International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:11, No:10, 2017

Use of Fruit Beetles, Waxworms Larvae and Tiger Worms in Waste Conditioning for Composting

Waleed S. Alwaneen

***Abstract*—**In many countries, cow dung is used as farm manure and for biogas production. Several bacterial strains associated with cow dung such as *Campylobacter*, *Salmonella* sp. and *Escherichia coli* cause serious human diseases. The objective of the present study was to investigate the use of insect larvae including fruit beetle, waxworms and tiger worms to improve the breakdown of agricultural wastes and reduce their pathogen loads. Fresh cow faeces were collected from a cattle farm and distributed into plastic boxes (100 g/box). Each box was provided with 10 larvae of fruit beetle, Waxworms and Tiger worms, respectively. There were 3 replicates in each treatment including the control. Bacteria were isolated weekly from both control and cow faeces to which larvae were added to determine the bacterial populations. Results revealed that the bacterial load was higher in the cow faeces treated with fruit beetles than in the control, while the bacterial load was lower in the cow faeces treated with waxworms and tiger worms than in the control. The activities of the fruit beetle larvae led to the cow faeces being liquefied which provided a more conducive growing media for bacteria. Therefore, higher bacterial load in the cow faeces treated with fruit beetle might be attributed to the liquefaction of cow faeces.

***Keywords*—**Fruit beetle, waxworms, tiger worms, waste conditioning, composting.

I. Introduction

LIKE other organisms, cows produce undigested residue of plant matter, which has passed through their gut. This dung material is rich in minerals and organic matter including undigested cellulose and lignin, originating from the cell walls of the plants and methane. In many countries dung is used as manure; it also may be collected and used to produce biogas methane [1].

Farm manure comprises the dung and urine of domestic animals, along with bedding material, as well as straw, peat, leaves, sawdust, shavings and other vegetable waste. Names for such material include manure, barn manure, stable manure, farmyard manure and barnyard manure [2]. Manure of various forms is one of the earliest materials to have been used for soil improvement. As well as providing small amounts of nutrients for plants, manure also supplies humus, greatly increasing the soil's water-holding capacity, whilst improving its physical character and making it a more suitable environment for the essential growth of bacteria [2].

An obvious problem with cow faeces when used as fertilizer relates to their potential microbial content, which might include pathogenic bacteria and parasitic worms, a problem which is clearly not found in relation to inorganic fertilizers. The average feedlot steer produces 1.62 kg of faeces (dry matter) per day, which results in more than 18 million metric tons of faeces (dry matter) per year in the United States alone. Enteric microbes of cattle affect animal health and food safety and can be used as an indicator of faecal pollution of drinking and recreational surface waters [3]. Pathogenic bacteria like *Escherichia coli* O157: H7 which are present in the bovine gastrointestinal tract have been linked to disease outbreaks due to the consumption of contaminated beef, milk, and drinking water. Pathogens are potentially released into the environment when bovine faecal waste is sprayed on farmland, or is accidentally discharged into the environment following severe storms, or onsite failure of waste management practices. Pathogens associated with such releases include *E. coli* O157:H7, *Campylobacter jejuni*, *Salmonella* spp., *Leptospira interrogans*, and *Cryptosporidium parvum* [4].

Faecal microbes play a critical role in animal health and productivity but also in food safety. Overall, the composition of the bacterial community correlated significantly with faecal starch concentrations, largely reflected in changes in the *Bacteroidetes*, *Proteobacteria*, and *Firmicutes populations* [3].

Comparatively few studies of the survival of enteric bacteria in cow pats on pasture have been reported [5]. Coliforms and faecal coliforms often survive for up to 18 weeks in cattle faeces in hot, dry summers and release of faecal coliforms and *Escherichia coli* from cattle faeces has been recorded for up to 30 days and 100 days. *Campylobacter* spp. have been detected in cattle faeces after incubation for 1 to 3 weeks at 5 °C and after incubation for 1 week at 30°C, while *Salmonella* spp. have been found for 12 weeks to 28 weeks after incubation at 5°C and for 4 weeks at 30°C. More recent survival studies have focused primarily on *E. coli* O157 and related strains because of the importance of cattle wastes as a major source of these pathogens. Most of these studies either have involved manure and slurries or have been laboratory based and have produced extinction times ranging from 24 h to 100 days, with faster inactivation generally associated with higher temperatures [5]. Few field-based studies of survival in cow pats have been conducted, although a 4- to 5-$\log_{10}$ decrease in *E. coli* O157:H7 within 50 days has been recorded in inoculated cow pats placed on grassland [5].

