polyethylene resistant to heat and covering of the soil with plastic or polyethylene; given that this method relates to high temperatures and relatively warm temperatures for several days, the term pasteurization also is justified (Katan, 1980; Jiménez, 1995). This technique of trap solar through transparent polyethylene with chemical resistance, hardness and flexibility also cost efficient and relatively low pathogenic inhabitants of the ground control. Therefore the general objetive of this study was:

The identification of Cryptosporidium parvum and Giardia lamblia in manure no solarized and solarized and to determine the effectiveness of solarization in different treatment, in order to know the most effective treatment, considering the conditions of temperature and moisture on the control of these pathogenic zoonotic.

MATERIALS AND METHODS

Location of the study: This study was developed between the years 2009-2010, in the Northern part of "Ejido El Fresno", located at km. 37.5 Torreón, San Pedro road, municipality of Francisco I. Madero, Coahuila-México and in the laboratory of Microbiology of the Juarez City University (UACJ), of Chihuahua Mexico and consisted of two stages:

Stage 1. Field Solarization Manure

There were nine treatments established to solarize the cattle manure using a padded plastic for high transmis-

Figure 1. Left: Geographical location of the experimental site of Northern Fresno Ejido. **Right;** Area of study in the same area of the Ejido El Fresno where there are piles of manure solarized.

Table 1. Factors in study to define treatments.

FACTOR A: % Moisture	FACTOR B: Kind of Pile
Level A1: witness (as it is the stable between 5-10% moisture)	Level B1: 1 m from the surface
Level A2: 25% moisture	Level B2: buried 25 cm.
Level A3: 50 % moisture	Level B3: buried 50 cm.

Table 2. Treatments to determine temperatures in different depths of piles of manure

TRRETMENTS	FACTOR A and B
T1 (A1,B1)	% moisture content y (*) + 1 m of the surface
T4 (A1,B2)	% moisture content (*) + buried 25 cm
T7 (A1,B3)	% moisture content (*) + buried 50 cm
T2 (A2,B1)	25% of moisture + 1 m of surface moisture.
T5 (A2,B2)	25% of moisture + buried 25 cm.
T8 (A2,B3)	25% of moisture + buried 50 cm.
T3 (A3,B1)	50% of moisture + 1 m of surface moisture.
T6 (A3,B2)	50% of moisture + buried 25 cm.
T9 (A3,B3)	50% of moisture + buried 50 cm.

Note: with a total of four repetitions
(*) Percentage of moisture as it comes from the stable manure between 5% and 10%

sivity of Sunlight to raise its temperatures (Misle and Norero, 2001; Juarez et al., 1991; Scaracia et al., 1994; Stapleton, 1986) and in order to confirm which treatments was which, these allowed a better raise in the temperature and eliminated the micro-organisms (Cryptosporidium parvum and Giardia lamblia) (Figure 1).

Two factors were studied: The type of solarization piles and the moisture in the same stack to define treatment (table1).

The distribution of treatments in the field was carried out using a randomized block design. There were 9 treatments established with four repetitions, which makes a total of 36 experimental cells (ex. Figure 1 and table 1). Data base was analyzed using SAS system method (SAS Institute Inc., 2009) Table 2.

Solarization Method

Method of solarization which consists of subjecting

(2)

(3)

Figure 2 and 3. Taking of samples at different depths for laboratory analysis **(2)** and . temperatures at diferent points **(3)** in the pails.

treatments to extreme temperatures, through the use of covered plastic and the rays of the Sun was used to increase the temperature in the piles of manure. Manure was used in the stable of Northern Fresno ejido Municipality of Fco. I. Madero Coahuila México, and was subjected to the process piled in mounds or piles with dimensions of 2 meters long by 1.5 m in width and 1.0 m high. The batteries were covered with plastic manufactured by the company Plastoza, s.a. of the State of Mexico, whose characteristic is: plastic type PLANAT 180 x 1000 / 100 of 1.80 m wide, 1000 m long, 100 microns in thickness and non-transport has albedo (Figure 3).

