

**BIOLOGY OF *XENYLLA* SP.  
(COLLEMBOLA: HYPOGASTRURIDAE)  
AND EFFECTS OF EFFECTIVE MICROORGANISM (EM™)  
ON ITS DEMOGRAPHY**

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OF THE REQUIREMENTS FOR  
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Thesis  
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**BIOLOGY OF *XENYLLA* SP. (COLLEMBOLA: HYPOGASTRURIDAE) AND EFFECTS OF EFFECTIVE MICROORGANISM (EM<sup>TM</sup>) ON ITS DEMOGRAPHY**

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THESIS ADVISORY COMMITTEE: VACHAROBON THIRAKHUPT, Ph.D.,  
BOONGEUA VAJARASATHIRA, Ph.D., CHARNWIT KOSITANONT, Ph.D.**ABSTRACT**

Currently, Effective Microorganism (EM<sup>TM</sup>) is widely used to increase crop yields in Thailand. However, there is little knowledge and no scientific proof on the influence of EM and the mechanisms on soil organisms, especially soil microarthropods which serve as bioindicators of soil fertility. A species of springtail in the genus *Xenylla* was extracted from Nakhon Pathom Province area and mass-cultured in bottles containing plaster of Paris-charcoal substrate and baker's yeast as food under 25-28 °C. Observations under stereo microscope were made and their life history as well as fecundity were recorded. The life history consists of three developmental stages – egg, juvenile and adult – which took an average of 21.67 days to complete. The egg stage lasted 6.44 days whereas the juvenile stages I to VI were 4.44, 2.14, 2.09, 2.21, 2.27 and 2.26 days, respectively. The first oviposition was as early as 12 – 16 days after hatching and the fecundity was 137.07 on average. The mean longevity of an adult was 64.27 days. Sexes could not be differentiated by morphological characters before the adult reached 30 days after hatching. That was when females were visibly larger in body size and the body color was bright yellow. Males of the same age had a darker body due to the distribution of pigments. Histological methods revealed a pair of ovaries in each female. Sex determination is an underlying problem for most springtails. For this species reared in the laboratory, it is possible to use the combination of body size and color to differentiate the sexes. Based on the life table, the means of life table statistics ( $R_0$ ,  $\lambda$ ,  $r_c$ ,  $r_m$ ,  $T$ ,  $T_c$  and  $D$ ) were obtained as 17.08, 1.09, 0.07, 0.08, 33.52, 37.12 and 8.54, respectively, and survivorship curve is of Type I. The influence of EM1 caused a decrease in percentage of eggs hatching and  $e_x$  and delayed developmental time from juvenile stage I to adult when compared to the control ( $p < 0.05$ ). This is probably due to a rapid growth of fungi that covered egg surfaces, in addition to the lower relative humidity (RH) and accumulation of metabolic wastes. The effects of EM1 and EM5 were not significantly different. Vinegar had no effects on the egg hatching, but promoted higher fecundity ( $R_0$ ), while effects of rice whiskey were similar to EM1. Based on this study, EM does not have any positive effects in the population of *Xenylla* sp., but this does not guarantee negligible effects in the field.

**KEY WORDS: *XENYLLA*/ COLLEMBOLA/ SPRINGTAIL/ DEMOGRAPHY/  
EFFECTIVE MICROORGANISM/ BIOLOGY/ LIFE TABLE**

163 pp.

ชีววิทยาของ *Xenylla* sp. (Collembola: Hypogastruridae) และผลของจุลินทรีย์ที่มีประโยชน์ (อีเอ็ม) ต่อ  
ค่าสถิติประชากร

(BIOLOGY OF *XENYLLA* SP. (COLLEMBOLA: HYPOGASTRURIDAE) AND EFFECTS  
OF EFFECTIVE MICROORGANISM (EM<sup>TM</sup>) ON ITS DEMOGRAPHY)

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### บทคัดย่อ

การนำจุลินทรีย์ที่มีประโยชน์ (อีเอ็ม) มาใช้ในการทำเกษตรกรรมเพื่อเพิ่มผลผลิตเป็นวิธีที่กำลังเป็นที่นิยม  
กันอย่างแพร่หลายในปัจจุบัน แต่งานวิจัยทางวิทยาศาสตร์เพื่อศึกษากลไกการทำงานหรือผลกระทบต่อสัตว์ในดินยังมี  
น้อยมาก โดยเฉพาะอย่างยิ่งผลต่อสัตว์ขาปล้องขนาดเล็กในดินซึ่งใช้เป็นดัชนีชีวภาพของความอุดมสมบูรณ์ของดิน  
แมลงที่ใช้ในการทดลองคือแมลงหางคืด *Xenylla* sp. ซึ่งสกัดจากดินในพื้นที่จังหวัดนครปฐมในขุดเลี้ยงที่มีซบส  
เตรตเป็นปุ๋ยพลาสเตอร์ผสมน้ำและถ่านกัมมันต์ และให้ยีสต์เป็นอาหารภายใต้อุณหภูมิ 25 – 28 องศาเซลเซียส พบว่า  
วัฏจักรชีวิตสมบูรณ์ภายในเวลาเฉลี่ย 21.67 วัน มีระยะการเจริญ 3 ระยะคือ ระยะไข่ (6.44 วัน) ระยะตัวอ่อนมี 6 ระยะ  
(4.44, 2.14, 2.09, 2.21, 2.27 และ 2.26 วัน) และระยะตัวเต็มวัย ซึ่งเริ่มวางไข่ครั้งแรกในวันที่ 12 ถึงวันที่ 16 หลังจาก  
ฟัก และสามารถวางไข่โดยเฉลี่ย 137.07 ฟองต่อตัว ตัวเต็มวัยมีอายุขัยเฉลี่ย 64.27 วัน และสามารถระบุเพศของตัวเต็ม  
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วัดจำนวนมากกระจายอยู่ตามลำตัว จากการตรวจสอบทางมิถุนวิทยาพบรังไข่หนึ่งคู่ในตัวเต็มวัยที่มีสีเหลืองและมี  
ลำตัวยาวกว่า 1 มม. ค่าเฉลี่ยของพารามิเตอร์ที่ประมาณจากตารางชีวิตได้แก่  $R_0$ ,  $\lambda$ ,  $r_c$ ,  $r_m$ ,  $T$ ,  $T_c$  และ  $D$  มีค่า  
เท่ากับ 17.08, 1.09, 0.07, 0.08, 33.52, 37.12 และ 8.54 ตามลำดับ และพบว่าเส้นกราฟการรอดชีวิตมีลักษณะคล้าย  
แบบที่ 1 ส่วนผลการศึกษาอิทธิพลของอีเอ็มต่อแมลงหางคืด พบว่า การฟักของไข่และค่า  $e_x$  ลดลงและระยะเวลาใน  
การพัฒนาจากตัวอ่อนเข้าสู่ระยะตัวเต็มวัยเพิ่มขึ้นอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับชุดการทดลองควบคุม ( $p < 0.05$ )  
ทั้งนี้เนื่องจากการเจริญอย่างรวดเร็วของเชื้อราบนไข่ และการเกิดสภาวะที่ไม่เหมาะสมต่อการเจริญของแมลงหางคืด  
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อีเอ็มอย่างมีนัยสำคัญ จากผลการทดสอบน้ำส้มสายชูกับเหล่าขาวซึ่งเป็นส่วนประกอบของอีเอ็ม 5 พบว่า น้ำส้มสายชู  
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สรุปในภาพรวม อีเอ็มไม่มีผลในการเพิ่มประชากรของแมลงหางคืด *Xenylla* sp. ในห้องปฏิบัติการแต่ผลการ  
ทดลองอาจแตกต่างในการปฏิบัติการในภาคสนาม

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**LIST OF ABBREVIATIONS**

%	percent
/	per
<i>et al</i>	(Latin) and others
C	degree Celsius
pH	per Hydrogen
cm <sup>2</sup>	square centimeter
ATP	adenosine triphosphate
cm	centimeter
mm	millimeter
μm	micrometer
g	gram
kg	kilogram
mg	milligram
μg	microgram
Ant.	antenna
Abd.	abdomen
Th.	thorax
SE	standard error of mean
EM	effective microorganism
EM5	Sutoju
V	vinegar
RW	rice whiskey

## **CHAPTER I**

### **INTRODUCTION**

The application of Effective Microorganism™ (EM™) is a natural practice in agriculture that does not use chemicals and was developed by Dr. Teruo Higa of Ryukyus University in Japan in 1980s. The main concept is to increase the beneficial microbial diversity in soil. As a result, microbial organisms added would help improve the soil conditions, suppress pathogenic microorganisms, and increase the efficiency of organic matter utilization by plants. Recently, EM has been widely used not only for agricultural purpose, but also in household, livestock and fishery for reducing notorious odor and improving health conditions. In Thailand, EM was introduced in 1989 and has been applied in agricultural soil by mixing with plant-extracted substances such as chili pepper, mint, neem and grass or mixing with vinegar and rice whiskey as a nutrient supplement to crops and to suppress pathogens and pests (Higa, 1999). In general, EM is used as the basic ingredient and various substances, mostly natural products, are added in order to show better performance in a particular way. Consequently, a number of formulae have been initiated for different purposes or different types of microorganisms. The names of formulae were designated without definite rules. For example, EM2, EM3 and EM4 are formulae of EM that mainly contain a group of fungi, photosynthetic bacteria as well as lactobacillus and yeasts, respectively, while EM-X was designed for improving health conditions of livestock and human. The users could purchase the stock of EM and produce their own formula or could purchase the ready-made ones of their choice.

In Thailand, there are a large number of different commercial formulae in the market for different applications. Public acceptance and satisfaction for the products are generally on the positive side, possibly based on the better performance of the crops. Rice, rubber trees, palm, cabbages, radish and cucumbers are examples of the target crops (O et al., 2008; Wood et al., 2008; Yadav, 2008).

One of the most widely-used locally is EM5 or Sutoju as it is commonly called in Japan. EM5 consists of stock EM mixing with vinegar and rice whiskey. It is advertised as the formula to be used for repelling pests (Higa, 1999; Higa & Chinen, 1998). Even though a number of works have been done to assess the impact of EM on agriculture, there has been no scientific proof of their efficiency and mechanisms. To date, no published research papers on the possible mechanisms of how it helps increase crop yields or how it promotes plant growth - among others. Or else, it might have indirect effects on the beneficial soil organisms that have significant roles in the turnover of organic matters. The question is whether the EM promotes the population growth of soil animals and, in turn, help increase the organic matters and improve soil quality. Better soil quality might lead to the improvement of the plants such as high yield, growth rate and resistance to pests. One group of animals which deserves such attention is the springtails which are generally believed to be a bioindicator of fertile soil.

Springtails are minute wingless hexapods that often have a jumping apparatus called furcula located on the abdomen. They belong to the order Collembola. Most species live in soil, leaf litter and refuse with high humidity. In nature, springtails are among the most numerous of forest soil invertebrates. Through their feeding activities, springtails and other soil animals contribute to the decomposition of litter and soil formation (Swift et al., 1979). Due to their high abundance and productivity, these groups are regarded as important links in biomass transfer in the forest soil (Ernsting & Joosse, 1974; Petersen & Luxton, 1982; Van Straalen, 1989). Furthermore, according to the evidences of gut analysis of springtails, fungal hyphae have been found to constitute a large fraction of the diet of many collembolan species (Moore et al., 1987; Newell, 1984). It was also found that springtails preferred some fungal species to others. There were evidences that those fed with favorable fungi had higher growth rate and reproduction.

Recently, study on the suitability of fungi as food for springtails has highlighted the importance of the species of fungus involved and its life stage as well. For example, Chen et al. (1995) reported that *Protaphorura armatus* and *Tullbergia granulata* preferred *Mucor* sp. and *Absidia* sp. over *Penicillium* sp., *Humicola* sp. and actinomycete and preferred foods resulted in higher egg production and survival.

Due to the characteristics of springtails and their habitats, changes in the soil ecosystem caused by insecticides are liable to influence the population dynamics of collembolans directly and indirectly. Collembolans *Folsomia candida*, *Sinella coeca*, *Paronychiurus kimi* and *Xenylla* spp. were used as test organisms when assessing the toxicity of contaminants to soil fauna (Crommentuijn et al., 1993; Fountain & Hopkin, 2001; Kang et al., 2001; Menta et al., 2006). As it is known, changes in the concentration of some pollutants such as metal can modify the species diversity and the density of collembolans. Apart from altering the density of collembolans, they can still influence the biology and reduce the survival potential, the rate of growth and the reproduction of species which are more sensitive to these elements. In many cases, this effect is dose-dependent. The growth rate and the survival of *Onychiurus armatus* decreased significantly with increasing Cu and Zn concentrations (Tranvik et al., 1993). The growth response of *Orchesella cincta* fed with food containing lead (Joosse & Verhoef, 1983) or iron (Nottrot et al., 1987) appeared to be significantly slower than control and the duration of the molting interval was found to be shorter in lead contaminated conditions (Joosse & Verhoef, 1983). Since there are a number of factors involved in collembolan survival, the impacts of insecticides in soil ecological systems cannot be fully understood by determining only the acute mortality. The study in more details of key parameters which are the components of a life table may reveal responses or changes in collembolan biology, even though the overall performance remain unchanged.

Consequently, this experiment aims to evaluate the influence of Effective Microorganism (EM<sup>TM</sup>) on the biology of a *Xenylla* sp. on the basis of demographic study under laboratory conditions. Two commercial formulae –EM1 and EM5– were used. The effects were compared between those two and compared to the control. However, as the identification of this species revealed the possibility of a new species, the description was made in details and the general biology was recorded. The data were used for comparison with the treated groups.

**The objectives of this study are as follows:**

1. To observe the biology of *Xenylla* sp. under laboratory conditions.
2. To test the effects of EM on the biology of *Xenylla* sp.
3. To test the effects of EM5 on the biology of *Xenylla* sp.
4. To compare the effects of EM and EM5 on the biology of *Xenylla* sp.

## CHAPTER II

### LITERATURE REVIEW

#### 2.1 Effective Microorganism™

EM™ is an acronym named by its developer, Dr. Teruo Higa, consisting of the initial letters of "Effective Microorganisms™". As a commercial product, it is marketed and sold as "EM•1®" by authorized licensees around the world. EM•1® is a liquid containing many co-existing microorganisms. The major groups of microorganisms in EM•1® are photosynthetic bacteria, lactic acid bacteria, yeast, actinomycetes and fungi.

- **Photosynthetic bacteria:** The phototrophic bacteria are distinguished from other bacterial groups by their ability to use light energy to drive the synthesis of ATP (Stanier et al., 1981).
- **Lactic acid bacteria:** The lactic acid bacteria are gram-positive rods and cocci that produce lactic acid as a major end product during the fermentation of carbohydrates. The members of this group consist of *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, and *Pediococcus*.
- **Yeasts:** Yeasts are single-celled fungi. They can be spherical, oval or cylindrical and are usually 3 to 5 µm in diameter. They are important because of their ability to ferment carbohydrates in various substances, especially genus *Saccharomyces*, the chief agents in alcoholic fermentation.
- **Actinomycetes:** The actinomycetes are a large group of filamentous, gram-positive bacteria that form branching, a ramifying network of filaments is formed, called a mycelium. They are widely distributed in nature and can comprise a significant proportion of the bacterial population in the soil, about 10% - 33 % (Alexander, 1958; Hattori & Hattori, 1973). Actinomycetes are relatively resistant to desiccation and can survive under conditions of drought in desert soils. They favor alkaline or neutral pH and are sensitive to acidity.

The main ecological role of actinomycetes appears to be the decomposition of organic matters in soil. Besides, many actinomycetes produce antibiotics, which in addition to their medical importance, may play role in ecological relationships among differing microbial populations.