An attempt at *Escherichia coli* and *Salmonella enterica* reduction by using black soldier fly in larval stage in chicken manure has been studied by Erickson et al. [6]. The obtained results showed that the larvae accelerated inactivation of *E. coli* and *Salmonella* in chicken manure, either on autoclaved

Waleed S. Alwaneen (Assistant Professor, Faculty Member) is with the National Center for Agricultural Technology (NCAT), King Abdulaziz City for Science and Technology (KACST), P. O. Box 6086 Riyadh, Kingdom of Saudi Arabia (phone: 00966-555443614; e-mail: alwaneen@kacst.edu.sa).

or non-autoclaved manure.

The aim of the work described was to investigate the use of insect larvae (Fruit Beetle (FBL), Waxworms (WW) and Tiger worms (TW) to improve the breakdown of agricultural wastes and reduce their pathogen loads, hopefully leading to an improved material for use as a fertilizer or potting compost.

II. Materials and Methods

A. Methods for Bacterial Isolation and Identification

1) A selective medium of HiCrome *E. coli* Agar was used. It is based on Tryptone Bile Agar and is used to detect *Escherichia coli* in foods, where recovery of *E. coli* has been shown to be faster, more reliable and accurate. Most *E. coli* strains can be recognized from other coliforms by the presence of the enzyme glucuronidase which is highly specific for *E. coli*. The chromogenic agent X-glucuronide used in this medium helps to detect glucuronidase activity. *Escherichia coli* cells absorb X-glucuronide and the intracellular glucuronidase splits the bond between the chromophore and the glucuronide. The released chromophore gives the blue coloration of the colonies.

The medium is made as follows:

- Powder (36.6g) is suspended in 1000 ml of distilled water and sterilized by autoclaving at 121°C for 15 minutes.
- It was the cooled to 50°C and poured into sterile Petri dishes.

2) XLT-4 (Xylose Lactose Tergitol-4) Agar is a highly selective plating medium used for isolation and identification of Salmonellae from clinical, environmental and food samples [7]. The amino nitrogen, essential nutrients and vitamins are obtained from peptones and the yeast extract present in this formulation, ensuring optimal growth of Salmonellae. The selective agent Tergitol-4 (also known as Niaproof 4 or sodium tetradecyl sulphate) is an anionic surfactant. This largely hinders the growth of unwanted background flora. Due to the inclusion of phenol red, background colour of the plate is red, which results from pH changes due to fermentation and decarboxylation reactions. The fermentation of xylose, lactose and sucrose as well as the decarboxylation of lysine facilitates the differentiation on the medium. Due to the ability of *Salmonella* to reduce thiosulphate to hydrogen sulphide, Salmonellae appear as black or red colonies.

- Powder (59 g) of XLT-4 Agar Base was suspended in 1000 ml of distilled water
- XLT-4 Selective Supplement (4.6 ml) was added and the medium was boiled (avoiding overheating and autoclaving).
- Finally, the medium was cooled to approximately 50 °C and poured into sterile Petri dishes [7].

3) Nutrient Agar is a basic culture medium used to subculture organisms for maintenance purposes or to check the purity of subcultures from isolation plates prior to biochemical or serological tests. In semi-solid form, agar slopes, the medium is used to maintain control organisms [8]. It contains 1.5% agar to permit the addition of up to 10% blood or other biological fluid, as required. The medium, without additions, may be used for the cultivation of organisms which are not exacting in their nutritional requirements.

- Nutrient Agar (23 g, pH 6.8) was suspended in 1000 ml of distilled water and boiled; it was then autoclaved at 121 °C for 15 minutes.