Sample and Temperature Readings

Temperatures in the different treatments and reruns of stacks of manure in three pintos to both sides to address the West 40 cm from the Centre and the Center were surveyed. The temperatures were recorded using analog thermometers at two different depths of exploration (0-7.5 cm and 7.5 - 15 cm); for the months of May, June in spring and July, August, September and October in summer-fall (Figure 8). Used statistical method General Linear Model (GLM) for Georeferencing the distribution of temperatures in batteries as well as be analyzed temperatures with the SAS (SAS Institute 2009) package.The LSD was calculated for mean separation when the F statistic was significant at the $P < 0.05$ level (Figure 2).

Stage 2: Lab Analysis

Laboratory work to determine Cryptosporidum parvum and Giardia lamblia took place in the Universidad Autónoma de Ciudad Juárez, in the laboratory of Microbiology of the Institute of biological sciences using the following method: Sedimentation Method to diagnosis of Giardia and Cryptosporidium:

1 Place approximately 1 g of stool through the help with a spoon in a glass and add 10-12 ml of physiological saline solution. Mixed with an applicator of wood and homogenized.

2 Filter the suspension through a strainer or mesh and receive filtering in a tube of glass of 14 ml.

3 Centrifuge centrifugal min at 1500 rpm, remove the supernatant and resuspend in saline solution. The previous step, two or three times to wash,

4 Repeat re-suspending sediment in saline and homogenized with a swab, to obtain clean sediment.

5 Add to the sediment 10 ml of formaldehyde to 10% in saline solution, mix and let rest for 5 minutes.

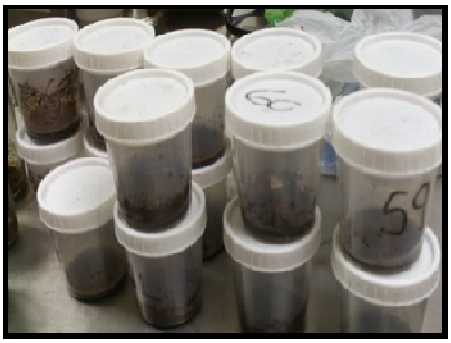
6 Add 3 ml of ether and shake vigorously 30 seconds.

7 Centrifuge 1 min at 1500 rpm

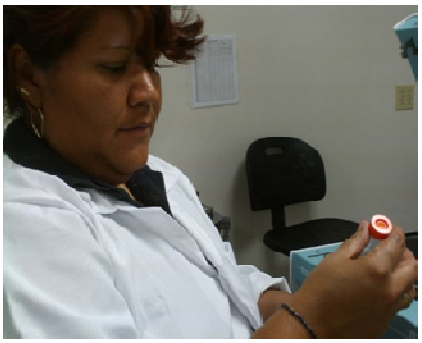
8 After centrifuging, there are 4 layers in descending ether and lipids on the surface b. a plug of fecal remains formaldehyde sediment at the bottom of the tube containing the parasites. Note: Check carefully with a wooden applicator a ring around the layer of fecal remains to liberate it from the sides of the tube, dump the supernatant liquid in a container. Drag the sediment, and remove it.

9. Search of Giardia, add one drop of sediment on a slide with a drop of lugol's, placed above a coverslip seen under a light microscope.

10 Cryptosporidium Applies to the stool sample at one end of a carrying objects and runs with the help of another holder objects. Dry in the open air. Repeats the smear on another slide.



(4)



(5)

Figures 4 and 5. Left: Preparation of samples of manure in saline solution; **right:** centrifuging samples.

(6)

(7)

Figures 6 and 7. Left: There are smears for subsequent staining; **right:** staining of the samples.

