

# METHANOL MACERATIVES OF PRE-PUPAL STAGES OF BLACK SOLDIER FLY, *HERMETIA ILLUCENS* L. (DIPTERA: STRATIOMYIDAE) FOR THE GROWTH INHIBITION OF SELECTED SPECIES OF BACTERIA

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## Abstract

The attempt of the studies was carried with the aim of determination of the inhibition of the growth of three bacterial species (*Salmonella typhimurium* L., *Escherichia coli* L. and *Pseudomonas aeruginosa* L.) through the use of whole-body extractives of pre-pupal stages of the black-soldier-fly, *Hermetia illucens* L. (Order: Diptera; Family: Stratiomyidae). The whole-body extractives of pre-pupal stages of the black-soldier-fly, *Hermetia illucens* L. (Order: Diptera; Family: Stratiomyidae) was prepared through the method of maceration. Completely Randomized Design (CRD) was followed for the experimentation. The attempt of experiments was consisting of six-groups of the treatment with three replications each. The groups of the treatment in the attempt include six different concentrations of whole-body extractives of pre-pupal stages of the black-soldier-fly, *Hermetia illucens* L. (Order: Diptera; Family: Stratiomyidae) (50 mg per Lit.; 100 mg per Lit.; 150 mg per Lit.; 200 mg per Lit.; 250 mg per Lit. and 300 mg per Lit.). The antibiotic compound: chloramphenicol (thirty micrograms per disc-paper) was served as a positive control. The dimethyl sulfoxide was served as negative control. The zone of inhibition of the growth of bacterial species, *Salmonella typhimurium* (L.) through the use of extractives of pre-pupal stages of the black-soldier-fly, *Hermetia illucens* L. (Order: Diptera; Family: Stratiomyidae) with concentration of 50; 100; 150; 200; 250 and 300 ppm in the present was 7.28 mm; 7.91 mm; 9.13 mm; 10.49 mm; 11.73 mm and 12.03 mm respectively. The zone of inhibition of the growth of bacterial species, *Salmonella typhimurium* (L.) through the use of antibiotic compound: Chloramphenicol was 14.76 mm. The zone of inhibition of the growth of bacterial species, *Escherichia coli* (L.) through the use of extractives of pre-pupal stages of the black-soldier-fly, *Hermetia illucens* L. (Family: Diptera; Family: Stratiomyidae) with concentration of 50; 100; 150; 200; 250 and 300 ppm in the present was 7.13 mm; 8.92 mm; 9.74 mm; 9.86 mm; 10.03 mm and 10.88 mm respectively. The zone of inhibition of the growth of bacterial species, *Escherichia coli* (L.) through the use of antibiotic compound: Chloramphenicol was 27.64 mm. The zone of inhibition of the growth of bacterial species, *Pseudomonas aeruginosa* (L.) through the use of extractives of pre-pupal stages of the black-soldier-fly, *Hermetia illucens* L. (Order: Diptera; Family: Stratiomyidae) with concentration of 50; 100; 150; 200; 250 and 300 ppm in the present was 7.18 mm; 9.96 mm; 11.47 mm; 12.56 mm; 13.44 mm and 14.69 mm respectively. The zone of inhibition of the growth of bacterial species, *Pseudomonas aeruginosa* (L.) through the use of antibiotic compound: Chloramphenicol was 21.34 mm. The zone of inhibition of growth of the bacterial species was found increasing significantly ( $P < 0.05$ ) according to the concentration of extractives of pre-pupal stages of the black-soldier-fly, *Hermetia illucens* L. (Order: Diptera; Family: Stratiomyidae). The concentration of 300 ppm of extractives of pre-pupal stages of the black-soldier-fly, *Hermetia illucens* L. (Order: Diptera; Family: Stratiomyidae) was found effective for the inhibition of growth of bacterial species in the attempt.

**Keywords:** Antimicrobial Proteins (AMPs); *Hermetia illucens* L.; *Salmonella typhimurium* L., *Escherichia coli* L. and *Pseudomonas aeruginosa* L.