B. Larvae, Worms and Cow Faeces: Collection and Incubation

Larvae and worms were purchased online from Ricks LiveFood and Original Organics Ltd. Fresh cow faeces were collected from one of Cattle Farm, and distributed in 12 plastic boxes (30×20×15 cm) with a lid perforated to allow for gas exchange, three control boxes and nine treatment boxes were set up. 100 larvae of Waxworms were then added. One hundred grams of cow faeces was added to each control box (three replicates). Fruit beetle larvae (10 larvae to 100 g cow faeces) were then added to each box (three replicates), and Tiger worms were then added, 40 worms to 100 g cow faeces in each box (three replicates). Approximately were used, as suggested by [9]. All samples were incubated at 25 ^{o}C throughout the incubation period.

C. Bacterial Isolation from Cow Faeces

Bacteria were isolated every seven days from both control cow faeces and cow faeces to which larvae were added; from zero time to 28 days in order to determine the populations of *E. coli, Salmonella* sp. and total bacteria count. Samples (1 g) were diluted in 9 ml of sterilized water then a serial dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) was performed and 0.1 ml of each diluted sample was spread onto *E. coli* agar, XLT4, and Plate Count Agar and incubated for between 18 h and 24 h at 37 ^{0}C.

D. Statistical Analyses

All observations were presented as means ±SE (standard error). Bacterial population numbers were converted to log CFU/g before statistical analysis. Statistical analyses were performed on experimental datasets using the t-test between two groups and comparing significance of means between treatment and control (P=0.05). Results were analysed using Sigma Plot 12.0© software.

III. Results and Discussion

Numbers of *E. coli* in the control samples declined steadily over the 28-day incubation period (Fig. 1 (A)). A decrease in the number of this bacterium also occurred in soils with added fruit beetle larvae (FBL), but from day 14 the number of *E. coli* was higher in the treated compared to control samples. The number of *Salmonella* fell sharply in the cow faeces at day seven and then decreased more slowly over the incubation period. From day seven, more *Salmonella* were found in the faeces treated with FBL than in the control (Fig. 1 (B)). The total heterotrophic bacterial count is shown in Fig. 1 (C). Numbers of bacteria declined in the untreated, control faeces, but remained higher in the FBL treated cow faeces over the

entire incubation period. The overall trend in these results was that the number of bacteria was higher in the cow faeces treated with fruit beetles than in the control.

The number of *E. coli* in the samples treated with Waxworms was less than the control over the 28 day incubation period (Fig. 2 (A)), but in day seven and day 14, the number of *E. coli* was less in the treated compared to control samples. The number of *Salmonella* fell sharply in the cow faeces at day 7 and the decreased more slowly over the incubation period. From day 7, less *Salmonella* were found in the faeces treated with Waxworms than in the control (Fig. 2 (B)). The total heterotrophic bacterial count is shown in Fig. 2 (C). Numbers of bacteria declined sharply in the cow faeces treated with Waxworms and control at day seven, and then decreased more slowly over the incubation period; however, throughout the incubation period, the number of bacteria in the control was higher than in the treatment. The overall trend in these results was that the number of bacteria was lower in the cow faeces treated with Waxworms than in the control.

The number of *E. coli* fell sharply in the cow faeces treated with Tiger worms was less than the control over the 28-day incubation period. In day seven, day 14 and day 21, the number of *E. coli* was very low in the treated compared to control samples (Fig. 3 (A)). Numbers of *Salmonella* fell sharply in the cow faeces at day seven and the decreased more slowly over the incubation period (Fig. 3 (B)). After day seven, less *Salmonella* were found in the faeces treated with Tiger worms than in the control. The total bacterial count is shown in (Fig. 3 (C)). Numbers of bacteria declined sharply in the cow faeces treated with Tiger worms and the control at day seven, and then decreased more slowly over the incubation period, but at day 14, the number of bacteria was close between treatment and control; however, throughout the incubation period the number of bacteria in the control was higher than in the treatment. The overall trend in these results was that the numbers of bacteria were higher in the cow faeces treated with Tiger worms than in the control.