- **Fungi:** Fungi are a group of diverse, unicellular and multicellular eukaryotic organisms, lacking chlorophyll, often filamentous and spore-producing. In the soil environment, most fungi are opportunistic and important as food sources, pathogens, beneficial symbionts, saprophytes to degrade crop residues, and biotic agents to improve soil structure and aeration. Some fungi are pathogenic to humans and other animals. Such diseases are called mycoses or fungal infections. Saprophytes are relatively unspecialized, producing enzymes that degrade complex organic compounds to simple sugars or other forms for absorption (Sylvia et al., 2005). In addition, some of these fungi can be used as biopesticides, like the ones that kill insects (entomopathogenic fungi). Specific examples of fungi that have been developed as bioinsecticides are *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus* and *Verticillium lecanii* (Alexander, 1958).

### 2.1.1 Application of EM

Currently, EM is used for several proposes, for example, water treatment, soil quality and health improvement such as reducing odor, decomposing wastes, and increasing the resistance to diseases and pests of animal husbandry. In Thailand, EM technology participates in sustainable agricultural development. Several products of EM as well as home-made EM were spread over the agricultural areas and wastewater treatment plants. EM can be applied in four ways as EM1 stock solution, EM5 solution, EM Bokashi and EM fermented plant extract. Only EM1 and EM5 will be reviewed here (Higa, 1999; Higa & Chinen, 1998).

#### 2.1.1.1 EM1 stock solution

This solution involves the proliferation of microorganisms by molasses. This is done by mixing EM, molasses and water in the ratio 1: 1: 20 by volume and

then fermenting at room temperature for seven days. It is used by spraying onto the plants or into the soils (1-2 times per week).

#### **2.1.1.2 EM5 or Sutoju**

EM5 is a fermented mixture of vinegar, rice whisky, molass and EM. It is used by spraying the plants to suppress pathogens and keep insect pests away. It is usually sprayed onto plants at a dilution of 1/200 – 1/500 in water once a week. The ingredient of EM5 is as follows: EM, molasses, rice whisky, vinegar and water in the ratio of 1: 1: 1: 1: 6 by volume. The mixed solution is stored at room temperature and is fermented 15 days before using.

#### **2.1.2 Researches on EM**

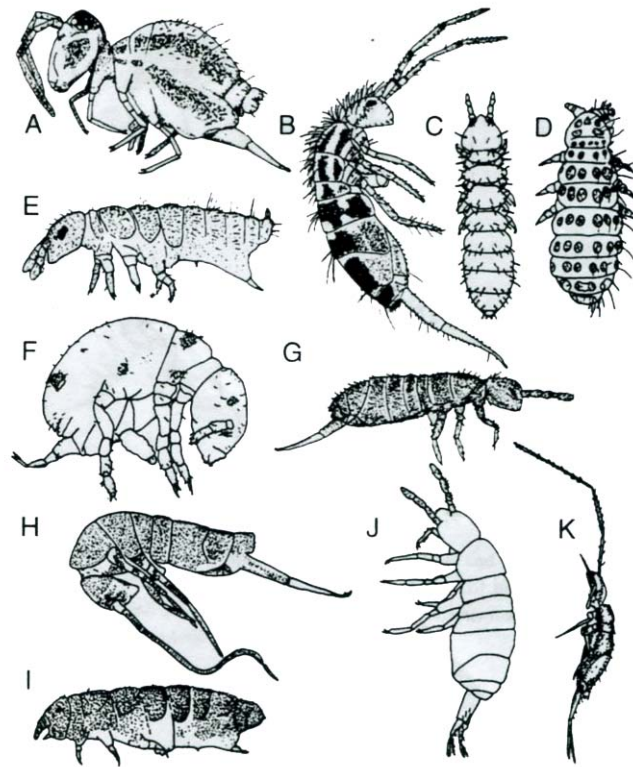
Although EM is widely used around the world, there have been very few researches on EM. Most researches emphasized on the effects on high yield of plants, animal health and environment. It was found that EM helps to increase yield, improve health and reduce waste (Ding et al., 2001; He et al., 2005; Javaid, 2006; Khaliq et al., 2006). However, from the quantity analysis of nutrients in EM, it was found that the contents of N, P and K were inadequate for using as fertilizer, while the amount of their elements in bokashi were similar to the other fertilizers (Jiwajinda et al., 1996). Moreover, the reports about the impacts of EM on insects are rare. Wood et al. (2008) reported that the applications of EM in organic cucumber resulted in a 47.75 % increase in yield over the controls and EM5 caused 88.75 % decrease of pickle worm infection when compared with control. Besides, Jiwajinda et al. (1996) reported that the effectiveness of pesticidal EM5 on mortality of *Plutella xylostella* larvae was higher than the controls, while EM without vinegar has no effects.

## **2.2 Springtails (Order Collembola)**

Springtails are among the most abundant of soil-dwelling arthropods. They are considered to be primitive insects on the basis of their having, like insects, a pair of antennae inserted into the head, a thorax with three pairs of legs, and a segmented abdomen with appendages. However, they differ from insects in numerous features,

for example, soft body, entognathous mouthparts (the mouthparts concealed into head capsule), wingless and lack of Malpighian tubules. Although they are minute insects of between 0.4 and 10 mm of body length, various body forms are shown in Fig. 2-1 (Christiansen & Bellinger, 1998, Heckman, 2001, Hopkin, 1997, Greenslade, 1991).

According to the body forms, springtails can be divided into two suborders: Arthropleona and Symphypleona. The former has a long and cylindrical body form (Fig. 2-2A). Members of this group (about 5,500 species) range from highly active surface-dwelling species to those that live out all their lives in the depths of the soil (Christiansen & Bellinger, 1998, Heckman, 2001). An example of this suborder is *Folsomia candida* which belongs to the family Isotomidae. It is familiar to ecotoxicologists as one of a suit of “standard test soil organisms”, which are used to assess the toxicity of new chemicals before they are released into the environment (Hopkin, 1997). The latter has a short and globular body form (Fig. 2-2B). They include about 1000 species and are mostly attractively patterned, surface-living species. A typical example is *Dicyrtomina ornata* (subfamily Dicyrtominae). Recently, the third suborder, Neelipleona, has been introduced, including only one family, Neelidae. They are very small soil-inhabiting springtails (typically 0.5 mm in length) with only about 25 species known around the world. They have a rounded body shape and bear a superficial resemblance to Symphypleona, but the globular body is formed largely by expansion of the thoracic rather than abdominal segments (Hopkin, 1997).



**Figure 2-1** Variety of collembolan body forms A. Sminthuridae B. Entomobryidae C. Onychiuridae D. Neanuridae E. Hypogastruridae F. Neelidae G. Isotomidae I. Odontellidae J. Oncopoduridae K. Paronellidae (Christiansen & Bellinger, 1998)

### 2.2.1 Morphology

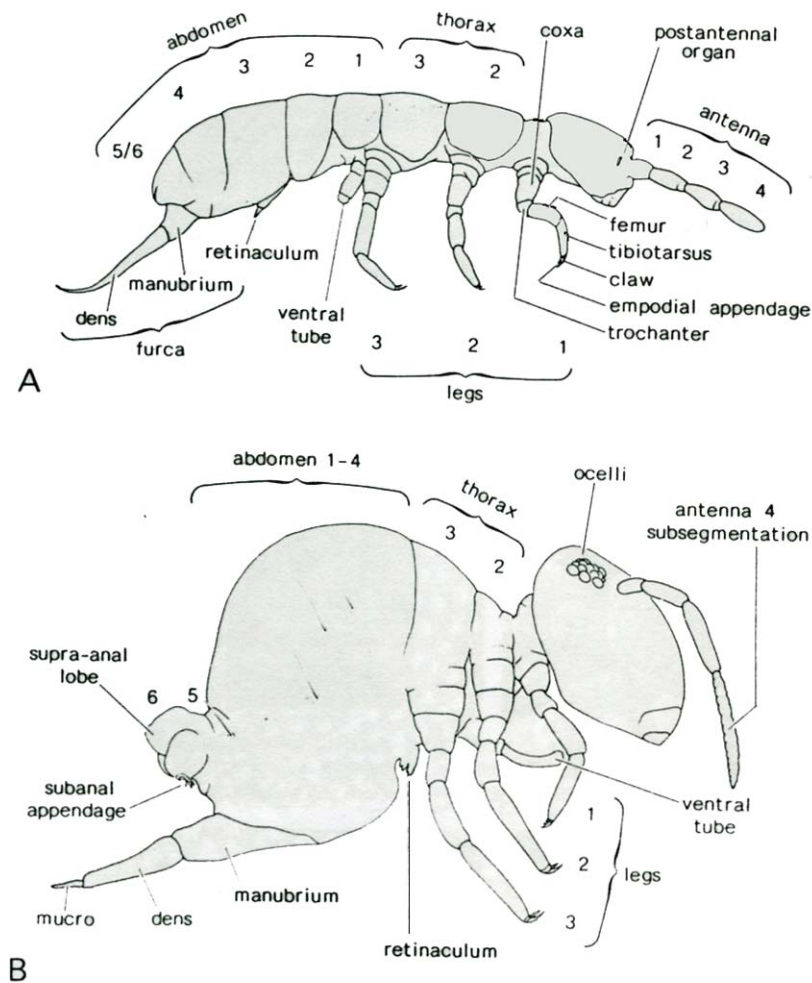
The body of springtails is divided into 3 parts: a head, a thorax with three segments, and an abdomen with six segments (Fig. 2-2A), but in Neelipleona and Symphypleona, some thoracic and abdominal segments fuse to give these springtails their characteristic globular appearance (Fig. 2-2B) (Christiansen & Bellinger, 1998, Heckman, 2001; Hopkin, 1997; Greenslade, 1991).

**Head:** It possesses of three main characteristics: a pair of antennae, a group of ocelli on each side and mouthparts. The antennae are a pair of segmented appendages located on the head above the mouthparts and usually sensory in function. An antenna is divided into four segments. In some species, one or more of the sections may be divided into subunits which give the antennae greater flexibility e.g. *Mastigoceras camponoti*. Additionally, in some species (e.g. the male of Sminthuridae), it is modified for clasping the female during courtship. Each group of

ocelli consists of a maximum of eight ommatidia. Some species are completely blind (*e.g.* Onychiuridae) (Hopkin, 1997). A postantennal organ is found in many species which may have an olfactory function. Mouthparts are composed of five main components: labrum, a pair of mandibles, a pair of maxillae, hypopharynx and labium. They vary enormously, but generally are somewhat elongate and always concealed within the head.

**Thorax:** Each segment of the thorax bears a pair of legs. Each leg is made up of a subcoxa, coxa, trochanter, femur, tibiotarsus and pretarsus with a claw and empodial appendage. The claws of some species are modified to enable them to walk more efficiently on wet surfaces. The tibiotarsus of others has one or more long hairs with sticky ends that allow these species to adhere to smooth surfaces.

**Abdomen:** This part of body consists of six segments and has the important structures. The first segment of the abdomen bears a ventral tube which plays an extremely important role in fluid and electrolyte balance. In addition, it may also be used to adhere to smooth surfaces. The third segment has the tenaculum, known as retinaculum, which functions as a 'catch' for the jumping organ or furcula which arises on the fourth abdominal segment. The ventral side of the fifth segment bears the inconspicuous male or female genital opening.



**Figure 2-2** External morphology of collembolans A. Cylindrical body form  
 B. Globular body form (Greenslade, 1991)

### 2.2.2 The genus *Xenylla*

The genus *Xenylla* was established by Tullberg for *X. maritima* Tullberg, 1869 as type species. It is one of the largest and most widespread genera in the family Hypogastruridae. This family differs from other families of Poduromorpha by having strong, chewing mouthparts with a molar plate. Members of this family are found in numerous habitats including soil, under bark, the sea shore, commercial mushroom beds and the percolating filters of sewage treatment works where they can be extremely abundant. They are those most often found in huge swarms (Greenslade, 1994; Hopkin, 1997). *Xenylla* sp. is cosmopolitan, containing about 126 species at present in the world. Species of this genus are known to have a well developed molar

plate, lacking a postantennal organ and unguiculus (a small claw). So far, the genus *Xenylla* sp. of Asia has been very poorly investigated in spite of its biogeographical importance. In Japan, about four species were recorded (Furuno et al., 2000) and one species was known to occur in China (Zhao et al., 1997). In Korea, four species of the genus *Xenylla* were recorded by Lee *et al* (2005) as *X. convexopyga*, *X. longistriata*, *X. dotata* and *X. marina*.

**Descriptions of *Xenylla* Tullberg, 1869** compiled from Dindal (1990) and Thibaud et al. (2004)

Pigment varying from pale to very dark blue-black. 5+5, rarely 4+4 ocelli. Postantennal organ and unguiculus absent. Mandible short with molar plate. Foot usually with two weakly to strongly clavate tenent hairs. In a few species the dens is strongly reduced or absent. Abd. V with  $p_3$  as sensilla. Uncleared specimens may be confused with the subgenus *Schoetella* of the genus *Hypogastrura*. There are 15 Nearctic species found everywhere except for the high arctic. Maximum sizes between 1 and 2 mm.

The chaetal designation of the body of *Xenylla* sp. was used to identify species as shown in Fig. 2-3 and Table 2-1 (Gama et al., 2001; Hopkin, 1997; Lee et al., 2005). The letters (a, m, p) refer to the positions of setae, for example,  $a_1$ ,  $m_1$ ,  $p_1$  are the first setae of the anterior, middle and posterior row, counted from the middle line, respectively. The letters (c, L and la) represent cervical setae, lateral setae on head and lateral anterior setae on thorax, *etc.* In addition, each derived characters was represented by special letter as follows:

**Dorsal chaetotaxy:**

- a:  $a_0$  on the head absent
- b:  $c_1$  on the head absent
- c:  $c_2$  on the head absent
- d:  $c_3$  on the head absent
- e:  $d_1$  on the head absent
- f:  $L_1$  on the head longer than  $L_3$
- g:  $L_3$  on the head longer than  $L_1$

h<sub>1</sub>: a<sub>2</sub> on Th. II-III displaced apically compared with a<sub>1</sub>

h<sub>2</sub>: p<sub>2</sub> on Th. II-III displaced apically compared with p<sub>1</sub>

i: la<sub>1</sub> on Th. II-III absent

i': la<sub>3</sub> on Th. II-III absent

j: la<sub>2</sub> on Th. II-III absent

k: m<sub>3</sub> on Th. II-III absent

l: p<sub>3</sub> on Th. II-III absent

n: p<sub>3</sub> on Abd. IV absent

o: m<sub>3</sub> on Abd. IV absent

q: a<sub>2</sub> on Abd. V absent

**Ventral chaetotaxy:**

r: p<sub>1</sub> on the head absent

s: m<sub>3</sub> on the head absent

t: the pair of setae on Th. II-III absent

v: p<sub>1</sub> and p<sub>2</sub> on Abd. II absent

w: p<sub>6</sub> on Abd. II absent

y: a<sub>6</sub> on Abd. II absent

a<sub>2</sub>: a<sub>6</sub> on Abd. III absent

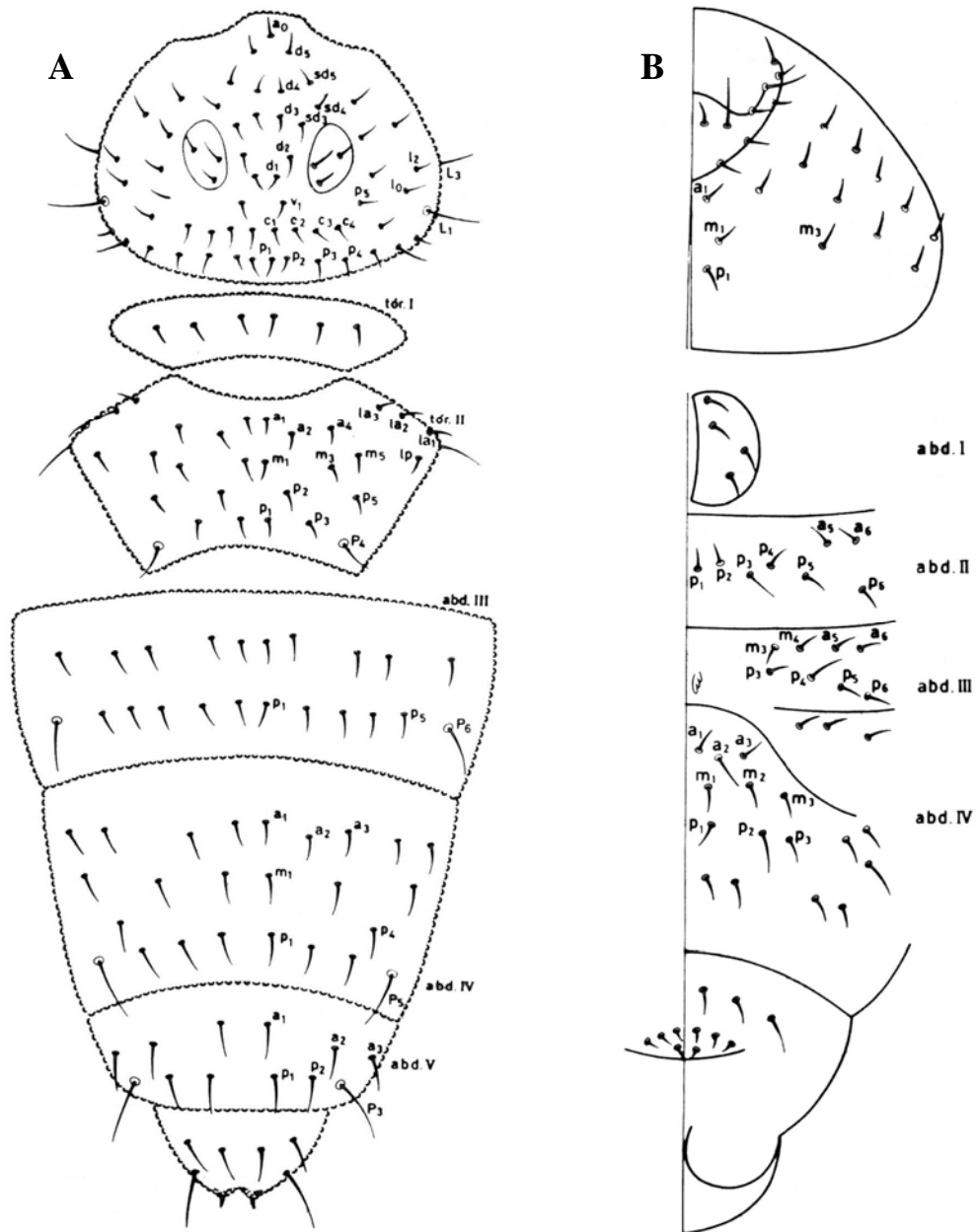
a<sub>3</sub>: p<sub>5</sub> on Abd. III absent

a<sub>4</sub>: m<sub>1</sub> on Abd. IV absent

### 2.2.3 Life history and development

Springtails are ametabolous or without metamorphosis. Eggs are generally laid in soil or leaf litter and hatch in a few days. The first juvenile stage closely resembles the adult in general appearance, but has fewer setae and a relatively undifferentiated cuticle. In general, the juveniles pass through 5 or 6 instars before maturity, usually becoming adult 3-5 weeks after hatching, depending on temperature. From the observation on biology of *Xenylla welchi* (Bandyopadhyaya & Choudhuri, 2002), eggs were generally laid in crevices, holes, and exuviae and hatch in five days at 26 °C to 30 °C. The total number of eggs per female varied between 134 and 148. The first instar closely resembled the adult and developed to adult within 10.5 days.

They usually continued to grow and molt throughout life. The individual life span was 83 days on average.



**Figure 2-3** The chaetotaxy of the genus *Xenylla* A. Dorsal view B. Ventral view (Gama et al., 2001)

**Table 2-1** The chaetotaxy of the genus *Xenylla* (adapted from Gama et al., 2001)

Species	a	b	c	d	e	f	g	h <sub>1</sub>	h <sub>2</sub>	i	j	k	l	n	o	q	r	s	t	v	w	a <sub>4</sub>
<i>X. acauda</i> Gisin, 1947			X				X	X	X										X			
<i>X. brevipina</i> Kinoshita, 1916								X									X	X	X	X		
<i>X. corticalis</i> Borner, 1901		X		X		X		X	X	X		X				X						X
<i>X. fernandesi</i> Gama, 1947		X				X		X	X	X						X						X
<i>X. franzi</i> Steiner, 1955			X		X		X	X	X		X											
<i>X. grisea</i> Axelson, 1900		X				X		X	X	X		X				X						X
<i>X. humicola</i> Fabricius, 1780								X									X		X			
<i>X. littoralis</i> Womersley, 1933						X								X					X	X	X	
<i>X. malasica</i> Gama, 1969	X	X						X	X	X			X			X			X			
<i>X. malayana</i> Salmon, 1951										X								X	X			
<i>X. marii</i> Tullberg, 1869		X				X		X	X							X						X
<i>X. mucronata</i> Axelson, 1903			X					X	X													
<i>X. tullbergi</i> Borner, 1903			X				X	X	X													
<i>X. welchi</i> Folsom, 1961																			X			
<i>X. yosii</i> Gama, 1971		X				X		X	X						X	X		X				
<i>Xenylla</i> sp. (studied)				X					X			X				X			X			

Note: x = Character found

#### **2.2.4 Habitats**

Springtails are found predominantly in the soil, leaf litter and other decomposition habitats such as logs and dung. Some species are found on grasses, in flowers and under the bark of trees. Springtails are cosmopolitan organisms, being found in all terrestrial biomes, including mountain tops, polar regions and deserts. In addition, they are found in caves, marine and freshwater littoral habitats. Relative humidity is one of the most important factors determining activities and distribution, but springtails can also be attracted to high carbon dioxide concentrations and other factors metabolites produced from microorganisms. Other factors influencing distribution are the location of food, temperature and soil pore space (Greenslade, 1991).

#### **2.2.5 Feeding behavior**

Springtails consume a wide variety of food materials such as fungi, bacteria, algae, yeasts and other forms of organic materials including other springtails. However, other experiments and observations have shown that some species have definite preferences for certain types of food and prefer fungal hyphae over other food types. Some species are carnivorous on protozoans, rotifers and nematodes *etc.* For example, Nayrolles (1990) found fragments of moths and springtails in gut of species of *Arrhopalites* (Katianninae) in Thai caves where decaying vegetation is scarce. A few species, including the clover springtail (or lucerne species) *Sminthurus viridis* feeds on plants, especially clover and lucerne, and *Onychiurus* sp. (Onychiuridae) can feed on plant roots (Hopkin, 1997).

#### **2.2.6 Reproductive system and fertilization**

Generally, springtails are dioecious. The male reproductive system consists of a pair of testes, two circumvoluted deferens ducts, and two seminal vesicles which join in the ejaculatory duct; this receives the secretion from two lateral accessory glands. The testes are the cylindrical sacs that have beak at apical region. The active spermatogenesis occurred into the apical and the middle region, and then formed and stored spermatids in the basal region of the gonad at the distal testes (Dallai et al., 2000). The female reproductive organ is a pair of ovary that is sac-shaped and not

divided into discrete ovarioles. Within ovary, there were two regions: a germarium and a vitellarium. The germarium are located laterally on an ovarian sac, whereas vitellarium has oocyte associated with the nurse cell (Biliński, 1993; Büning, 1994).

Fertilization usually takes place indirectly. The male deposits the spermatophore on the ground for the female to take up. The specific mating behavior ensures females to find and take sperm into the genital aperture. In Hypogastruridae and some other families, simple aggregation occurs and pheromones play a part. However, in some Sminthuridae (Bourletiellinae and Sminthurinae) there may be an elaborate dance, in which the male grasps the female by specialized spines and hairs on the antennae and directs her to the spermatopore. Some soil-dwelling springtails are parthenogenetic. After oviposition, springtails e.g. *Sminthurus*, *Arrhopalites* and some other Symphypleona protect eggs against dehydration and fungal attack by covering its eggs with freshly eaten soil mixed with a rectal fluid voided through the anus. In other species (e.g. *Folsomia candida*), eggs are kept free of fungal hyphae by grazing of the adults (Greenslade, 1991).

### **2.2.7 Role of springtails in the decomposition processes**

Springtails are numerous in terrestrial ecosystems and play several important roles in decomposition processes. Consumption of dead vegetation and its subsequent excretion as partially-decomposed fecal pellets increases the surface area and suitability of the material for microbial and fungal attack (Hasegawa & Takeda, 1994; Takeda, 1988; Van Amelsvoort et al., 1988). In addition, grazing of mycorrhizae on roots can stimulate growth of the symbiont and improve plant growth (Lussenhop, 1996). In other situations, springtails may reduce plant disease by consuming fungus pests. For example, grazing by *Sinella curivseta* on the cucumber fungus *Fusarium oxysporum* suppressed the infection and produced healthy plants in comparison to ungrazed controls which wilted badly (Nakamura et al., 1992).

### 2.3 Effects of fungal species on fitness of springtails

The importance of food quality for the growth and the reproduction of animals is widely recognized (Fiennes, 1972; Watson, 1970). In decomposer systems, fungi are highly efficient at penetrating plant remains and within a short period from the onset of decay, a major proportion of the labile nutrients are incorporated in microbial tissue (Swift 1976). Microbial tissues may, therefore, represent a high quality food resource for soil invertebrates, which may be grazed directly by small animals or indirectly by the larger animals feeding on higher plant debris. Springtails appear to be generalist feeders ingesting a wide range of fungal taxa, algae and detritus (Chen et al., 1995; Pong, 2000). Although springtails have been regarded as general feeders, however, in laboratory food choice experiments, it has been documented that springtails preferentially ingest certain fungal taxa (Klironomos & Kendrick, 1995; Maraun et al., 2003). They also found that food preference has effects on fitness parameters, *i.e.* growth and reproduction of springtails.

Booth and Anderson (1979) reported that *Folsomia candida* exhibited trends of increased exuviae and egg laying rates when feeding on Basidiomycete fungi, *Coriolus versicolor* and *Hypholoma fasciculare*. Besides, they showed that the nitrogen content of food can marked effects on survivorship and reproduction.

Chen et al. (1995) reported that collembolan species had different food preferences. As a result to its preferred foods, it has higher egg production and survival rate than on non-preferred foods. However, shorter life spans were compensated for by higher survival and egg production during early instars.

Klironomos et al. (1999) reported that *Folsomia candida* preferred common saprobic fungi over a variety of arbuscular mycorrhizal fungi. Coincidentally, fecundity levels across two *Folsomia* generations were higher when springtail fed exclusively on the preferred fungus, *Alternaria alternata*. On the other hand, when fed with less palatable fungi, fecundity was greatly reduced.

Walsh and Bolger (1990) showed that each collembolan species had significant preference of food and *Onychiurus furcifer* achieved largest population size and fastest growth rates when fed on *Trichoderma viride*, one of its least preferred food.

## 2.4 Life and fecundity table

In general, the change of population of organisms comes from the four factors - birth, death, emigration and immigration. From this basic knowledge, biologists used it to invent a technique for studying population or demography. Demography began as the study of human population and the word was derived from the Greek root *demos*, meaning “the people”. Although early period of demography used only census or estimating expected-time of human life, it was recently brought to use in many fields such as sociology, business, ecology, forestry and entomology (Carey, 1993; Krebs, 2009).

A life table is the convenient tool for describing the mortality of a population giving the probability of dying and various other statistics at each age (Carey, 1993; Stiling, 1999). There are two general forms of the life table. The first is the time-specific life table. It was useful in examining populations of long-lived animals as herds of elephants, where following a cohort of individuals from birth to death would be impractical. In addition, it could prepare from a collection of skulls of known ages, for example, Dall mountain sheep skulls. The latter is the cohort life table that constructed from data of a group of individuals (a cohort) from birth until all members of the cohort have died. Besides the life table, the reproductive rate of population could be estimated by fecundity table. Fecundity table was constructed from combination of reproduction and mortality (Carey, 1993; Hendrick, 1984; Krebs, 2009; Neal, 2004; Stiling, 1999). The parameters of life and fecundity table were composed of  $R_0$ ,  $\lambda$ ,  $r_c$ ,  $r_m$ ,  $e_x$ ,  $T$ ,  $T_c$ , and  $D$ .

- The net reproductive rate ( $R_0$ ) is the average production of female offspring per female during her lifetime (Neal 2004). This parameter showed trend of population. For example, if  $R_0$  is below 1.0 or upper 1.0, the population is declining or increasing, respectively.

- The finite rate of increase ( $\lambda$ ) is the number of times of population that could multiply itself per time interval.

- Capacity for increase ( $r_c$ ) is an approximation of intrinsic rate of natural increase ( $r_m$ ) that is the measure of the rate of increase of a population under controlled conditions (Stiling, 1999).

- Life expectancy ( $e_x$ ) is an estimated length of time that an average animal is expected to live.

- Generation time (T) is the time between the birth of a parent and the birth of its offspring. For  $T_c$ , this estimated an average parental age at which all the offspring are born and used to estimate T and calculated  $r_c$ .

- Doubling time (D) is the time taken for the population to double its size.

The benefits of life table are not only used to summarize the mortality and reproduction of a population, but also, in toxicology, it was used to assess the impacts of chemicals and influences of important factors on the biology of animals.

Kang et al. (2001) reported that glufosinate-ammonium was found to have impacts on life table of *Paronychiurus kimi* in artificial soil. The reproduction of *P. kimi* was significantly reduced at 0.5 mg/kg and the adult survival rate decreased at 50 mg/kg, whereas there were no effects on springtails in plaster-charcoal substrate, except at high concentration (1572.70  $\mu\text{g}/\text{cm}^2$ ).

Menta et al. (2006) showed that reproduction and survival of *Sinella coeca* and *Folsomia candida* were highly sensitive to cadmium when exposed to 50  $\mu\text{g g}^{-1}$ . Moreover, their egg hatching was affected by cadmium concentrations and by the time period that the eggs stayed in contact with cadmium, while lead was not found to have any effects on both species, except at the concentration up to 1000  $\mu\text{g g}^{-1}$ .

## CHAPTER III

### MATERIALS AND METHODS

#### 3.1 Materials

##### 3.1.1 Effective Microorganism (EM<sup>TM</sup>) (EX-M<sup>®</sup>)

Effective Microorganism (EM) is a mixture of many species of microorganisms. It has been used to improve soil property and is believed to help increasing nutrients into soil by microbial activity. In this study, two commercial formulations of EM were used as EM1 and EM5. Both were formulated by mixing stock solution of commercial EM (EX-M<sup>®</sup>) (Fig. 3-1) with different components according to the labeled instructions. The first formulation contains EM stock and water in the ratio of 1: 1000 by volume.



Figure 3-1 Stock solution of EM (EX-M<sup>®</sup>)

The second formulation is called EM5 or sutoju. It is an EM derivative that was developed for repelling insects. The EM5 stock consists of EM stock, molasses, rice whiskey, vinegar and water in the ratio of 1: 1: 1: 1: 6 by volume. A commercial formulation of EM5 used in this study contains EM5 stock and water in the ratio of 1: 200 – 500 by volume.