11 One of the slide is stained with Ziehl Neelsen modified for identification of coccidian bowel, such as Cryptosporidium: a small sample of feces on a slide is put through a wooden applicator and prepare a fine extension of fecal matter. Let dry at room temperature and is fixed with methanol for 3 minutes. Smears with the dye but Kinyoun Fuchsina was stained for 10 minutes. Became a careful with water washing to remove the excess dye. I didhloro with acid alcohol for 2 minutes and rinse with water, and it slid. Then rub with the contrast, Malachite Green dye, was covered for 3 minutes. Make a final wash, he slid and let dry at room temperature. Stained smears were observed under a light microscope with the goal of 40 and X 100. Of the coccidia oocysts noted Fuchsia red color in contrast with a green background. This technique was based on the content of fatty acids of long chain on the cell walls of some parasites and bacteria, acquiring the red hue in the technique and resisting the bleaching with alcohol-ácid, after staining with dyes Basic (Siuffi, et al., 2006; Savala and Sanchez, 2002)

12 Technique of Acrifluor: another slide is fixed with methanol for a minute. Acri-Fluor dyes applied for 15 minutes. Washed with deionized water and drain. Applies the bleaching sliding on the sample 30 seconds. You wash, drain and allowed to dry in the open air. Looking at the fluorescence microscope, the oocyst are seen fluorescent orange color (Figures 4, 5, 6 and 7)

Note: Common sources of error: too laden stool suspension, poor fecal suspension, centrifuged badly balanced, time and speed of centrifugation inadequate.

No onscreen manure (control) was conducted to a homogeneous mixture of the stable of Northern Fresno Ejido, immediately took four samples of manure type in plastic Ziplock bags, identifying the sample with a marker on the outside of the bag, to be transferred to the laboratory of biological sciences of the UACJ for immediate analysis.

Table 3. Present *Cryptosporidium and Giardia* on solarized and not solarized manure.

PROTOZOA	Control (no solarized)	T1	T2	T3	T4	T5	T6	T7	T8	T9
Cryptosporidium parvum	F	NF	NF	NF	NF	NF	NF	NF	NF	NF
Giardia lamblia	F	NF	NF	NF	NF	NF	NF	NF	NF	NF

F= Found NF= No Found

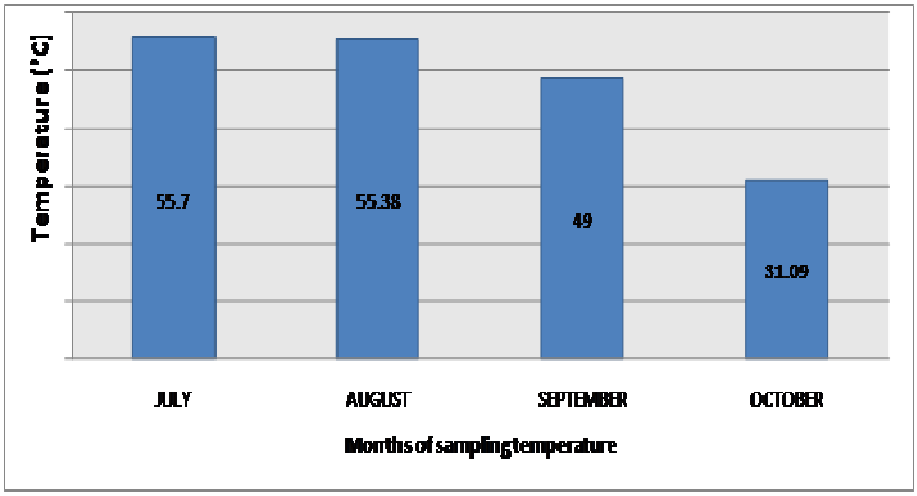


Figure 8. Temperatures recorded during the months of sampling.

RESULTS

Analysis of Cryptosporidium and Giardia lamblia. The results of the identification of cryptosporidium and Giardia lamblia in the UACJ turning negative in nine treatments, and the two sampled depths for 0-7.5 cm depth both for 7.5 to 15 cm. not sample witness (manure without solarized) where you could see lots of cysts of cryptosporidium parvum and Giardia lamblia. As shown in table 3.