It is not immediately apparent why the number of bacteria in cow faeces treated with fruit beetle larvae should be higher than the control, when the opposite is true of the Waxworm and Tiger worm treatments. It was noticeable, however, that the fruit beetle larvae were extremely active and moved around in the faeces much more so than did the larvae of the other two beetles. The activity and overall metabolism of the fruit beetle larvae led to the faeces being liquefied, an effect not seen with the other larvae. This liquefaction effect will be discussed in more detail below, as it may have important biotechnological implications. We assume the liquefaction was the main reason why bacterial numbers were higher in cow faeces treated with fruit beetle larvae, as this would have encouraged aeration and the tendency for bacterial numbers to be higher in liquid compared to solid faeces.

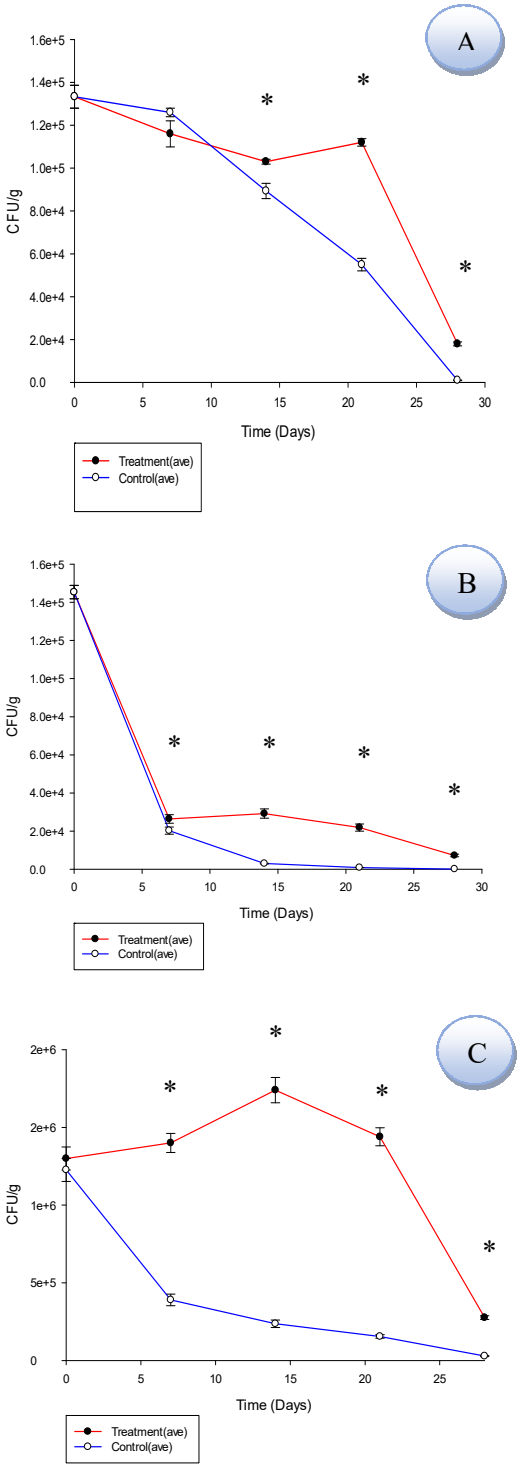

Fig. 1 Bacterial average numbers isolated from cow faeces treated with Fruit beetle, bacteria grown on; A. HiCrome *E. coli* medium B. *Salmonella* sp. medium and C plate count medium (*Significantly different from control)