## Introduction

With reference to the antimicrobial proteins, the larval life stage and pre-pupal life stage of black-soldier-fly BSF, *Hermetia illucens* (L.) are potential source. According to Veldkamp, *et al.* (2012), the amino acids composition of proteins in the body of the larval life stage and pre-pupal life stage of black-soldier-fly BSF, *Hermetia illucens* (L.) are exhibiting resemblance to composition of the amino acids in the meal of soybean meal and the meal of fish. In addition to the significantly high content of the proteins, the larval and pre-pupal stages of black-soldier-fly BSF, *Hermetia illucens* (L.) are reported for suppressing the growth of bacteria. This bacterial growth suppressing action of black-soldier-fly is through the peptides of

“Antimicrobial Proteins (AMPs)” (Park, *et al.*, 2014). Kim and Rhee (2014) reported high content of lauric acid in the body of the larval and pre-pupal stages of black soldier fly BSF, *Hermetia illucens* (L.). The fatty acid, lauric acid, is recognized as the natural antimicrobial compound. Bovera, *et al.* (2016) opined the possibility of the content of chitin (polysaccharides) for the fortification of immunity in animals. Harlystiarini (2012) demonstrated the activity of suppressing the growth of the “Gram-negative bacteria” (*E. coli*) through the use of extractives of the body of the larval and pre-pupal stages of black-soldier-fly BSF, *Hermetia illucens* (L.). Jayanegara, *et al.* (2017) reported the possibility of use of the insect body as potential ingredient to be used through the

feed for ruminant animals: Biochemical constituents, in vitro fermentation in the rumen part of stomach and emission of methane gas. The activity of bio-compound pertaining “antibacterial” category deserve significant action in wealthy-healthy status and role in the development of organs of digestion in poultry birds. The activity of bio-compound pertaining “antibacterial” category help for the absorption of nutrients in the animals. The antibiotic compounds used in the animal feeds for the optimum growths are recognized as “Antibiotic Growth Promoters”. Prohibition of the utilization of “Antibiotic Growth Promoters” is due to their potentials of causing resistance. Increased microbial resistance to antibiotic compound is going to provide a grand opportunities for the purpose of obtaining antimicrobial compound, may be in the form of antimicrobial proteins (AMPs) through the use of the larval life stage and pre-pupal life stage of black-soldier-fly BSF, *Hermetia.illucens* (L.) like insects. The present attempt is planning to determine the potential of larval and pre-pupal stages of black-soldier-fly BSF, *Hermetia.illucens* (L.) for antimicrobial proteins (AMPs).

As the industry of black soldier fly (BSF) progresses into the scale of commercial production, there is possibility of continuity of the trend of increasing sophisticated and controlled growth. It is utmost important to note that, the facility of advanced nature with capability of prevention of infection from entering and to stop any spread of infections within facility. The defense against the insect pathogens and the solutions of the control established may eventually trickle down in part to the facilities of “less-advanced” category. However, the facilities of “Semi-Open” category may be preferable provided that, it should be designed properly. The insects use to multiply at higher rate in simple, semi-open facilities. The most significant and immediate responsibility of the farmers rearing the black soldier fly (BSF) is management of possible diseases through the garbage (waste material). The farmers rearing the black soldier fly (BSF) must send the samples of waste material and the samples of black soldier fly to the laboratory for diagnosis of pathogenic situations and further specific advices.

### Material And Method

(A). Nurturing (Rearing) of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae):

The present attempt on the rearing of the black soldier fly, *Hermetia.illucens* (Linnaeus) (Diptera: Stratiomyidae) in local environmental conditions of Baramati (India) has biology of was carried during

