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Laboratory Evaluation of *Bacillus subtilis* Bioactivity on *Musca domestica* (Linn) (Diptera: *Muscidae*) Larvae from Poultry Farms in South Western Nigeria

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Abstract**—**Muscid flies are known to be vectors of disease agents and species that annoy humans and domesticated animals. An example of these flies is *Musca domestica* (house fly) whose adult and immature stages occur in a variety of filthy organic substances including household garbage and animal manures. They contribute to microbial contamination of foods. It is therefore imperative to control these flies as a result of their role in Public health. The second and third instars of *Musca domestica* (Linn) were infected with varying cell loads of *Bacillus subtilis in vitro* for a period of 48 hours to evaluate its larvicidal activities. Mortality of the larvae increased with incubation period after treatment with the varying cell loads. Investigation revealed that the second instars larvae were more susceptible to treatment than the third instars treatments. Values obtained from the third instar group were significantly different ($P<0.05$) from those obtained from the second instars group in all the treatments. Lethal concentration (LC_{50}) at 24 hours for 2nd instars was 2.35 while LC_{50} at 48 hours was 4.31.This study revealed that *Bacillus subtilis* possess good larvicidal potential for use in the control of *Musca domestica* in poultry farms.

Keywords**—***Bacillus subtilis*, larvicidal activities, *Musca domestica*, poultry farms.

I. Introduction

MUSCA DOMESTICA (Linn) (housefly) is a domestic and well known cosmopolitan pest of both farms and homes. Vernacularly, it is called "Esinsin", "Kuda" and "Ezeze" in the three major languages in Nigeria (Yoruba, Hausa and Ibo respectively). Housefly is the most widely distributed insects found all over the world, most common of all domestic flies accounts for about 90% of all flies in human habitation and considered a pest that can transmit serious diseases. House flies are grey, approximately 6mm (1/4 inch) long, with four dark longitudinal stripes on top of the thorax, or middle body region [2]. The mouth parts of house fly are adapted for sponging up liquids.

Houseflies feed on faeces, open sore, sputum and moist decaying organic matter such as spoiled food, eggs and flesh [3]. The flies feed on liquid or semi liquid substances beside solid materials and pass it again to the abdomen. They deposit faeces constantly, one of the factors that make the insect a dangerous carrier of pathogens. The most important damage related with this insect is the annoyance and the indirect damage produced by the potential transmission of pathogens (viruses, bacteria and fungi) associated with it. Insect can pick up pathogenic organisms from garbage, sewage and other sources and then transferred on their mouthparts, through their vomits, faeces and other sources and then transferred to human and animal food, hence, are of medical importance [1]. Houseflies are most commonly linked to outbreak of food poisoning, diarrhea, shigellosis, anthrax, cholera, dysentery and parasitic worms such as *Ascaris lumbricoides* [18], [6], [15]. The breeding site suitability of this fly include horse manure, human excrement, cow manure, fermenting vegetables, kitchen waste and structures containing poultry, swine, sheep, cattle also incompletely composted manure are highly favoured sites for breeding [14].

The control of housefly is vital to human health and comfort in many areas of the world [7]. The control depends on their economic threshold density and tolerance. In sensitive environments such as restaurants, hospital small numbers of flies cannot be tolerated whereas in livestock or poultry production some flies are inevitable. Serious problems occur when cities or suburban development occur near poultry production facilities, as residents usually will not tolerate the large number of flies emanating from such facilities [7]. The chemical ways of controlling this fly is by application of adulticide or larvicides to directly or indirectly suppress adult densities, resistance to permethrin developed more rapidly in fly population from farm on a continuous permethrin regime [16]. Microbial insecticides offer effective alternatives for the control of many insect pests. Their strength is their safety, as they are essentially non-toxic and non-pathogenic to non-target organisms [19]. Microbial insecticides can be used successfully in place of more toxic insecticides to control insect-pests [10].

The use of biological control in fly management is still at a relatively early stage [9]. The objective of this study is *in vitro* assessment of the bioactivity of *Bacillus subtilis* on the different instars of *Musca domestica* larvae.