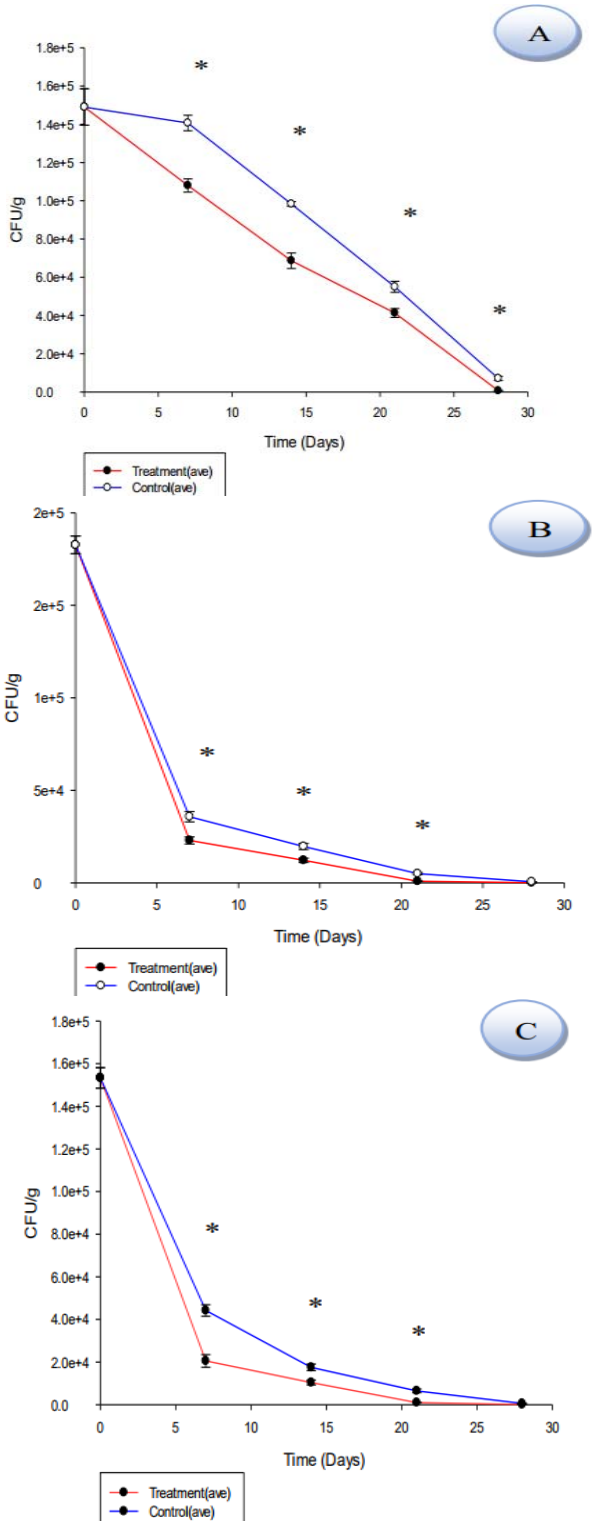

Fig. 2 The effect of Waxworm larvae on numbers (Average) of A *E. coli*, B *Salmonella* sp. and C bacterial total account in cow faeces (*Significantly different from control)