4 November, 2020 –28 February, 2021 in the insectary (Green House) of Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Tal. Baramati, Pune, India. The culture was initiated through keeping household organic waste (Kitchen Waste). The content of the organic waste (Kitchen waste) was with sour milk, waste tea powder, vegetable waste (cabbage and fruits of papaya). This content of the organic waste (Kitchen waste) was taken in a box and labelled as “tray with rearing bed” (or Larval Rearing Bin). This box (Larval Rearing Bin) was designed in the shape of a rectangular wooden box with the dimensions of 2x1.5x1.5 feet with ventilation holes on the top lid, and a rectangular plank was placed at an inclined position making an angle of 45° with the bottom so as to facilitate the process of harvesting (auto-harvesting) of full grown (matured) larvae. The fully grown (matured) larvae use to convert into next life stage (pre-pupa). Little amount of water was used to spray on the contents in a tray. Spraying the water on organic waste initiates the process of decomposition through bacteria. After a few days as the wastes began to decompose (Pedro, *et al.*, 2014). The fertilized egg mass of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) was procured from Mangal Agro Farm Miri Rd, Maka, Maharashtra 414501 India. The egg mass was kept suspended over fresh food (slices of fruits of papaya, *Carica papaya* L.). For uniform hatching, it requires a humid and cool place with fresh airflow. Hatching of the eggs take place within twenty-four of hours of provision of favourable conditions to the fertilized eggs.

On fifth day after hatching, the larvae from incubation box were transferred to box with rearing bed (Larval Rearing Bin). The larvae were allowed for feeding and their development. The mature stages of prepupa of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) were collected from this stock culture. The mature stages of prepupa of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) were transferred to the rearing cages once in three days. This transfer of the mature stages of prepupa of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) is to the rearing cages observe different life stages. The cages with the mature stages of prepupa of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) were placed inside the insectary (Green House) of Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Tal. Baramati, Pune, India. After three days, the stages of prepupa of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera;

Family: Stratiomyidae) from the rearing cages were placed in a small plastic bucket containing soil and kept inside the rearing cage in order to provide a place for pupation. The condition of humidity of the cage was maintained at 70-80 %. This was achieved through keeping a water source with sponges soaked in it as well as by spraying water three to four times a day (Briscoe and Chittka, 2001; Pedro, *et al.*, 2014; Zhang, *et al.*, 2010). The source of lighting was provided daily for twelve hours. The light provision is to stimulate adult mating. The card boards were made hung in various locations. The provision of the card boards is to mimic sites for laying the eggs by the adult female black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) (Pedro, *et al.*, 2014; Sheppard, *et al.*, 1994; Sheppard, *et al.*, 2002; Shields, 1982; Tomberlin Jeffery and Craig Sheppard, 2002). The observations were recorded on egg hatching; the period of development of the larval, pupal and adult stages and the morphology of the life stages. The sex ratio was determined through random sampling performance and observations of the genitalia of randomly collected adults.

For the purpose of determination of frequency of mating of newly emerged adult flies of the-black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) and egg laying capacity (fecundity), the sets of experimentation were ten in number. The sets of experimentations were identical and in the form plastic containers (capacity: 2 L). Newly emerged adult male and adult female were kept in pairs plastic containers. All these setups were then placed in the insectary (Green House) of Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Tal. Baramati, Pune, India. They were provided with artificial lighting (60W) and humidity (70-80 %) (Briscoe and Chittka, 2001; Zhang, *et al.*, 2010; Savonen Carol, 2005; Spranghers, *et al.*, 2017; Lalander, *et al.*, 2018; Wang, *et al.*, 2017). The observation on the determination of the egg laying sites (ovipositional sites); egg laying period (ovipositional period) and the life span was carried every twelve hours. The eggs were collected from this set up. The eggs were allowed to hatch under varying conditions. This attempt was for the purpose to determine period of incubation and the ability of the eggs to tolerate unfavourable temperatures. The eggs and larvae were collected daily from the rearing bin. Larvae were taken back to laboratory. The larvae were washed thoroughly to remove impurities. The larvae were knocked out by freezing and measured using coulometer to record total body length, body width, and length of mouth hook. The Dyar's rule was followed for the purpose of determination of

the morphometry of the larvae. It was carried through the determination of number of larval instars in its life cycle of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) (Craig Sheppard, *et al.*, 2002; Goldson, *et al.*, 2001; Mohammed, 2011; Bonelli Marco, *et al.*, 2020; Bruno Daniele, *et al.*, 2018; Holmes, *et al.*, 2013). The olfactometer was utilized for the determination of behaviour of the feeding and the preference of food waste by the larval stages of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae). For the purpose to get the consistency for the data processing for the results, each attempt was repeated at least for three times. The collected data was subjected for analysis through the statistical method.