II. Materials and Methods

A. Breeding of the Insect Larvae

Chicken dung samples were collected into a sterile plastic container from the poultry farm of Department of Animal Production and Health of Federal University of Technology, Akure, Ondo – State. The dung samples were kept in a moist condition inside aluminium trays, placed in a cages constructed with little modification as described by [12]. The

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cages were 70x40x40cm with 2.8cm diameter ventilation ports and a 28x20cm access opening at the front. The ventilation ports were left unmeshed for easy access by house flies and the access opening was covered with wired net. The cages were located behind the Microbiology laboratory of Federal University of Technology, Akure, Ondo–State for easy accessibility of houseflies to enable them lay their eggs on the dung samples.

The cages were left undisturbed for 5 days after which the samples were visually observed for housefly larvae starting with the first instars to the third instars. Collection of the larvae instars were done inside sterile aluminium trays for bioassay.

B. Identification and Cultivation of the Entomopathogenic Bacterium

This study was conducted at the Department of Microbiology, Federal University of Technology Akure, Nigeria. *Bacillus subtilis* used in this study was isolated according to [13]. Identification of the bacterial isolate was done using cultural, morphological and biochemical characteristics according to the methods of [8]. A basal medium containing K_2HPO_4 (17.4g), NH_4SO_4 (1.98g), $MgSO_4$ (0.48g), $FeSO_4.7H_2O$ (0.0025g) and glucose (2.0g) in 100mL of sterile distilled water was used for the cultivation of this bacterium. The isolate was inoculated into10mL of sterile basal medium, incubated at 37^oC for 24h. The cells were centrifuged at 12.168x10^{3}g for 15min (Centrifuge MSE Minor 35) and re-suspended into 2mL sterile water. The cells were counted and diluted to obtain different concentrations.

C. Susceptibility of Musca domestica larvae to Bacillus subtilis

One hundred *Musca domestica* larvae were used for each concentration in this experiment. The larvae were surface sterilized in separate Petri dishes using 75% alcohol and sterile distilled water was used to rinse it three times to ensure total removal of the alcohol. There were four replicates and control per treatments with 25 housefly larvae in each container. Each larva was inoculated with the cells of bacterial isolate at varying cell loads. Incubation was carried out for 48h. Mortality of the larvae was physically monitored by checking their movement at 12 h interval using an applicator stick. The cadavers were rd.

D. Statistical Analysis

The second and third instars larvae data were analyzed using analysis of variance (ANOVA), and in the lethal concentration bioassay, the LC_{50} values were determined using the probit analysis for correlated data [4].

III. Results and Discussion

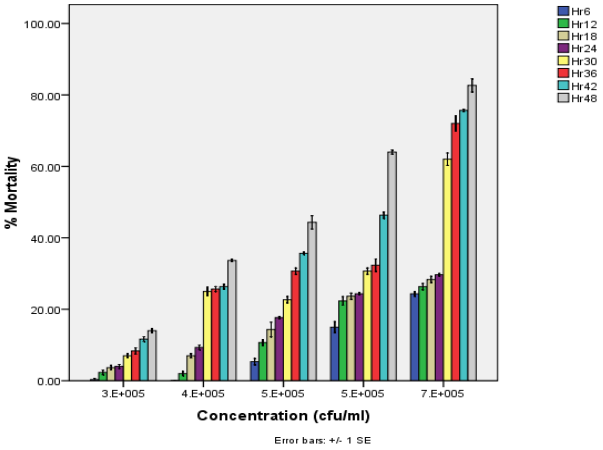

Fig. 1 Effect of different concentrations of *Bacillus subtilis* on the 2nd instars of housefly larvae at varied exposure time of post infection *Percentage mortality (%) against concentration (cfu/ml) *E - exponential