Determination of temperature for using the behavior of the temperatures recorded was in descending order, showing that I may record the average temperature of 71 ° C, (spring-fall) and in summer autumn lent a measure of 57.09 July 55.38 for August and September of 49.58. The month of October was less hot month with a measure of 31.09 ° C respectively. July temperatures were lower from the month of May, given that in the month of July rains that occur in this desert region do is temperatures decrease with respect to the other months of spring - summer (Figure 8).

The statistical analysis finds treatments and depths to show significant differences with a Pv = 0 0001. The treatments have two conditions, the first condition refers to the percentage of moisture present in the battery; the second condition relates to the beds of manure. With regard to the last condition analysis of variance carried out shows that there is no significant difference. What leaves clear buried batteries does not affect to increase or decrease the temperature. For the first condition carried out an analysis of variance with the method of Global Linear Model (GLM) to check if the temperature behaved in a uniform manner in the pailes of manure to the level 1 moisture (as it brings the barn manure), level 2; 25% moisture and level 3; 50% moisture being this very similar in higher unemployment.

The statistical analysis verifies that there are significant differences with a Pv = 0. 04, checking that the factor of moisture at 50% is the component that more increases temperatures averaging in spring-summer of 71.5 ° C and 56.41 ° C for summer-fall following higher than 25%, moisture level with an average of 54.43 ° C and also for moisture at stable conditions level with 52.66 ° C. This results demonstrate the effectiveness of the

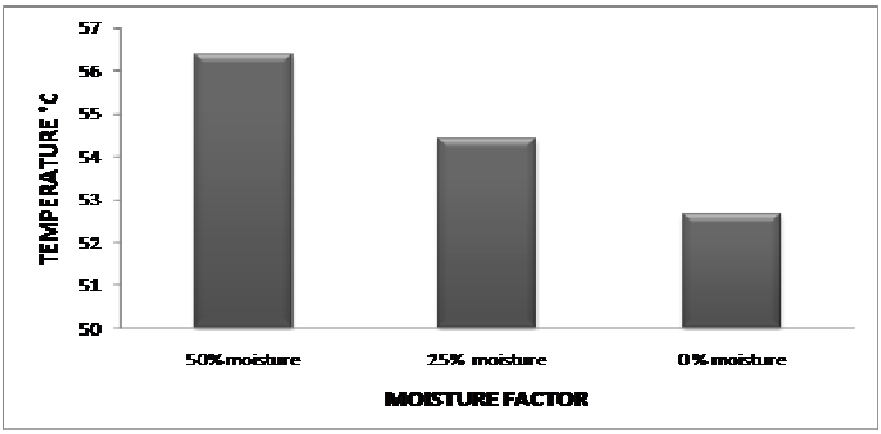


Figure 9. Means testing for temperature level moisture in the pile of solarization for summer fall.

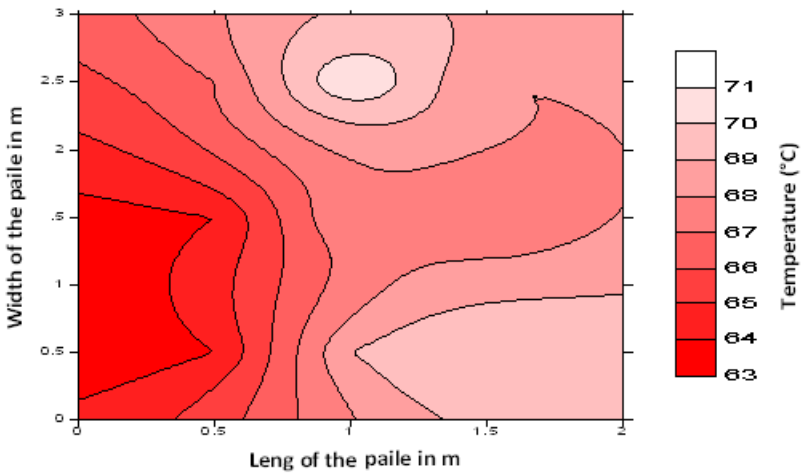


Figure 10. Average temperatures from May to June in 2009 at a depth of 15 cm.