(B). Preparation of Extractives of the Pre-pupal Life Stages of the Black-Soldier-Fly (BSF), *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae):

The mature pre-pupal life stages of the Black-Soldier-Fly (BSF), *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) were selected randomly from the stock culture. They were kept in freezer at -35°C for twenty-four hours. After twenty-four hours of freezing, they were subjected for thawing followed by washing thoroughly. The content was then processed for drying for forty-eight hours in oven (60 °C). Through the use of blender, the oven dried pre-pupal stages of the Black-Soldier-Fly (BSF), *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) were subjected for grinding until smooth. The content thus obtained was titled as, "Black-Soldier-Fly-Meal" (BSF Meal). For the purpose to prepare the extractives from "Black-Soldier-Fly-Meal" (BSF Meal), methanol was selected as solvent. Ten milligrams of "Black Soldier Fly Meal" (BSF Meal) were mixed in hundred millilitres of methanol. The contents were kept for twenty-four hours at room temperature for maceration. The method of obtaining extractives through the maceration belong to Choi, *et al.* (2012). After twenty-four hours of maceration, the content was filtered through the use of common laboratory filter paper. For the purpose to obtain extractives in concentrated form, the filtrate was subjected for evaporation. Rotary evaporator was utilized. This evaporator was with a reduced pressure and temperature of 40°C.

(C). Assay of Antimicrobial (Antibacterial) Activity of the Extractives of the Pre-pupal Life Stages of the Black-Soldier-Fly (BSF), *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae):



The assay of antimicrobial (antibacterial) activity of the extractives of the pre-pupal life stages of the Black-Soldier-Fly (BSF), *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) was carried-out in vitro. The bacterial species selected for the study include: *Salmonella typhimurium* (L.), *Escherichia coli*(L). and *Pseudomonas aureginosa* (L.). The cultures of bacterial species selected for the study (*Salmonella typhimurium* L., *Escherichia coli* L. and *Pseudomonas aureginosa* L.) are from Medical Microbiology Laboratories of Government Medical College and General Hospital, Baramati (Plot no. P-107, MIDC Area, Tal - Baramati, Dist - Pune - 413133, Maharashtra, India). The method explained by Nagappan, *et al.* (2011) was followed for the assay of antimicrobial (antibacterial) activity of the extractives of the pre-pupal stages of the Black-Soldier-Fly (BSF), *Hermetia-illucens* L. (Order: Diptera; Family: Stratiomyidae) through the method of agar diffusion by using paper discs. The bacterial species to be tested for assays were subjected for the subculturing first on the medium of "Tryptic Soya Agar" (TSA). The incubation of petri-plates at 35°C ( $\pm 1^\circ\text{C}$ ) for twenty-four hours. The bacterial species that have been subcultured that have grown on the medium of "Tryptic Soya Agar" (TSA) were converted into the suspensions. The bacterial population in suspensions was of 107 Colony-Forming-Unit (CFU / cfu).

Through the use of volumetric pipette, total suspension of 0.1 ml of was taken. The suspension of bacterial culture was transferred into the growth medium of "Muller-Hilton-Agar" (MHA). The suspension of bacterial culture was made into flat thickness on the surface of the growth medium of "Muller-Hilton-Agar" (MHA). For the purpose of flattening the bacterial suspension, separate bent glass rod was used. The "Muller-Hilton-Agar" (MHA) medium was kept at room temperature for about fifteen. Through the process of dissolving appropriate quantity of extractives of the pre-pupal stages of the Black-Soldier-Fly (BSF), *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) in known volume of dimethyl sulfoxide (DMSO), solution of six different strength were prepared. The desired strengths (concentrations) of the extractives of pre-pupal stages of the black-soldier-fly, *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) in the attempt include: 50 mg per Litre.; 100 mg per Litre.; 150 mg per Litre.; 200 mg per Litre.; 250 mg per Litre. and 300 mg per Litre. Sixty microliters of the extractives of pre-pupal stages of the black-soldier-fly, *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) from each strength were dripped on each disc-paper with the help of