### 3.1.2 Chemicals

1. 70%, 80 %, 95 % and 100 % ethyl alcohol
2. 10 % KOH
3. Bouin's fixative
4. Hematoxylin and eosin stain
5. Xylene
6. Hoyer's mounting media

### 3.1.3 Equipment

1. Berlese-Tullgren funnel
2. pH-meter
3. Compound microscope and stereo microscope (Olympus Model S2X-ILLB200<sup>®</sup>)
4. Ocular micrometer and stage micrometer
5. Digital camera (DSC-F717) (Sony DSC-F717<sup>®</sup>)
6. Plastic bottles (1.8 and 4.5 cm diameters)
7. Activated Charcoal
8. Plaster of Paris
9. Baker's yeast
10. Rice whiskey (หงส์สีทอง<sup>™</sup>)
11. Distilled vinegar (Golden Mountain<sup>®</sup>)
12. Molasses
13. Fine soft brushes and needles
14. Microscope slides and cover glasses
15. Pasteur pipettes
16. 10 ml, 100 ml and 1000 ml glass cylinders

17. 250 ml and 500 ml glass bottles

## **3.2 Methods**

### **3.2.1 Springtail culture and identification**

Springtails were collected from soil at Salaya Campus, Mahidol University, in Nakhon Pathom province, Thailand. They were extracted live by using Berlese-Tullgren funnel (Fig. 3-2). The bottles of 4.5 cm diameter and 6 cm tall with a base of plaster of Paris-charcoal substrate (a mixture of plaster-charcoal and water in the ratio of 8: 4: 5.5 by volume) (Fig. 3-3) were used to maintain stock cultures at 25 – 28 C (Fig. 3-4). Baker's yeasts were given as food (Fig. 3-5). In order to keep the culture bottles moist, water was added in drops every three days depending upon the dryness of the culture medium.

For identification, springtails were preserved in 70 % ethanol and discolored with 10 % KOH solution. Hoyer's mounting media was used to prepare specimen slides. The specimens were examined under compound microscope and identified using keys by Heckman (2001). The chaetal designation of the body was according to the system by Gama et al. (2001) and Lee et al. (2005).

### **3.2.2 Sex determination**

#### **3.2.2.1 Body length**

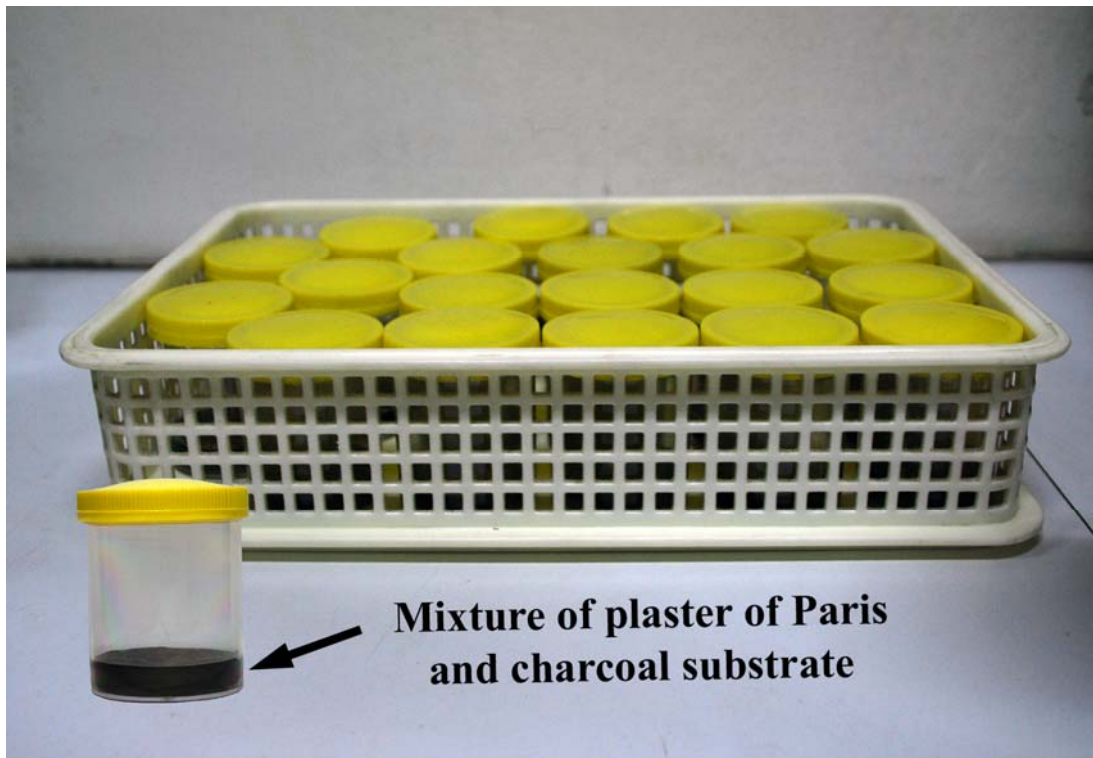
Thirty one-day-old juveniles were removed from a culture bottle and each individual was placed in separate bottles. Digital photographs were taken daily for a month and the body length of the springtails, from the end of the posterior abdominal segment to the anterior margin of head, were measured.



**Figure 3-2** Extraction of springtails by Berlese-Tullgren funnel



**Figure 3-3** Ingredients of plaster of Paris-charcoal substrate



**Figure 3-4** Culture bottles (4.5 cm diameter)



**Figure 3-5** Baker's yeast

### **3.2.2.2 Histological study**

Springtails were fixed in Bouin's fixative for 15 minutes. Then, they were washed in 70 % ethyl alcohol in order to remove the fixative. After dehydration through a graded series of ethyl alcohol (70 %, 80 %, 95 % and 100%) for 30 minutes each, they were infiltrated with xylene and embedded in paraffin wax, sectioned at 5µm thick and finally stained with hematoxylin and eosin (H&E). The specimens were examined and photographs of the reproductive system of *Xenylla* sp. were taken under the compound microscope.

### **3.2.3 Life history of *Xenylla* sp.**

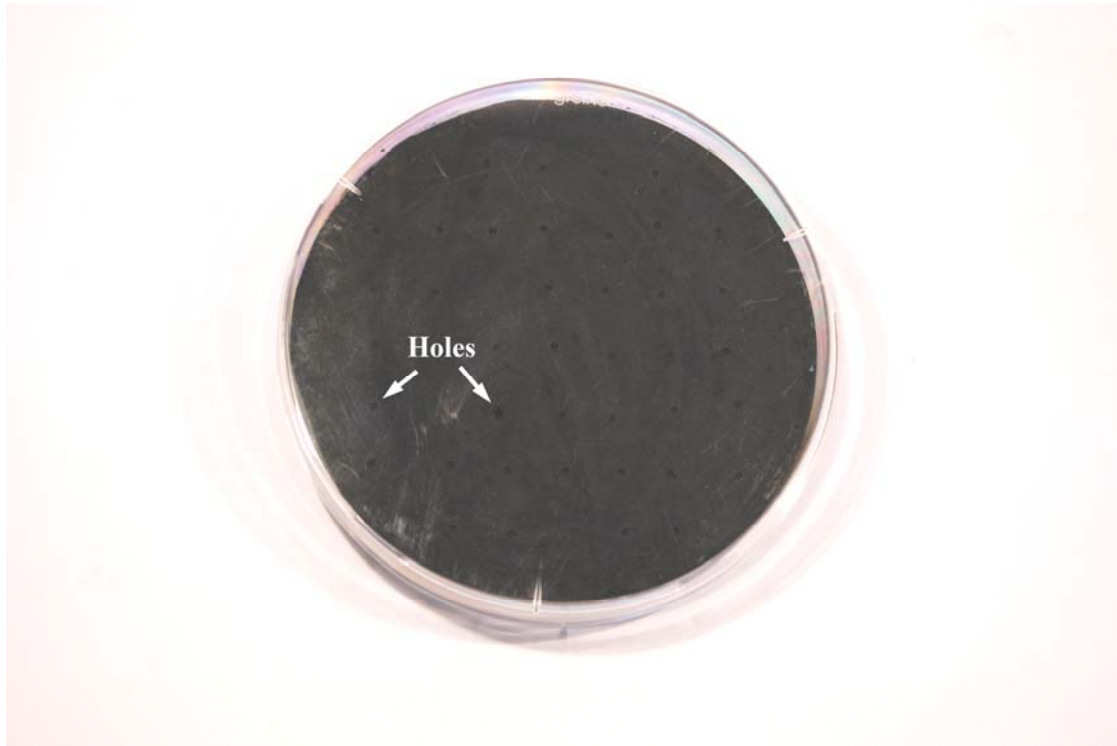
Observations on the stages of life history and fecundity were done under stereo microscope. One hundred one-day-old eggs were transferred by fine soft brush into the bottles with a base of plaster of Paris-charcoal substrate. After hatching, the juveniles were transferred to the new bottles of 1.8 cm diameter and 4.7 cm tall with 10 individuals per bottle. The numbers of dead bodies, exuviae and eggs were recorded daily to the last surviving individuals.

### **3.2.4 Comparisons of biology of *Xenylla* sp. when reared in substrate with EM1 and EM5**

The biology of egg, juvenile and adult stages was studied and compared.

#### **3.2.4.1 Effects of EM1 on *Xenylla* sp.**

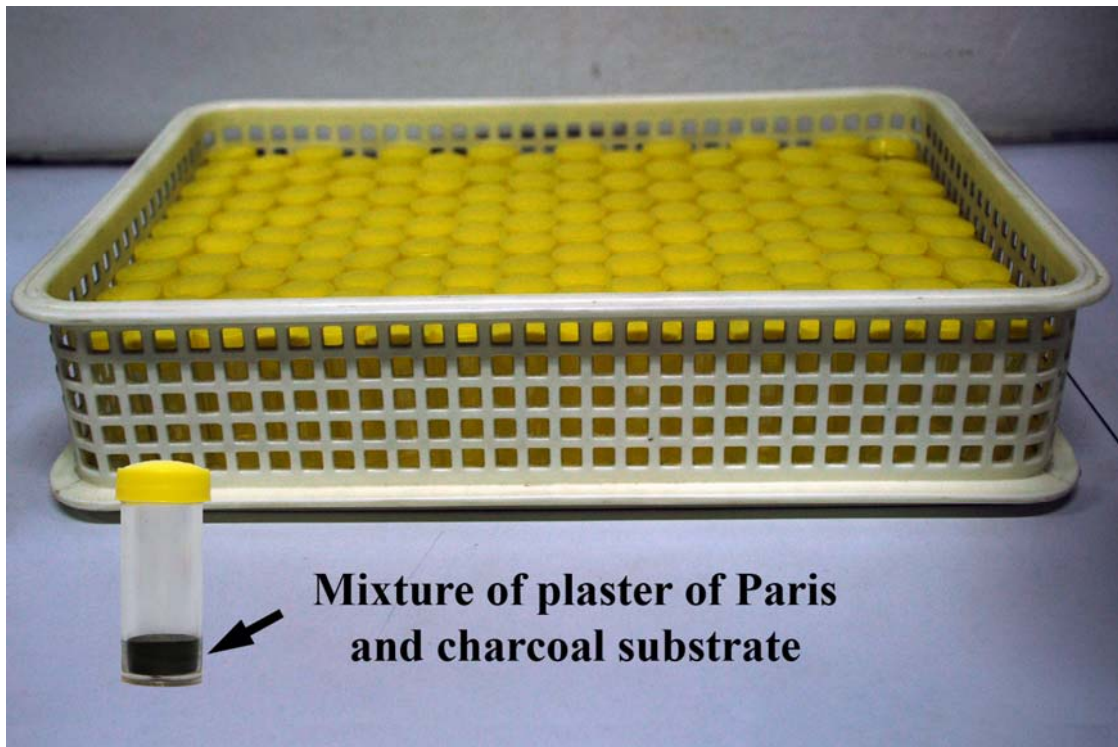
To evaluate the percentage of egg hatching of the springtails, thirty one-day-old eggs were placed in a petri dish (9 cm diameter) (Fig. 3-6) with a base of plaster of Paris-charcoal substrate. The petri dishes were treated with water (control), EM1 and molasses. The number of juveniles hatched was recorded daily until it was clear that no more eggs would hatch. Each treatment was repeated five times.



**Figure 3-6** Petri dish (9 cm diameter) with holes provided for eggs

To evaluate the developmental time of juvenile springtails, ninety one-day-old juveniles were transferred by fine soft brush to the bottles that were treated with water (control), EM1 and molasses with one individual per bottle (Fig. 3–7). The juveniles were observed daily and the numbers of dead bodies and exuviae were recorded up to the sixth instar. During experiment, baker’s yeasts were given as food every three days and treatments were supplied once a week.

To evaluate the survival rate and the reproduction of adult springtails, ten 12-day-old juveniles were transferred by fine soft brush into each bottle of which the substrate was treated with water (control), EM1 and molasses. There were five bottles for each treatment, making a total of 15 bottles for one replicate. The experiments were repeated three times. The numbers of dead bodies and eggs were recorded and removed daily. During experiment, baker’s yeasts were given as food every three days and treatments were supplied once a week.



**Figure 3-7** Test bottles (1.5 cm diameter) for juveniles and adults

#### **3.2.4.2 Effects of EM5 on *Xenylla* sp.**

To evaluate the percentage of egg hatching of the springtails upon exposure to EM5, thirty one-day-old eggs were placed in a petri dish (9 cm diameter) with a base of plaster of Paris-charcoal substrate. The petri dishes were treated with water (control), EM5, vinegar and rice whiskey. For EM5, vinegar and rice whiskey, they were diluted in volume ratio 1: 200, 1: 300, 1: 400 and 1: 500 (Fig. 3-8). The number of juveniles hatched was recorded daily until it was clear that no more eggs would hatch. Each treatment was repeated five times.



**Figure 3-8** Ingredients of EM5

To evaluate the developmental time of juvenile springtails, ninety one-day old juveniles were transferred by fine soft brush to the bottles that were treated with water (control), EM5, vinegar and rice whiskey at the concentrations described above with one individual per bottle. The juveniles were observed daily and the numbers of dead bodies and exuviae were recorded up to the sixth stage. During experiment, baker's yeasts were given as food every three days and treatments were supplied once a week.

To evaluate the survival rate and the reproduction of adult springtails, ten 12-day-old juveniles were transferred by fine soft brush into each bottle of which the substrate was treated with water (control), EM5, vinegar and rice whiskey at the concentrations described above. There were five bottles for each treatment, making a total of 15 bottles for one replicate. The experiments were repeated three times. The numbers of dead bodies and eggs were recorded and removed daily. During experiment, baker's yeasts were given as food every three days and treatments were supplied once a week.

### 3.2.5 Estimation of life table statistics

On basis of the observed data, a life table was constructed. The following parameters were calculated: Mean life expectancy ( $e_x$ ), intrinsic rate of natural increase ( $r_m$ ), net reproductive rate ( $R_0$ ), finite capacity of increase ( $\lambda$ ), generation time (T), doubling time (D), capacity for increase ( $r_c$ ) and cohort generation time ( $T_c$ ) (Neal 2004).

The mean life expectancy is the estimated length of time that an average animal is expected to live by calculating from the equation:

$$e_x = T_x / l_x$$

Where  $l_x$  = The age specific survival rate

$T_x$  = Sum of the number of survivors in age class (x) and older age classes

The intrinsic rate of natural increase is estimated from the data using Lotka's equation:

$$1 = \sum_0^{\infty} \exp(-rx) l_x m_x$$

Where  $l_x$  = The age specific survival rate

$m_x$  = The age specific fecundity

$x$  = Age (day)

Net reproductive rate is the number of female offspring produced per female and could calculate from the equation:

$$R_0 = \sum l_x m_x$$

Finite capacity of increase is the number of times the population that will multiply itself per unit of time. The equation is:

$$\lambda = \exp(r_m)$$

Capacity for increase was an approximation of  $r_m$ . The equation was:

$$r_c = \ln R_0 / T_c$$

Generation time is the time between the birth of a parent and the birth of its offspring. This parameter could calculate by equation:

$$T = \ln R_0 / r_m$$

For cohort generation time ( $T_c = \sum x l_x m_x / \sum l_x m_x$ ), it was an average parental age at which all the offspring are born and an estimating parameter of T

Doubling time was the number of days required for the population to double its numbers. It was estimated from the equations:

$$D = \ln 2 / r_m$$

### 3.2.6 Data analysis

Data were analysed by single factor ANOVA ( $P < 0.05$ ) in order to detect the effects of Effective Microorganism on biology of *Xenylla*. For comparison of means, Tukey's honestly significant difference was used. Data are log-transformed prior to the analyses to improve homogeneity of variances if necessary. For non parametric data, there were analysed by Kruskal Wallis test ( $p < 0.05$ ) and the Mann-Whitney test was applied to detect the significant difference between treatments.