solarization and moisture on the pailes that you know in advance that at the center of the pile effect of the microorganisms temperature increases to more than 70 ^{0}C under conditions of not solarization and under these circumstances the temperature in the first layers near surface temperature decreases depending on the region to less than the environment temperature (between 25 and 35 ^{0}C) so the solarization is necessary to kill pathogens (Salazar et al., 2010b)

As reported in the experiment is consistent with that reported by (Berwick, 2003) in which used a model to measure the variation of soil temperature under different factors of moisture in the soil, finding the maximum temperatures are recorded to the extent that the soil moisture is increased, was also marked by Mahrer (1979), where checks the solarization is a hydrothermal method and its success depends on the moisture available for greater transfer heat, maximum heat reaching the soil increases with the increase moisture of the same. Moisture levels showed have significant differences with a Pv = 0 0001 for what a mean test was performed to verify the behavior of their temperatures at the two depths of (0-7.5 Cm and 7.5 - 15 Cm) checking that the temperatures on the surface of the mounds varies sligthly of major minor but always in condition suitable for micro-organism kill pathogens (more than 55 0C).

Above is verified with what it describes (Salazar 2010ª) where reports onscreen manure have thermal cycles higher (more than 55 ° C) 15 cm deep (Figure 9, 10 and 11).

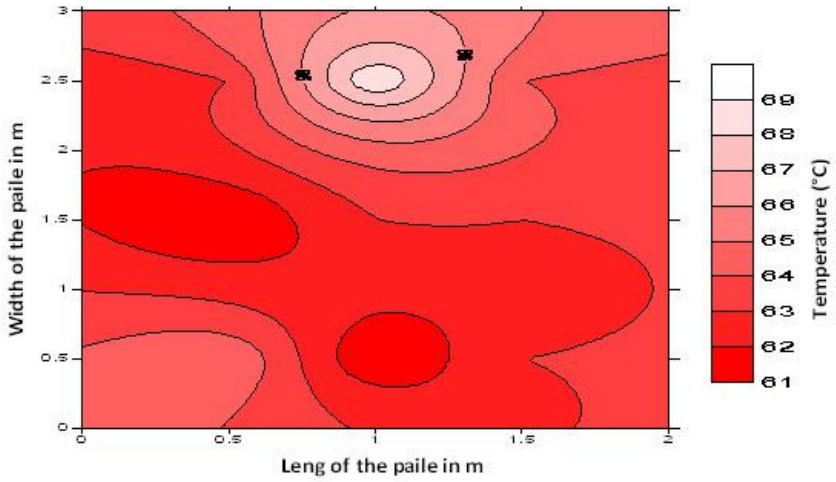


Figure 11. Average temperatures from May to June in 2009 at a depth of 30 cm.

CONCLUSION

The identification of Cryptosporidium and the Giardia Lamblia were negative in nine treatments and replicates in the two sampled depths, both for the depth of 0-7.5 cm to 7.5 to 15 cm. yet for the sample witness without solarized where you could see lots of cysts of cryptosporidium parvum and Giardia lamblia.

The depths do not interfere on the batteries' temperature to increase or decrease. The results are robust where employment of the solarization stands one acceptable temperature higher than 55 ° C and up to more than 71 ° C in spring - summer and were thus eliminated the two Protozoan present in manure not onscreen.

Moisture at 50 per cent factor is the component that more increases temperatures with an average of 71 ° C for spring-summer and summer-autumn 56.41 ° c following in this same period the 25% moisture with a 54.43 ° C average and finally the moisture factor witness (such as this newly brought manure in stable between 5 and 10% moisture content) with an average of 52.66 ° C.

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