micropipette and placed in petri-dish with sterile "Muller-Hilton-Agar" (MHA). Antibiotic: chloramphenicol (thirty micrograms per disc paper) was utilized as a group of positive control. The "Muller-Hinton-Agar" (MHA) in each petri dish was subjected to a bacterial suspension (of about  $1.5 \times 10^8$ ) to the entire surface. Care was taken for uniformness. Then all the petri-dishes were allowed to become dry through waiting for about an hour. All the petri-dishes were then subjected for incubation at 37 °C for 24 hours. The observations on antimicrobial (antibacterial) activity of the extractives of the pre-pupal life stages of the Black-Soldier-Fly (BSF), *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) were carried out at twenty-four hours after the period of incubation. Sensitivity of the microbial (bacterial) species to the extractives of the pre-pupal stages of the Black-Soldier-Fly (BSF), *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) was made confirmed with the observation. Sensitivity of the microbial (bacterial) species to extractives of the pre-pupal stages of the Black-Soldier-Fly (BSF), *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) was expressed by the wide diameter of zone of inhibition. The diameter of inhibitory zone diameters was measured through the use of digital calipers in millimeters (mm). For the purpose to get consistency in the results, the whole experimentation was repeated for three times.

#### (D). Statistical Analysis of the data:

The whole experimentation was repeated for three times. The data was collected and subjected for analysis through the statistical methods. In vitro-antibacterial activity of the extractives of pre-pupal life stages of the black-soldier-fly, *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) test data was analyzed through the use of "Analysis of Variance" (ANOVA). The level of confidence for the "Analysis of Variance" (ANOVA) was ninety-five percent ( $\alpha = 0.05$ ). Duncan test was followed for the data exhibiting significant difference.

### Results And Discussion

The results on the invitro antimicrobial (antibacterial) activity of the extractives of pre-pupal life stages of the black-soldier-fly, *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) are summarized in table-1 and presented in fig.1, fig. 2 and fig.3.

The diameter (mm) of zone of inhibition of the growth of bacterial species, *Salmonella typhimurium* (L.) through the use of extractives of pre-pupal life stages of the black-soldier-fly, *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) with concentrations of 50 ppm; 100

ppm; 150 ppm; 200 ppm; 250 ppm and 300 ppm in the present was 7.28 mm; 7.91 mm; 9.13 mm; 10.49 mm; 11.73 mm and 12.03 mm respectively. The diameter (mm) of zone of inhibition of the growth of bacterial species, *Salmonella typhimurium* (L.) through the use of antibiotic compound: Chloramphenicol was 14.76 mm (table-1 and Fig.1).

The diameter (mm) of zone of inhibition of the growth of bacterial species, *Escherichia coli* (L.) through the use of extractives of pre-pupal life stages of the black-soldier-fly, *Hermetia illucens* L. (Order: Diptera; Family: Stratiomyidae) with concentrations of 50 ppm; 100 ppm; 150 ppm; 200 ppm; 250 ppm and 300 ppm in the present was 7.13 mm; 8.92 mm; 9.74 mm; 9.86 mm; 10.03 mm and 10.88 mm respectively. The diameter (mm) of zone of inhibition of the growth of bacterial species, *Escherichia coli* (L.) through the use of antibiotic compound: Chloramphenicol was 27.64 mm (table-1 and Fig.2).

The diameter (mm) of zone of inhibition of the growth of bacterial species, *Pseudomonas aeruginosa* (L.) through the use of extractives of pre-pupal stages of the black-soldier-fly, *Hermetia illucens* L. (Order: Diptera; Family: Stratiomyidae) with concentration of 50 ppm; 100 ppm; 150 ppm; 200 ppm; 250 ppm and 300 ppm in the present was 7.18 mm; 9.96 mm; 11.47 mm; 12.56 mm; 13.44 mm and 14.69 mm respectively. The diameter (mm) of zone of inhibition of the growth of bacterial species, *Pseudomonas aeruginosa* (L.) through the use of antibiotic compound: Chloramphenicol was 21.34 mm (table-1 and Fig.3).