## CHAPTER IV

### RESULTS

#### 4.1 General Biology of *Xenylla* sp.

A species of *Xenylla* was extracted from Salaya soil and has been maintained continuously since 2005 using a plaster of Paris-charcoal substrate and baker's yeast as food supply (Fig. 4-1). Early population occasionally collapsed due to predatory mite infestations. The bottles with mite infection were discarded whenever they were detected. After a few months, all stock cultures were mite-free and the population grew very well and established itself as a stock culture for this study (Fig. 4-2).



**Figure 4-1** *Xenylla* sp. feeding on baker's yeast in a culture bottle



**Figure 4-2** Mass culture of *Xenylla* sp. in the laboratory

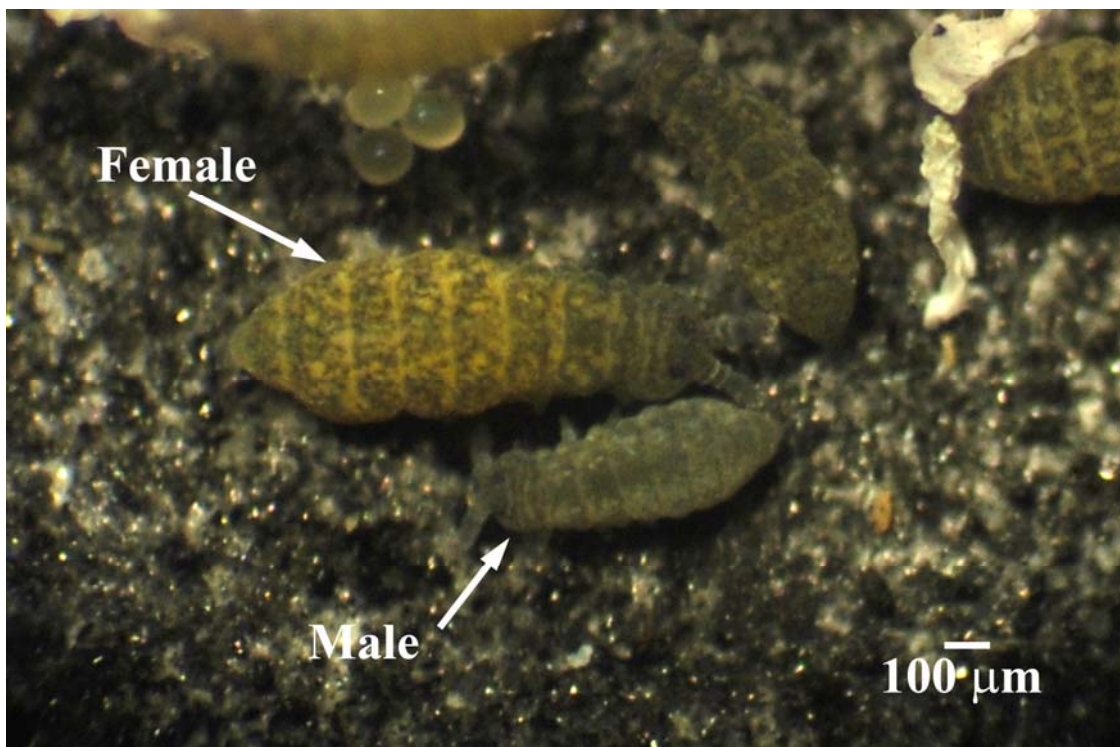
#### 4.1.1 Description of *Xenylla* sp.

The observation of prepared slides of *Xenylla* sp. adult specimens under compound light microscope revealed the following morphology and chaetotaxy.

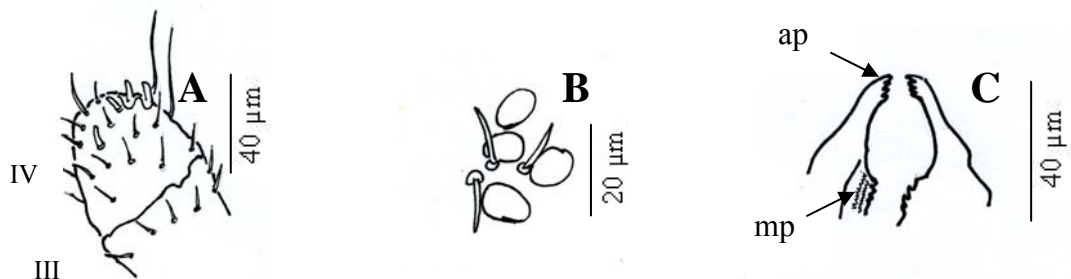
**Type data:** 10 males and 10 females collected from the soil at Salaya Campus, Mahidol University in Nakhon Pathom province, Thailand.

**Diagnosis:** Body length 710 to 1170  $\mu\text{m}$ . Color yellow in female and yellowish gray in male, covered with fine granules over the whole body except inter-segmental portions and the ventral side. Body cylindrical, being narrower abruptly at Abd.V (Fig. 4-3). Head length 146 - 175  $\mu\text{m}$ . Antenna shorter than head, 0.75 in ratio of head; ratio of length of antennal segments I: II: III: IV is 1: 1.44: 1.63: 2.37, respectively. Ant. IV with a simple apical bulb and four thick curving sensory setae (Fig. 4-4A). Antenna I, II, and III with 6, 11, and 17 setae, respectively. Eye 4 + 4, eye patch with 3 setae (Fig. 4-4B). Postantennal organ (PAO) absent. Mandible with 4 apical teeth (Fig. 4-4C). Thorax length 229 - 270  $\mu\text{m}$ . Each leg with 7, 5, 10, and 19 setae on coxa, trochanter, femur and tibiotarsus, respectively; Unguiculus absent.

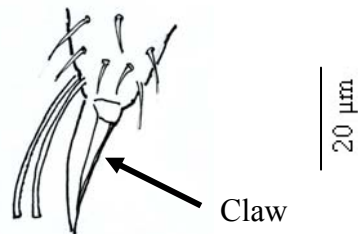
Tenant hair very long and slender, 2, 2, 2 in number upon each leg (Fig. 4-5). Abdomen length 463 - 945  $\mu\text{m}$ . Ventral tube with 4 + 4 setae (Fig. 4-6A). Tenaculum bearing 3+3 teeth (Fig. 4-6B). Furcula shorter than Abd. IV. Mucro well separated from the dens with 2 posterior setae (Fig. 4-6C). Abd. V bears 2 anal spines (Fig. 4-6D); with genital plate on the ventral side (Fig. 4-7).



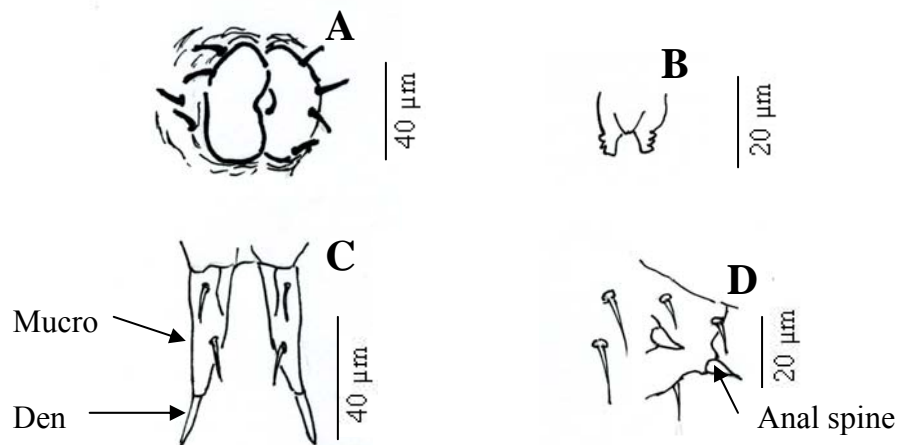
**Figure 4-3** Male and female of *Xenylla* sp. at approximately 60 days after hatch



**Figure 4-4** The organs on the head of *Xenylla* sp. A. Antennal segments III and IV (dorsal view) B. Four ocelli with three setae C. Mandible with apical teeth (ap) and mandibular plate (mp)



**Figure 4-5** The tibiotarsus of the hind leg of *Xenylla* sp. with claw



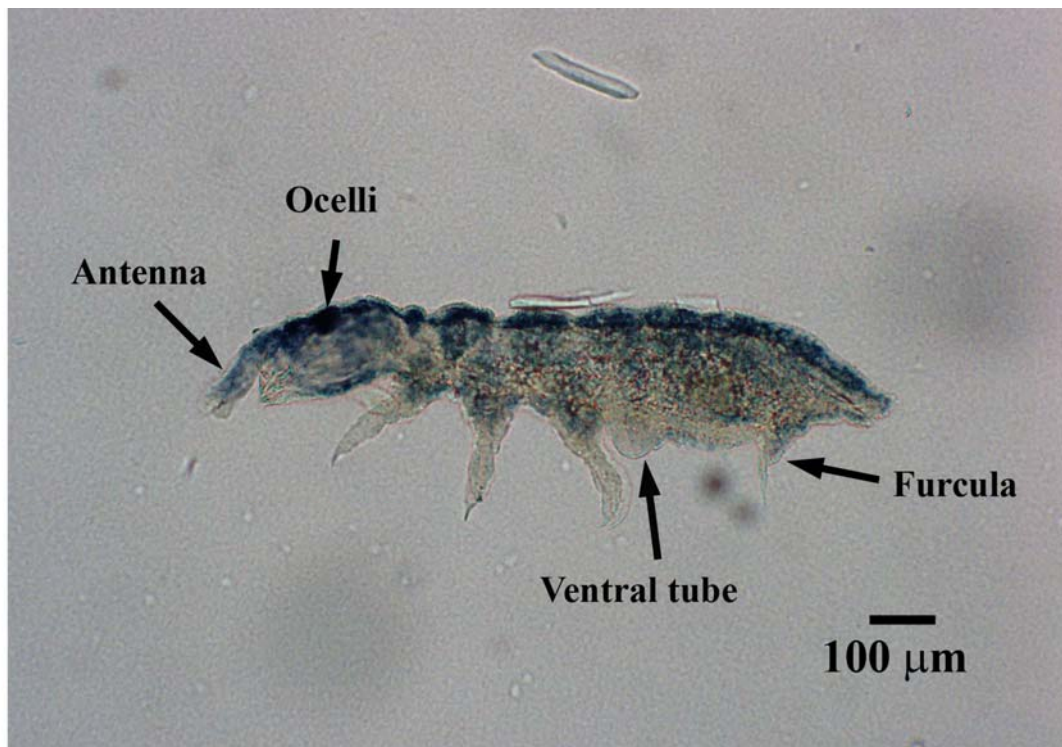
**Figure 4-6** The abdominal organs of *Xenylla* sp. A. Ventral tube B. Tenaculum  
C. Furcula D. Anal spine



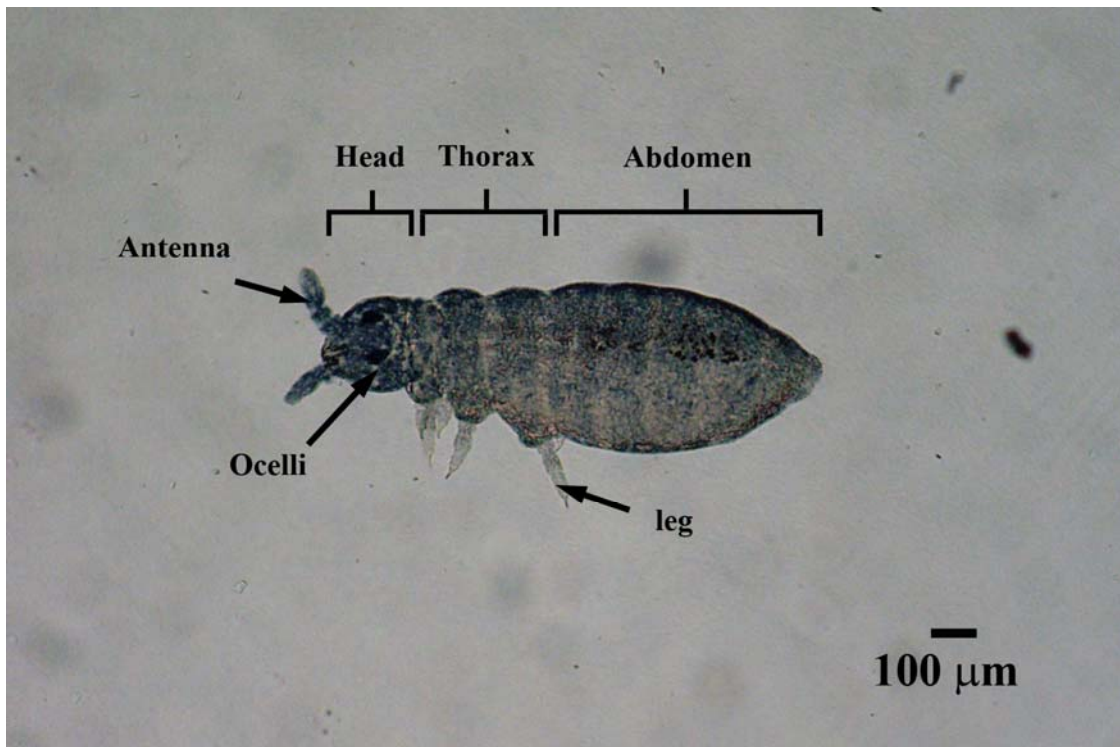
**Figure 4-7** Genital plate of *Xenylla* sp. A Male B. Female

**Chaetotaxy.** It is the most widely-used taxonomic method of all. The technique involves mapping the distribution of setae on the surface of the cuticle and looking for similarities and differences between species.

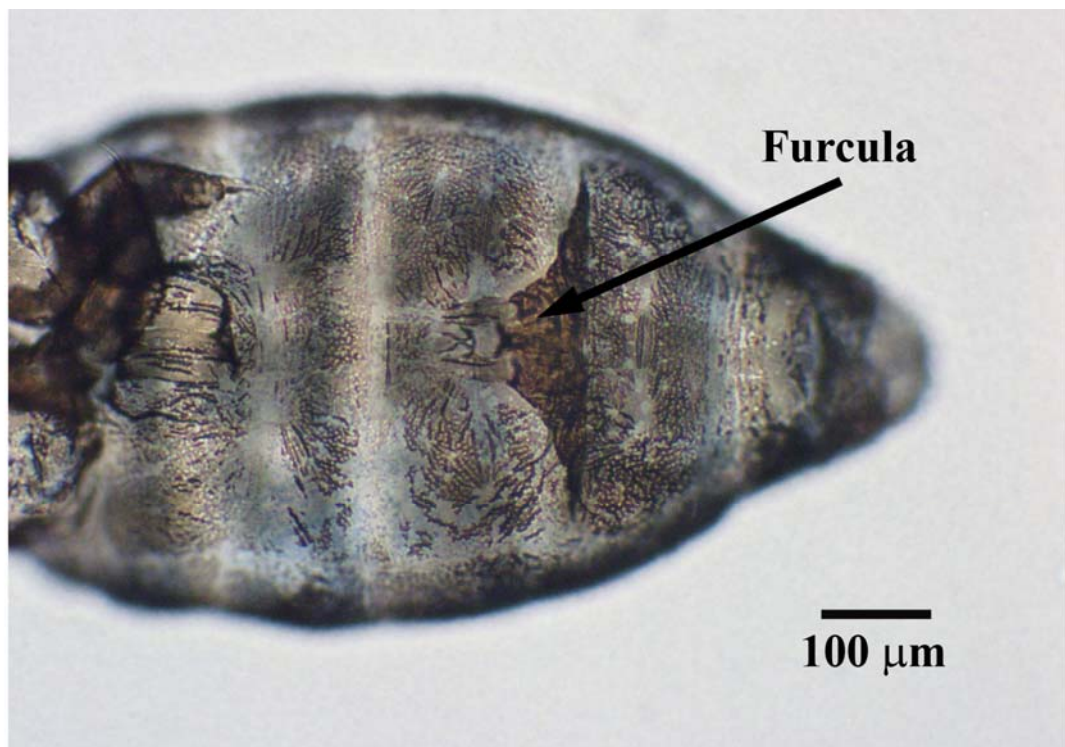
The specimens were examined from the prepared slides under compound microscope. The lateral and dorsal sides of the body (Fig. 4-8 and 4-9, respectively) were observed at 40X. The 400X magnification was used to study the ventral abdominal segment (Fig. 4-10). The 1000X was necessary to examine the mouthparts (Fig. 4-11).