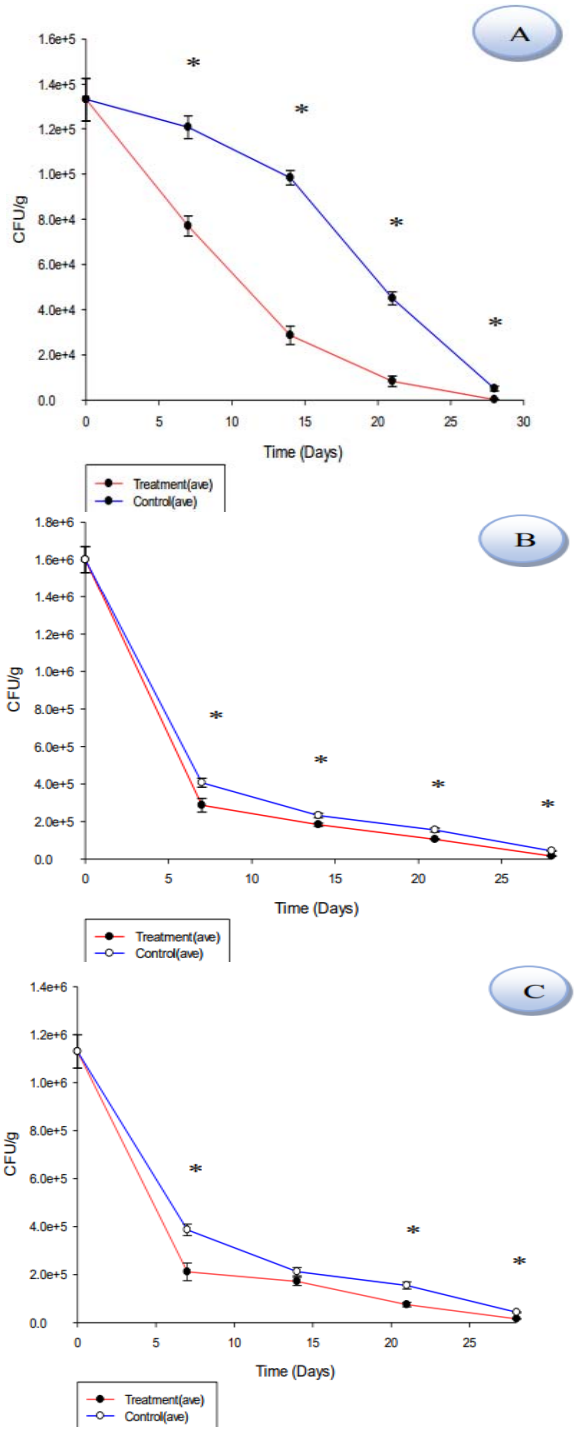

Fig. 3 Bacterial numbers (Average) isolated from cow faeces, bacteria grown on; A. E. coli selective media B. Salmonella Sp selective media and C plate count medium, treated with Tiger worms (*Significantly different from control)

IV. Conclusion

Numbers of all three bacteria were higher in the cow faeces treated with fruit beetles than in the control, while the number of all three bacteria was lower in the cow faeces treated with

Waxworms than in the control. Numbers were lower in cow faeces treated with Tiger worms than in the control. Liquefaction appears the main reason why bacterial numbers were higher in cow faeces treated with Fruit Beetle larvae.

ACKNOWLEDGMENT

The author gratefully thanks King Abdulaziz City for Science and Technology (KACST) for providing financial and other facilities for conducting the experiment.

REFERENCES

[1] Adegunloye, D. V., Adetuyi, F. C., Akinyosoye, F. A. and Doyeni, M. O. (2007). Microbial Analysis of Compost Using Cowdung as Booster. *Pakistan Journal of Nutrition*. 6: 506-510.
[2] Westover, H. L. (1926) *Farm Manures* p. 193-196.
[3] Shanks, O. C., C. A. Kelty, S. Archibeque, M. Jenkins, R. J. Newton, S. L. McLellan, S. M. Huse, and M. L. Sogin (2011). Community structure of cattle fecal bacteria from different animal feeding operations. *Applied and Environmental Microbiology*. 77:2992-3001.
[4] Gannon, V. P. J. *(*2004*)* Control of zoonotic waterborne pathogens in animal reservoirs. *In Waterborne Zoonoses: Identification, Causes and Control ed.* Cotruvo, J. A*,* Dufour, A.*,* Rees, G.*,* Bartram, J.*,* Carr, R.*,* Cliver, D. O*,* Craun, G. F*,* Fayer, R. *and* Gannon, V. P. J. *pp*. 380–408. *London, UK: IWA Publishing.*
[5] Sinton, L. W., R. R. Braithwaite, C. H. Hall, and M. L. Mackenzie. 2007. Survival of indicator and pathogenic bacteria in bovine faeces on pasture. *Applied and Environmental Microbiology*. 73:7917-7925.
[6] Erickson, M. C., M. Islam, C. Sheppard, J. Liao, and M. P. Doyle. 2004. Reduction of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar enteritidis in chicken manure by larvae of the black soldier fly. *Journal of Food Protection.* 67(4):685–690.
[7] Miller, R. G. and Tate, C. R. (1990). XLT4: A highly selective plating medium for the isolation of *Salmonella*. *Maryland Poultryman*, April p. 2-7.
[8] Lapage, S. P., Shelton J. E. and Mitchell T. G. (1970) *in Methods in Microbiology' Eds. Norris J. R. and Ribbons D. W. Vol.3A. Academic Press. London. p.* 116.
[9] Li, Q., Zheng, L., Qiu, N., Cai, H., Tomberlin, J. K. and Yu, Z. (2011). Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. *Waste Management* 31, 1316-1320.