The zone of inhibition of growth of the bacterial species was found increasing significantly ( $P < 0.05$ ) according to the concentration of extractives of pre-pupal life stages of the black-soldier-fly, *Hermetia illucens* L. (Order: Diptera; Family: Stratiomyidae). The concentration of 300 ppm of extractives of pre-pupal stage stages of the black-soldier-fly, *Hermetia illucens* L. (Order: Diptera; Family: Stratiomyidae) was found effective for the

inhibition of growth of bacterial species in the attempt.

The diameter of zone of inhibition of growth of bacterial species, *Salmonella typhimurium* (L.); *Escherichia coli* (L.) and *Pseudomonas aeruginosa* (L.) for the negative control group of the present attempt exhibited significant differences with reference to the positive control group. The negative control group in the present attempt exhibited the absence of zone of inhibition of growth of the bacterial species. The positive control group in the present attempt exhibited the most significant of zone of inhibition of growth of the bacterial species.

Choi, *et al.* (2012) reported higher levels of sensitivities of the methanol extractives of black soldier fly (BSF) by the bacterial species of the "Gram Negative" group in comparison with the bacterial species of the "Gram Positive" group. The possibility of presence of specific ability of interaction of the wall of bacterial species to the active compounds present in the methanol extractives of black soldier fly (BSF) is maximum.

### Conclusion

The extractives of the pre-pupal stages of the black soldier fly (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae) has antibacterial activity against the growth of bacterial species in the attempt of studies (*Salmonella typhimurium* L., *Escherichia coli* L. and *Pseudomonas aeruginosa* L.). Increase in the concentration of extractives of the pre-pupal stages of the black soldier fly (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae) resulted in the tendency in increase in the diameter of zone of inhibition of growth of the bacterial species. The extractives of the pre-pupal stages of the black soldier fly (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae) with the concentration of 300 ppm was found most significant and effective for inhibition of growth of the bacterial species: *Salmonella typhimurium* (L.), *Escherichia coli* (L.) and *Pseudomonas aeruginosa* (L.).

Bacterial Species→ Group ↓ (Strength of BSF Extractives) (ppm)	<i>Salmonella typhimurium</i> (L.)	<i>Escherichia coli</i> (L.)	<i>Pseudomonas aeruginosa</i> (L.)
000 (Negative Control)	00.00	00.00	00.00
050	07.28(± 0.16 <sup>a</sup> )	07.13(± 0.27 <sup>a</sup> )	07.18(± 0.63 <sup>a</sup> )
100	07.91(± 0.23 <sup>b</sup> )	08.92(± 0.48 <sup>b</sup> )	09.96(± 0.77 <sup>b</sup> )
150	09.13(± 0.42 <sup>c</sup> )	09.74(± 0.53 <sup>c</sup> )	11.47(± 0.82 <sup>c</sup> )
200	10.49(± 1.07 <sup>d</sup> )	09.86(± 0.71 <sup>d</sup> )	12.56(± 1.23 <sup>d</sup> )
250	11.73(± 1.38 <sup>e</sup> )	10.03(± 1.89 <sup>e</sup> )	13.44(± 1.86 <sup>e</sup> )
300	12.03(± 1.79 <sup>f</sup> )	10.88(± 1.63 <sup>f</sup> )	14.69(± 2.07 <sup>f</sup> )
Antibiotic Compound: Chloramphenicol (Positive Control)	14.76 (± 0.49)	27.64 (± 0.57)	21.34(± 0.63)

- Each figure is the mean of the three replications.
- Figure with  $\pm$  sign in the bracket is standard deviation.
- The numbers followed by different letters (a,b,c,d,e,f) in the same column mean significantly different ( $P < 0.05$ ) extracts of BSF based on the Duncan test.

Figure-1: The zone of inhibition of growth (mm) of bacterial species, *Salmonella typhimurium* (L.) by the different concentrations (ppm) of the extracts of the pre-pupal stages of the Black-Soldier-Fly, *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae).