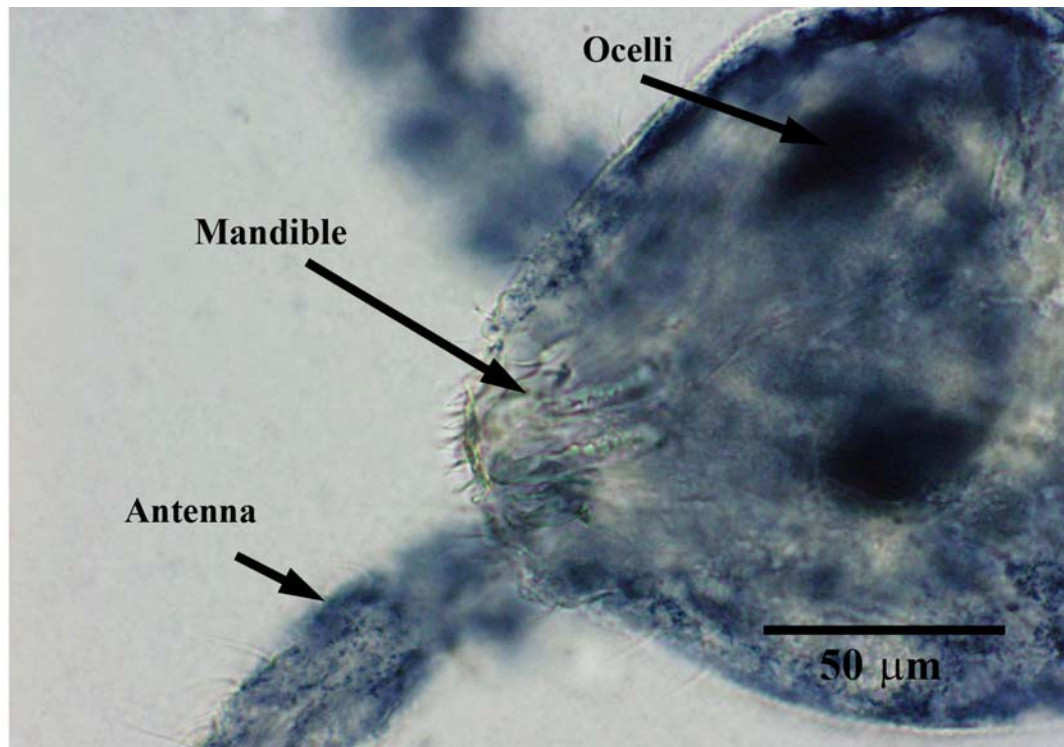
**Figure 4-8** Lateral view showing key characteristics of *Xenylla* sp. (40X)



**Figure 4-9** Dorsal view of *Xenylla* sp. (40X)



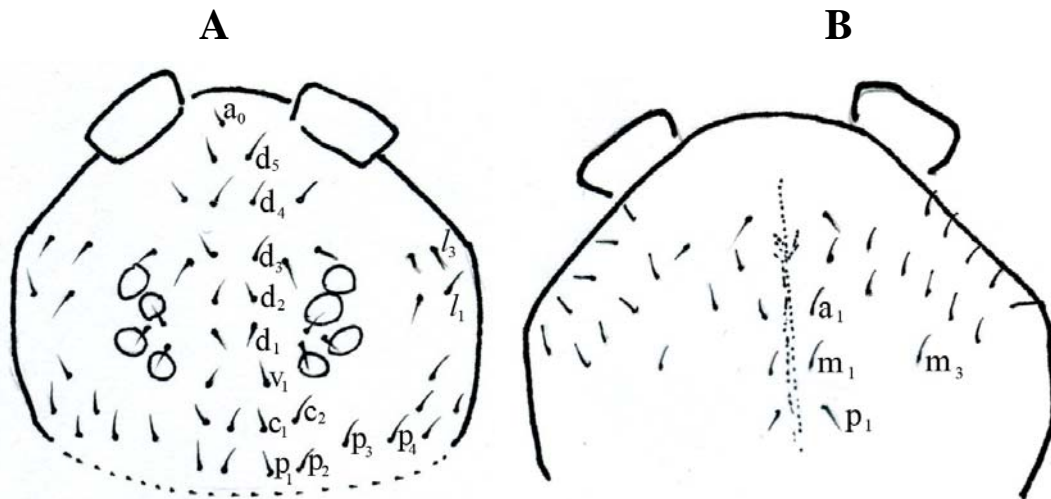
**Figure 4-10** Ventral view of abdomen of *Xenylla* sp. (400X) showing position of furcula



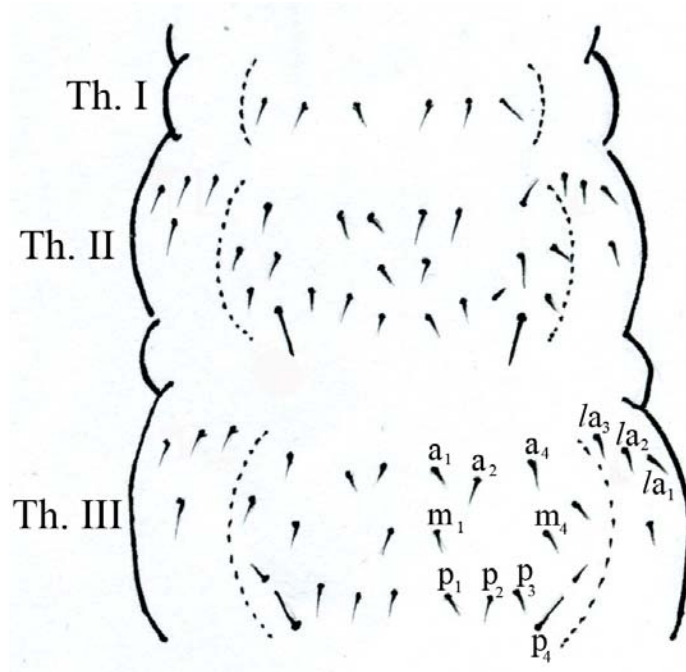
**Figure 4-11** Head and mouthparts of *Xenylla* sp. (1000X)

The letters (a, m, p) indicate the positions of setae, for example, a<sub>1</sub>, m<sub>1</sub>, p<sub>1</sub> are the first setae of the anterior, middle and posterior rows, counted from the middle line, respectively. The letters (c, L and la) represent cervical setae, lateral setae on head and lateral anterior setae on thorax, respectively.

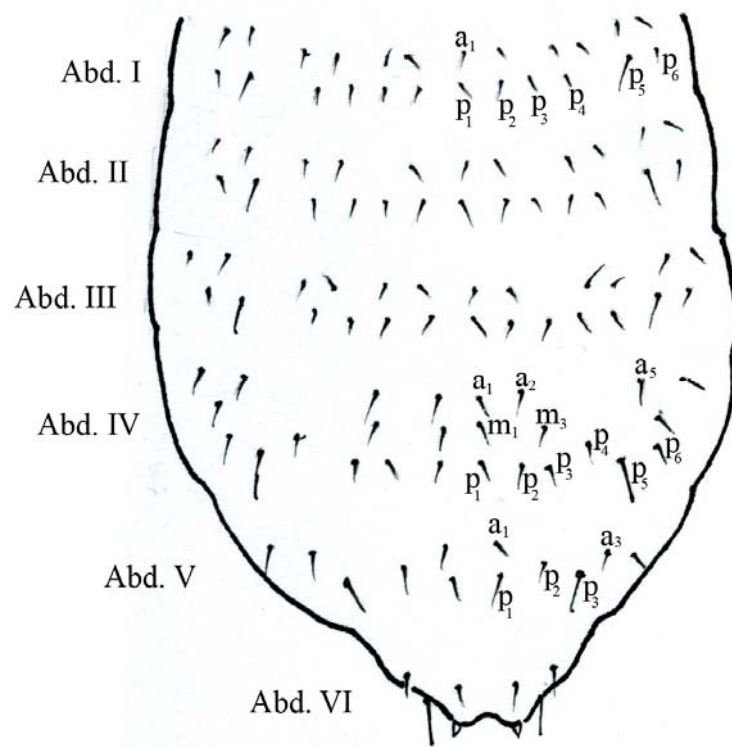
Dorsal: Head without c<sub>6</sub>; with l<sub>1</sub> as long as l<sub>3</sub> (Fig. 4-12A). Th. I with 3 + 3 setae in row. Th. II and III composed of 3 rows of setae, without a<sub>3</sub>, m<sub>2</sub>, m<sub>3</sub>; with la<sub>1</sub>, la<sub>2</sub>, la<sub>3</sub>; p<sub>4</sub> longer than other. Th. II a<sub>2</sub> displaced distally compared with a<sub>1</sub>. Th. II and III p<sub>2</sub> displaced apically compared with p<sub>1</sub> (Fig. 4-13). Abd. I – III covered with 2 rows of setae, p<sub>5</sub> sensory setae and longer than other. Abd. IV with 3 rows of setae; without a<sub>3</sub>, a<sub>4</sub>, m<sub>2</sub>, m<sub>4</sub>. p<sub>5</sub> sensory setae and longer than other. Abd. V with 2 rows of setae without a<sub>2</sub>, a<sub>4</sub>; p<sub>3</sub> sensory setae and longer than other (Fig. 4-14). Ventral: Head with a<sub>1</sub>, m<sub>1</sub>, m<sub>3</sub>, p<sub>1</sub> (Fig. 4-12B). Th. II and III without a pair of medial setae. Abd. III composed of 3 rows of setae; without setae above the tenaculum.



**Figure 4-12** Chaetotaxy of *Xenylla* sp. head. A. Dorsal view B. Ventral view



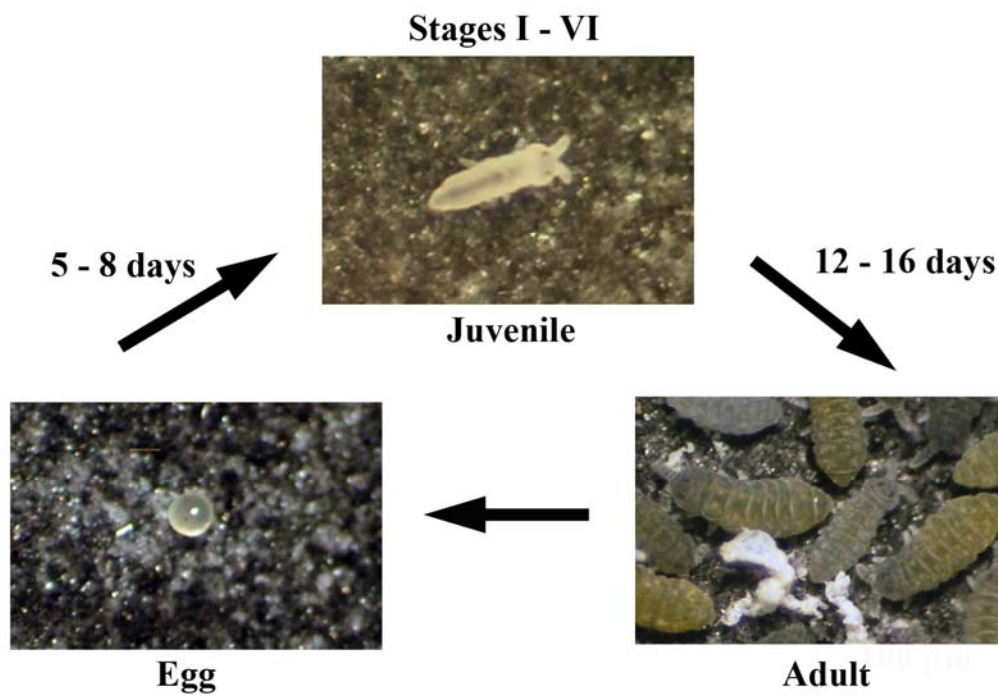
**Figure 4-13** Chaetotaxy of *Xenylla* sp. thorax (Dorsal view)



**Figure 4-14** Chaetotaxy of *Xenylla* sp. abdomen (Dorsal view)

#### 4.1.2 Life cycle of *Xenylla* sp.

When reared on plaster of Paris-charcoal substrate and the life cycle was recorded, this *Xenylla* species demonstrated typical key characteristics of collembolans with some variations. It is an ametabolous insect (or having no metamorphosis). They must be aggregated and usually laid eggs in batches in small holes on the substrate surface, in exuviae and on the side wall of the culture bottles as well. The life history consists of three developmental stages as egg, juvenile and adult stages (Fig. 4-15). The number of stages of a juvenile was determined by counting the number of exuviae before becoming adult. Six exuviae were recorded, indicating six juvenile stages before reaching adult stage. The adult stage was recognized when eggs were found in the bottles.



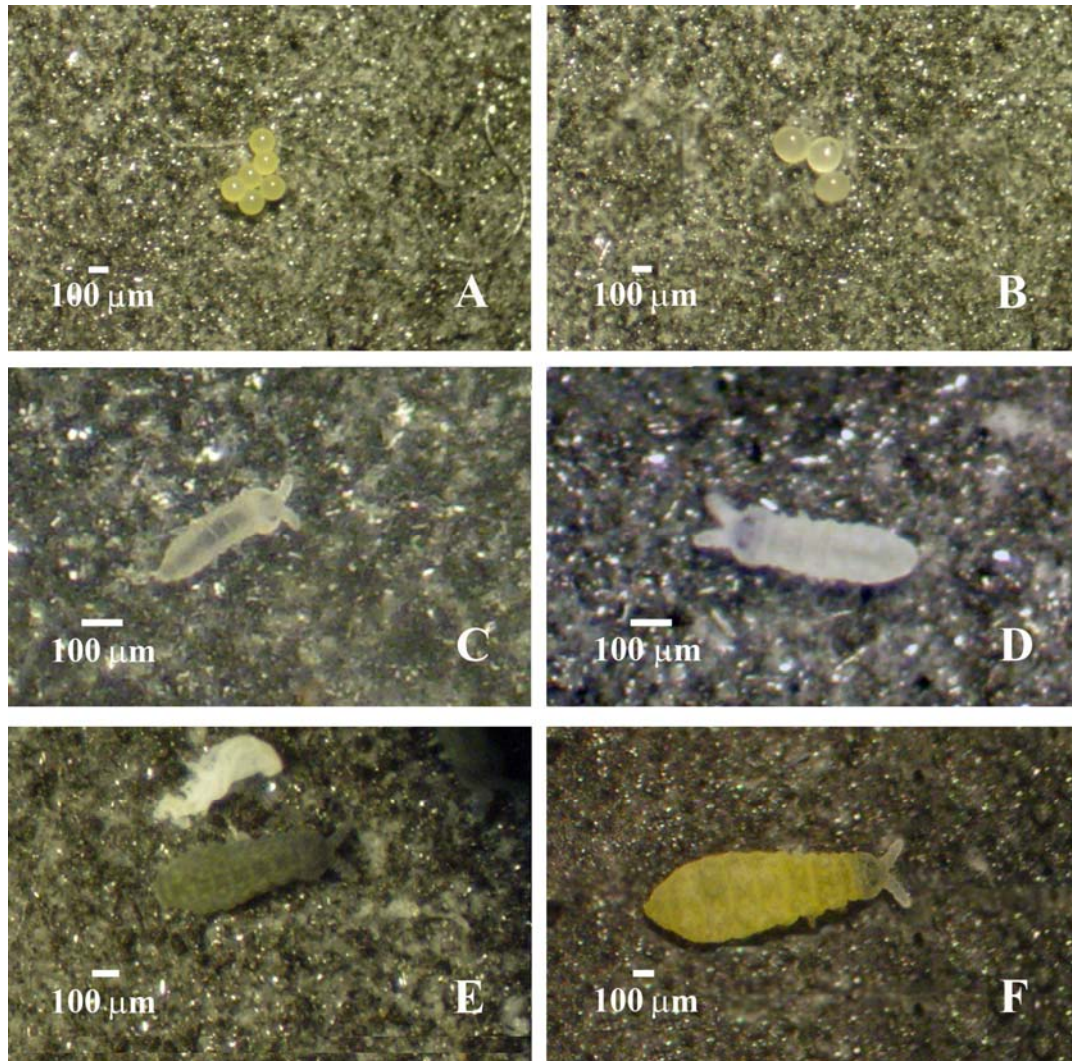
**Figure 4-15** Stages in life cycle of *Xenylla* sp.