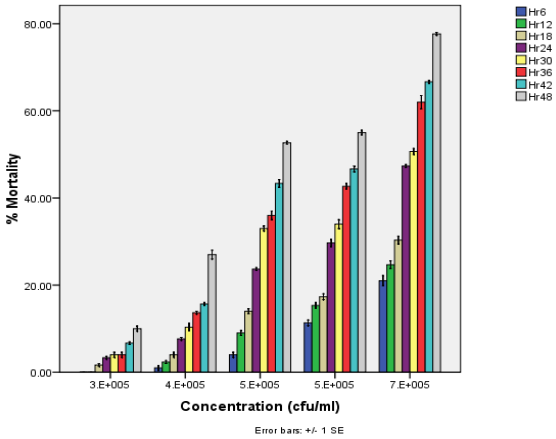

Fig. 2 Effect of different concentrations of *Bacillus subtilis* on the 3rd instars of housefly larvae at varied exposure time of post infection *Percentage mortality (%) against concentration (cfu/ml) *E - exponential

TABLE I
THE LC_{50} AND RESISTANCE RATIO OF 2ND AND 3RD INSTARS OF HOUSEFLY LARVAE AT 24 HR AND 48 HR OF POST INFECTION

Housefly instars	LC_{50}at 24 hours	LC_{50} at 48 hours	RR	RR*
2nd instars	2.35	4.31	1	1
3rd instars	5.89	9.36	2.50	2.17

LC_{50} – lethal concentration at which the bacterium kills 50% of the housefly larvae at a given time.
RR – Resistance ratio at 24 hours
RR*- Resistance ratio at 48 hours

The susceptibility of housefly larvae to *Bacillus subtilis* at varying concentration and time affect the morbid effect recorded in this study (Figs. 1 and 2). The percentage mortality recorded varied with the concentration of the bacteria cells. At the lowest concentration, 15% mortality was

recorded at 48 hrs of post infection while the highest concentration of 7.2 x 10^7cfu/ml used in this study was able to cause 85 % mortality at 48 hours of post treatment on the 2nd instars treatment (Fig. 1). On the 3rd instars treatment at both low and high concentrations, morbid effect was seen but not as high as that of the 2nd instars treatment. The highest percentage mortality was 78% mortality. This might be as a result of reduction in the feeding rate of the larvae in preparation for metamorphosis into the pupa stage. In Fig. 2, decrease in percentage mortality of the inoculated house fly larvae observed could be as a result of decrease in the ingestion rate due to the age of the larvae. This observation was in line with the fact that mature larvae stop feeding and burrow into drier surrounding areas where it pupates. The studied revealed that the second and the third instars were the feeding stages and thus ingestion of metabolic products that was made of spores and associated toxins from *Bacillus subtilis* disrupting the gut of the larvae that contained specific receptor which aided the activation of the toxin [11].

The larviciding period also contributed to the percentage mortality of the larvae. Figs. 1 and 2 showed that with increase in the hours of larviciding, the morbid effect was seen to increase, that is the larviciding period is directly proportional to percentage mortality. At 48h of exposure in both treatments (2nd and 3rd instars larvae treatments), gradual increase in percentage mortality was recorded and results from each treatment was significantly different from each other at $P \leq 0.005$ (Figs. 1 and 2). This might be as a result of variation in the feeding rate of the larvae which is in agreement with [5].

The relative potency of *Bacillus subtilis* on second and third instars of *Musca domenstica* was reported in Table I. The lethal concentration (LC) was seen to increase with time of exposure. In the second instars treatment, two folds of *Bacillus subtilis* was necessary to induce the same effect of 50% mortality (LC_{50}) on the larvae at 48 hrs of post treatment when compared to treatment after 24 hrswhile more than one folds of *Bacillus subtilis* concentration will cause the same effect after 48 hr of treatment in the third instars larvae. The observed difference in the susceptibility might be due to their ingestion rate [17]. Hence more mortality of the mosquito larva was recorded in the second instars treatments. Therefore, from the data obtained in this study, it could be inferred that the application of *Bacillus subtilis* on housefly larvae could significantly reduce the population of fly and consequently control the havoc they cause in disease transmission.

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