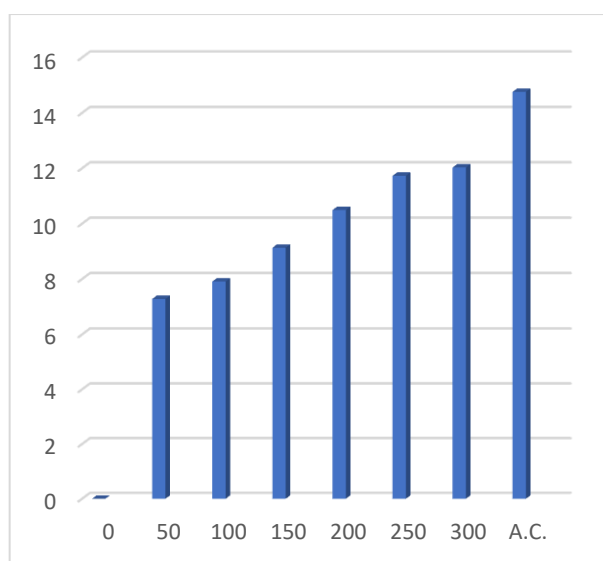


Figure-2: The zone of inhibition of growth (mm) of bacterial species, *Escherichia coli* (L.) by the different concentrations (ppm) of the extracts of the pre-pupal stages of the Black-Soldier-Fly, *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae).

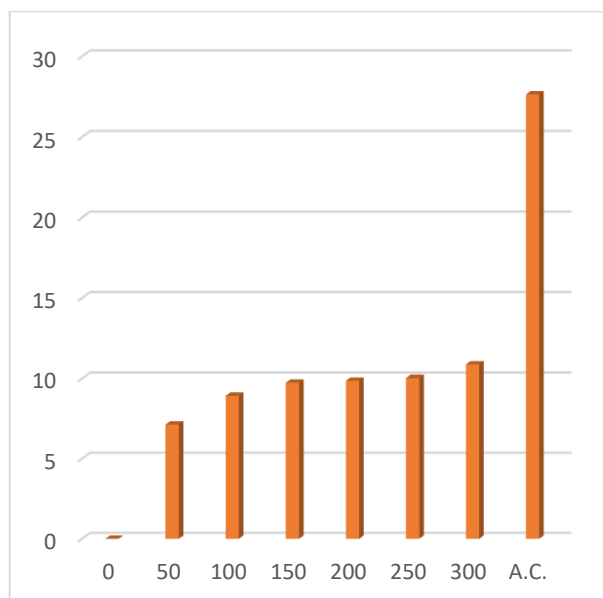
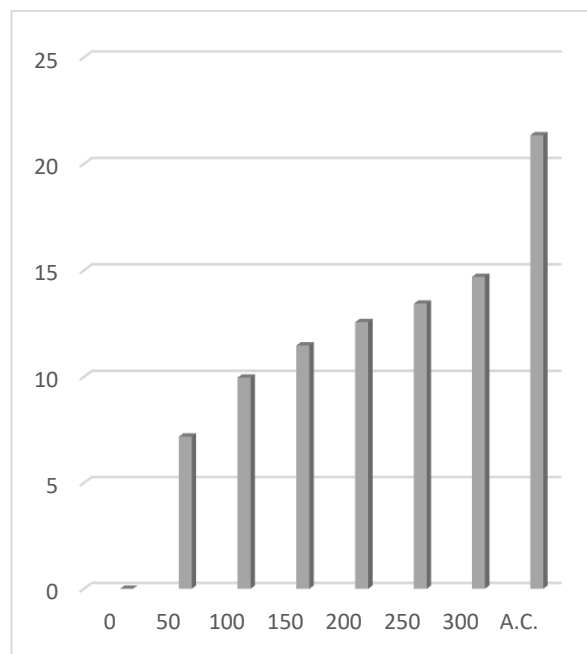


Figure-3: The zone of inhibition of growth (mm) of bacterial species, *Pseudomonas aureginosa* (L.) by the different concentrations (ppm) of the extracts of the pre-pupal stages of the Black-Soldier-Fly, *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae).



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