The body size of all stages and the time spent (in days) for each stage are shown in Table 4-1. The average times from egg to juvenile and juvenile to adult were 6.44 and 15.23 days, respectively. An adult lived for the average of 64.27 days and molted throughout life.

**Table 4-1** Mean developmental time (days  $\pm$  SE) and the body size (mm) of *Xenylla* sp. in each stage reared at 25 – 28  $^{\circ}$ C

<b>Stage</b>		<b>Size (mm)</b>	<b>Time (days <math>\pm</math> SE)</b>
Egg		0.120 (diameter)	6.44 $\pm$ 0.01
Juvenile	Stage I	0.349 – 0.412	4.44 $\pm$ 0.12
	Stage II	0.459 – 0.506	2.14 $\pm$ 0.01
	Stage III	0.484 – 0.607	2.09 $\pm$ 0.03
	Stage IV	0.552 – 0.700	2.21 $\pm$ 0.07
	Stage V	0.586 – 0.753	2.27 $\pm$ 0.08
	Stage VI	0.640 – 0.836	2.26 $\pm$ 0.14
Total			15.23 $\pm$ 0.20
Adult		0.708 – 1.170	64.27 $\pm$ 1.36

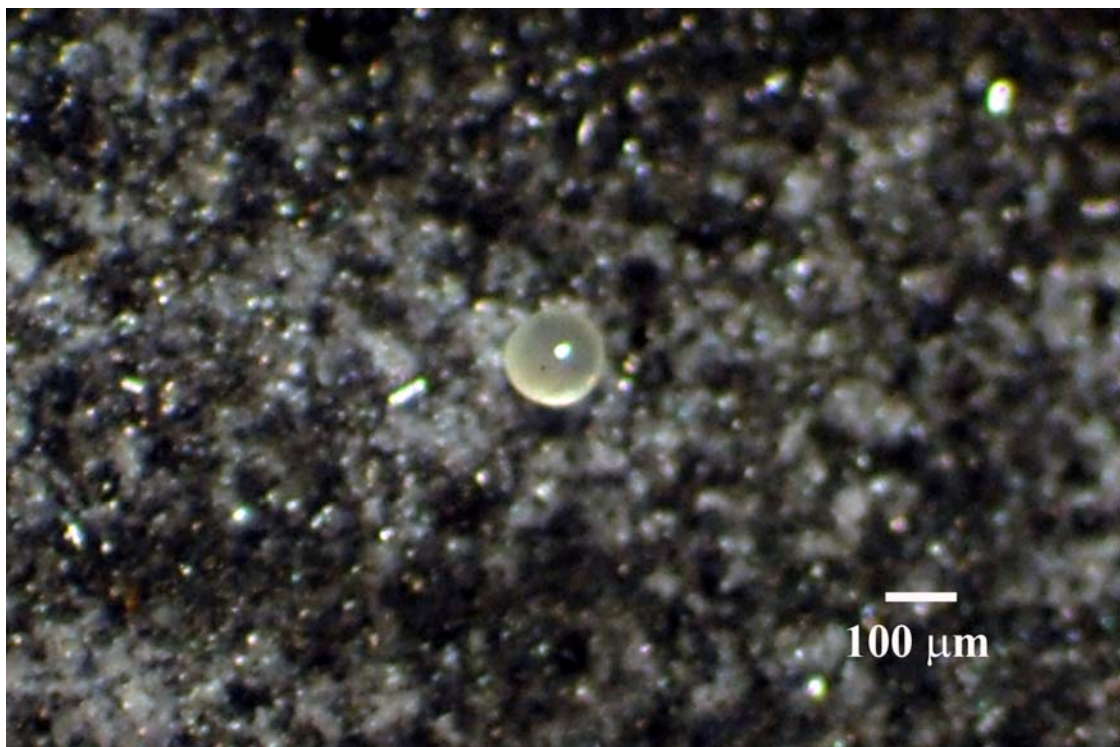
The general appearances of all development stages are summarized in Figure 4-16.



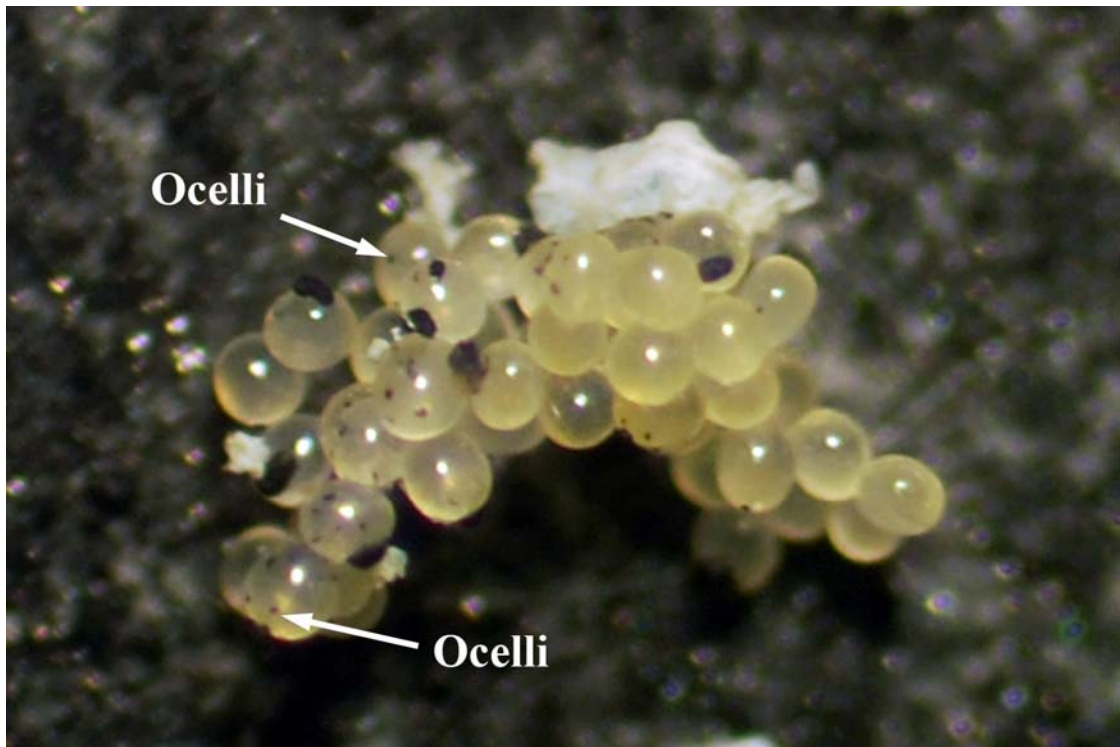
**Figure 4-16** Developmental stages of *Xenylla* sp.

- A. Newly-laid eggs
- B. Five-day-old eggs
- C. Newborn juvenile
- D. Seven-day-old juvenile
- E. Adult male
- F. Adult female

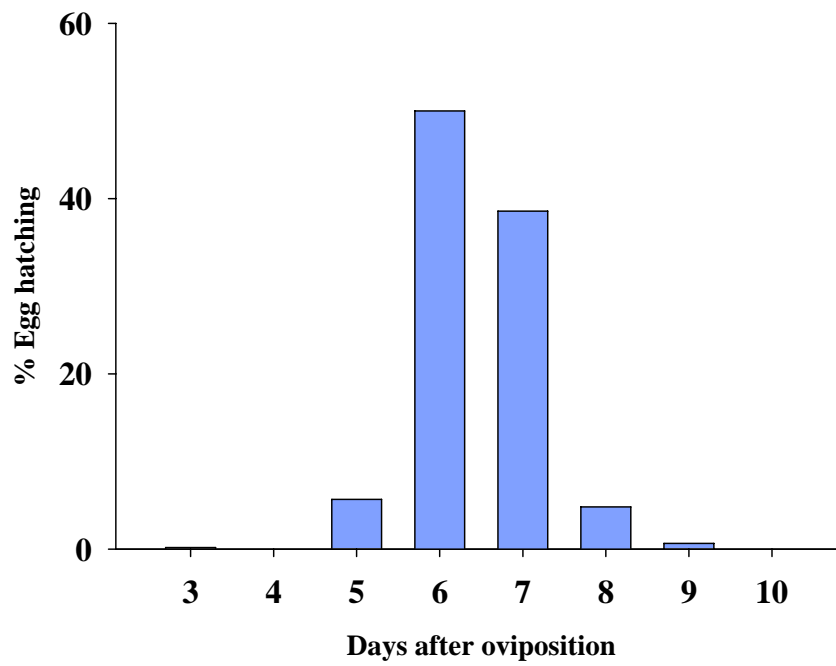
**Egg** - The newly-laid eggs of *Xenylla* sp. were spherical in shape, white in color, transparent and had a smooth surface (Fig. 4-16A). They were 120  $\mu\text{m}$  in diameter. After a few hours, the eggs became opaque (Fig. 4-16B). As the embryo began to develop, the size of the egg became gradually larger (Fig. 4-17), the shape changed from round to elliptical and eyes were present (Fig. 4-18). The time required for embryonic development was five to eight days and the percentage of egg hatching was highest (50 %) on the sixth day (Fig. 4-19).



**Figure 4-17** *Xenylla* sp. egg a few hours after oviposition



**Figure 4-18** *Xenylla* sp. eggs four to five days after oviposition



**Figure 4-19** The percentage of daily hatching of *Xenylla* sp. eggs

**Juvenile** (Fig. 4-16C and D) – A juvenile consisted of six stages. The body color of new-born juveniles was white and the overall appearance resembled the adult. The body consisted of three parts: a head, a thorax with three segments, and abdomen with six segments. They had a pair of ocellus groups and no trace of pigmentation on the body (Fig. 4-20). However, the degree of pigmentation gradually increased when they developed toward maturity. The first molting was about three to four days after hatch.



**Figure 4-20** *Xenylla* sp. juveniles one day after hatch

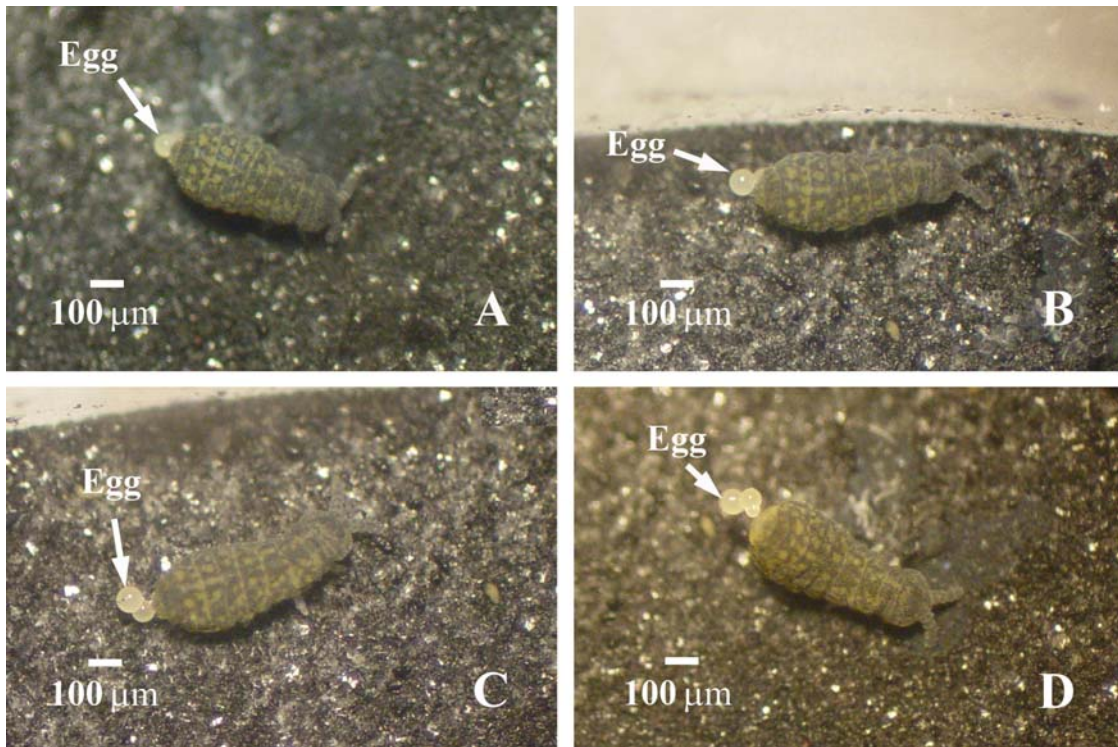
**Adult** (Fig. 4-16E – F) – The body length ranged from 708 to 1170  $\mu\text{m}$ . The sexes could be determined by using the color and size of the body. In general, female was bright yellow in color and the size was bigger than male while male was smaller with yellowish gray body and had visible pigmentation on the dorsal side of the body (Fig. 4-21). The developmental time from an egg to the adult ranged from 21 to 24 days at 25 – 28  $^{\circ}\text{C}$ . The first oviposition was 12 – 16 days after hatch and the number of eggs per individual averaged  $137.07 \pm 30.8$ . The mean adult longevity was

$64.27 \pm 1.355$  days. Adults were found to molt even after reaching maturity and continued throughout their life span.



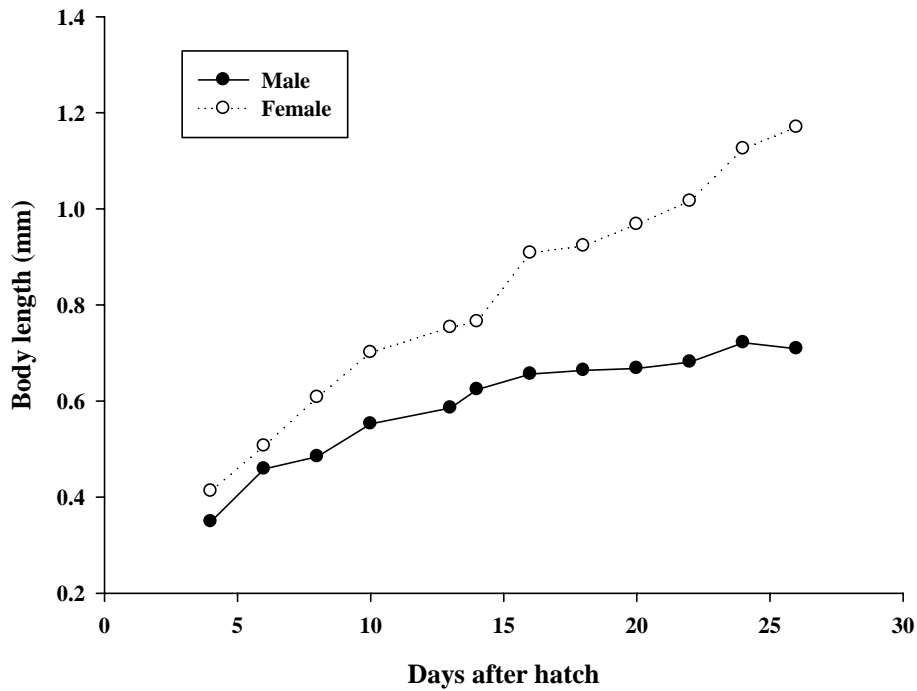
**Figure 4-21** Male and female *Xenylla* sp. sixty days after hatch

It was observed that the oviposition took place during the day and night and that eggs were usually found in small holes on the surface of the substrate, if any. During oviposition, the female remained immobile. Eggs were laid singly. The first egg was released out of the genital opening on the fifth abdominal segment and was deposited on the substrate through a little jerking of the abdominal tip. The second egg then followed immediately and was deposited over or adjacent to the first egg (Fig. 4-22). It appeared that parthenogenesis was not established because no eggs were found in the bottles that kept females alone throughout the experiment.



**Figure 4-22** Sequence of the oviposition of *Xenylla* sp. (in order from A to D) on plaster of Paris-charcoal substrate

After reaching the adult stage, the external features of newly-emerged male and female were similar. The sexes could be determined when they grew older and reached a certain age. From the observations, the body length of the two sexes became visibly different at 15 to 17 days after hatch (Fig. 4-23) while the measurement of body length in each day showed that the sexes of this species of *Xenylla* sp. could be determined as early as on the eighth day (Table 4-2). The pigmentation gradually increased in male, causing darker body color. The body color of females at this age was bright yellow without any visible dark pigmentation. The body of a female could reach 1.17 mm long at 25 days after hatch compared to 0.7 mm in male. In addition, histological process was used to confirm the sex. Oocytes were found in *Xenylla* sp. with bright yellow body and the body length over 1 mm. Figure 4-24 shows the oocytes in the ovaries of the *Xenylla* sp. composing of red yolk granules. This is consistent with the observation that females had yellow body and was larger in size than males.



**Figure 4-23** The body length of male and female *Xenylla* sp.

**Table 4-2** Comparison of body length between two sexes of *Xenylla* sp.

Day	Body length (mm)				Sig.	P-value
	Male		Female			
	mean	S.E.	mean	S.E.		
4	0.35	0.017	0.41	0.031	NS	0.146
6	0.46	0.02	0.51	0.039	NS	0.319
8	0.48	0.016	0.61	0.037	S	0.016
10	0.55	0.04	0.7	0.049	S	0.049
13	0.59	0.024	0.75	0.051	S	0.018
14	0.62	0.006	0.77	0.026	S	0.001
16	0.66	0.02	0.91	0.047	S	0.001
18	0.66	0.012	0.92	0.04	S	0.000
20	0.67	0.017	0.97	0.051	S	0.000
22	0.68	0.017	1.02	0.069	S	0.001
24	0.72	0.036	1.13	0.01	S	0.000
26	0.71	0.016	1.17	0.035	S	0.001

Note: The data were statistically analyzed using T-test ( $p < 0.05$ )

S indicates the significant differences of body length between sexes

NS indicates no significant differences of body length between sexes



**Figure 4-24** Oocytes in the ovaries of female *Xenylla* sp. (X-section, 400x, HE stain)

#### 4.1.3 Life table of *Xenylla* sp.

At 25 - 28 °C, fifty springtails were reared in the bottles (ten individuals per a bottle) with the mixture substrate of plaster of Paris, activated charcoal and water in the ratio of 8:4:5.5 by volume. The baker's yeasts were supplied as food. The life tables were constructed from the collection of data on the mortality of *Xenylla* sp. over one-day interval in the laboratory as shown in Table 4-3 to Table 4-5. The replicates demonstrate the variation in the longevity of the members. Data were collected for as long as 100 days but not shorter than 45 days. Using the information from these tables, the life table statistics were calculated and shown in Table 4-6. From three replicates, a female *Xenylla* sp. laid the average of 17.08 eggs per life time ( $R_0$ ) and increased at the rate of 0.0817 ( $r_m$ ) and 0.0739 ( $r_c$ ) per individual day<sup>-1</sup>. The mean of the finite rate of increase ( $\lambda$ ) was 1.0851 individuals day<sup>-1</sup> whereas the life expectancy of a newly-hatched juvenile ( $e_x$ ), generation time ( $T$ ), the cohort generation time ( $T_c$ ) and the doubling time ( $D$ ) were 45.81, 33.52, 37.12 and 8.54 days, respectively.

**Table 4-3** The abridged life table and fecundity table of female *Xenylla* sp. (Rep1, N = 25)

Stage	Age interval (n = days)		Parameter						
	n	days	$l_x$	$d_x$	$L_x$	$T_x$	$e_x$	$m_x$	$l_x m_x$
*Egg	6	0 - 6	1.0000	0.0667	5.7999	45.2796	45.2796	0.0000	0.0000
**Juvenile	12	6 - 18	0.9333	0.1036	10.5781	39.4797	42.3012	0.0000	0.0000
Adult	7	18 - 25	0.8297	0.0000	5.8080	28.9016	34.8333	2.1143	1.7542
Adult	7	25 - 32	0.8297	0.0000	5.8080	23.0936	27.8333	5.6571	4.6938
Adult	7	32 - 39	0.8297	0.0790	5.5314	17.2857	20.8333	3.8095	3.1608
Adult	7	39 - 46	0.7507	0.1976	4.5634	11.7542	15.6579	1.8526	1.3908
Adult	7	46 - 53	0.5531	0.2371	3.0423	7.1908	13.0000	0.8857	0.4899
Adult	7	53 - 60	0.3161	0.0790	1.9360	4.1486	13.1250	0.4000	0.1264
Adult	7	60 - 67	0.2371	0.0790	1.3829	2.2126	9.3333	0.0000	0.0000
Adult	7	67 - 74	0.1580	0.1185	0.6914	0.8297	5.2500	0.0000	0.0000
Adult	7	74 - 81	0.0395	0.0395	0.1383	0.1383	3.5000	0.0000	0.0000
Adult	4	81 - 85	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Note:  $l_x$  = Proportion at birth of female being alive at age interval  
 $d_x$  = Proportion of the female died at age interval  
 $L_x$  = Average number of individuals alive during the age interval x to x+1  
 $T_x$  = Sum of  $L_x$  during age interval x to finish  
 $e_x$  = Mean expectation of life for animals alive at birth of age x  
 $m_x$  = The number of female offspring per female per day  
 $l_x m_x$  = Egg curve

\* the values were estimated from the data of the percentage of egg hatching

\*\* the values were estimated from the data of the developmental time of juvenile

**Table 4-4** The abridged life table and fecundity table of female *Xenylla* sp. (Rep2, N = 17)

Stage	Age interval (n = days)		Parameter						
	n	days	$l_x$	$d_x$	$L_x$	$T_x$	$e_x$	$m_x$	$l_x m_x$
*Egg	6	0 - 6	1.0000	0.0667	5.7999	42.9245	42.9245	0.0000	0.0000
**Juvenile	12	6 - 18	0.9333	0.1037	10.5774	37.1246	39.7778	0.0000	0.0000
Adult	7	18 - 25	0.8296	0.0000	5.8072	26.5472	32.0000	2.2857	1.8962
Adult	7	25 - 32	0.8296	0.1185	5.3924	20.7400	25.0000	4.6000	3.8162
Adult	7	32 - 39	0.7111	0.0593	4.7702	15.3476	21.5833	4.1333	2.9392
Adult	7	39 - 46	0.6518	0.1778	3.9406	10.5774	16.2273	2.6182	1.7066
Adult	7	46 - 53	0.4741	0.2963	2.2814	6.6368	14.0000	0.6000	0.2844
Adult	7	53 - 60	0.1778	0.0000	1.2444	4.3554	24.5000	0.6667	0.1185
Adult	7	60 - 67	0.1778	0.0593	1.0370	3.1110	17.5000	1.7333	0.3081
Adult	7	67 - 74	0.1185	0.0593	0.6222	2.0740	17.5000	0.4000	0.0474
Adult	7	74 - 81	0.0593	0.0000	0.4148	1.4518	24.5000	4.4000	0.2607
Adult	7	81 - 88	0.0593	0.0000	0.4148	1.0370	17.5000	4.0000	0.2370
Adult	7	88 - 95	0.0593	0.0000	0.4148	0.6222	10.5000	6.0000	0.3555
Adult	7	95 - 102	0.0593	0.0593	0.2074	0.2074	3.5000	0.0000	0.0000
Adult	4	102 - 106	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Note:  $l_x$  = Proportion at birth of female being alive at age interval

$d_x$  = Proportion of the female died at age interval

$L_x$  = Average number of individuals alive during the age interval x to x+1

$T_x$  = Sum of  $L_x$  during age interval x to finish

$e_x$  = Mean expectation of life for animals alive at birth of age x

$m_x$  = The number of female offspring per female per day

$l_x m_x$  = Egg curve

\* the values were estimated from the data of the percentage of egg hatching

\*\* the values were estimated from the data of the developmental time of juvenile

**Table 4-5** The abridged life table and fecundity table of female *Xenylla* sp. (Rep3, N = 23)

Stage	Age interval (n = days)		Parameter						
	n	days	$l_x$	$d_x$	$L_x$	$T_x$	$e_x$	$m_x$	$l_x m_x$
*Egg	6	0 - 6	1.0000	0.0667	5.7999	49.2382	49.2382	0.0000	0.0000
**Juvenile	12	6 - 18	0.9333	0.1036	10.5780	43.4383	46.5427	0.0000	0.0000
Adult	7	18 - 25	0.8297	0.0000	5.8079	32.8603	39.6050	1.6632	1.3799
Adult	7	25 - 32	0.8297	0.0000	5.8079	27.0524	32.6053	7.8947	6.5502
Adult	7	32 - 39	0.8297	0.0437	5.6550	21.2445	25.6053	10.2316	8.4891
Adult	7	39 - 46	0.7860	0.0437	5.3493	15.5895	19.8333	2.5778	2.0262
Adult	7	46 - 53	0.7424	0.1747	4.5852	10.2402	13.7941	8.2353	6.1135
Adult	7	53 - 60	0.5677	0.3057	2.9039	5.6550	9.9615	3.3846	1.9214
Adult	7	60 - 67	0.2620	0.1310	1.3755	2.7511	10.5000	2.6000	0.6812
Adult	7	67 - 74	0.1310	0.0437	0.7642	1.3755	10.5000	2.1333	0.2795
Adult	7	74 - 81	0.0873	0.0437	0.4585	0.6114	7.0000	1.8000	0.1572
Adult	7	81 - 88	0.0437	0.0437	0.1528	0.1528	3.5000	0.4000	0.0175
Adult	7	88 - 95	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Note:  $l_x$  = Proportion at birth of female being alive at age interval

$d_x$  = Proportion of the female died at age interval

$L_x$  = Average number of individuals alive during the age interval x to x+1

$T_x$  = Sum of  $L_x$  during age interval x to finish

$e_x$  = Mean expectation of life for animals alive at birth of age x

$m_x$  = The number of female offspring per female per day

$l_x m_x$  = Egg curve

\* the values were estimated from the data of the percentage of egg hatching

\*\* the values were estimated from the data of the developmental time of juvenile

**Table 4-6** Life table statistics of female *Xenylla* sp. reared on a substrate at 25 – 28 °C

Rep	$R_0$	$\lambda$	$r_c$	$r_m$	$e_x$	T	$T_c$	D
1	11.6144	1.0811	0.0736	0.0780	45.2796	31.4391	33.3088	8.8865
2	11.9699	1.0787	0.0655	0.0758	42.9245	32.7493	37.8911	9.1444
3	27.6475	1.0956	0.0827	0.0913	49.2382	36.3586	40.1548	7.5920
Mean	17.0773	1.0851	0.0739	0.0817	45.8141	33.5157	37.1182	8.5410
S.E.	5.2861	0.0053	0.0050	0.0048	1.8421	1.4709	2.0137	0.4803

Note:  $R_0$ : Net reproductive rate (females per female)

$\lambda$ : Finite rate of increase (females per female day<sup>-1</sup>)

$r_c$ : Capacity for increase (females per female day<sup>-1</sup>)

$r_m$ : Intrinsic rate of natural increase (females per female day<sup>-1</sup>)

$e_x$ : Life expectancy (days)

T: Generation time (days)

$T_c$ : Cohort generation time (days)

D: Doubling time (days)

From the life table,  $l_x$  was used to plot the survivorship curve and it was found to resemble Type I. It indicates the population of *Xenylla* sp. with very little loss for most of the life span and high losses of older organisms. The fecundity of *Xenylla* sp. averaged 137.07 eggs per individual and eggs laid were highest on the second to the third week and gradually decreased when they were older (Fig. 4-25).

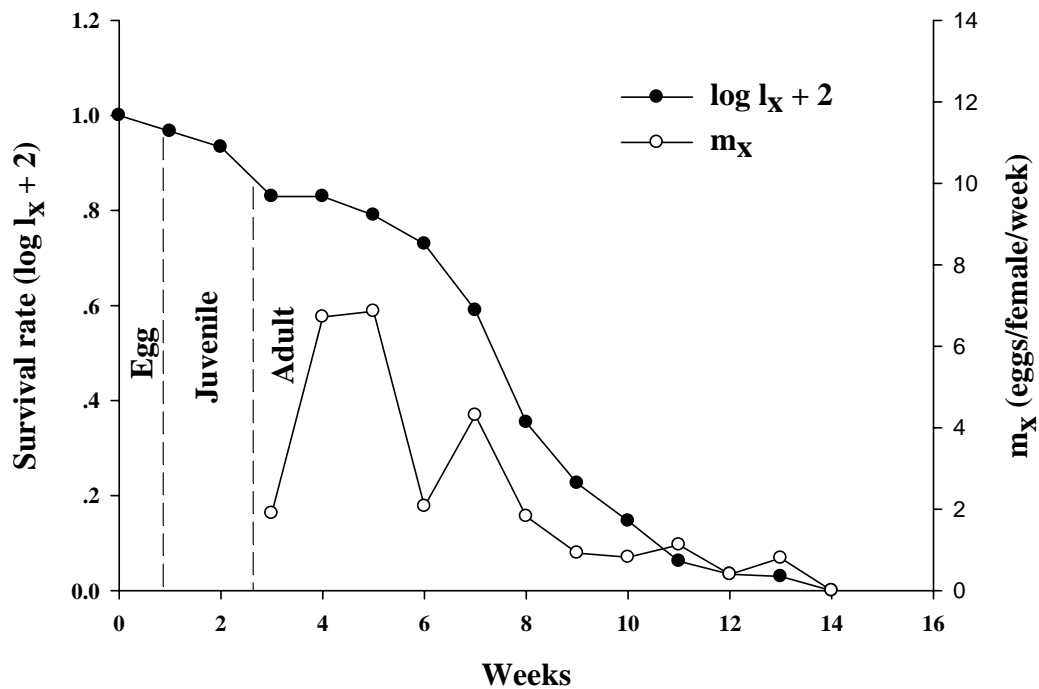


Figure 4-25 Survival rate and fecundity of *Xenylla* sp.

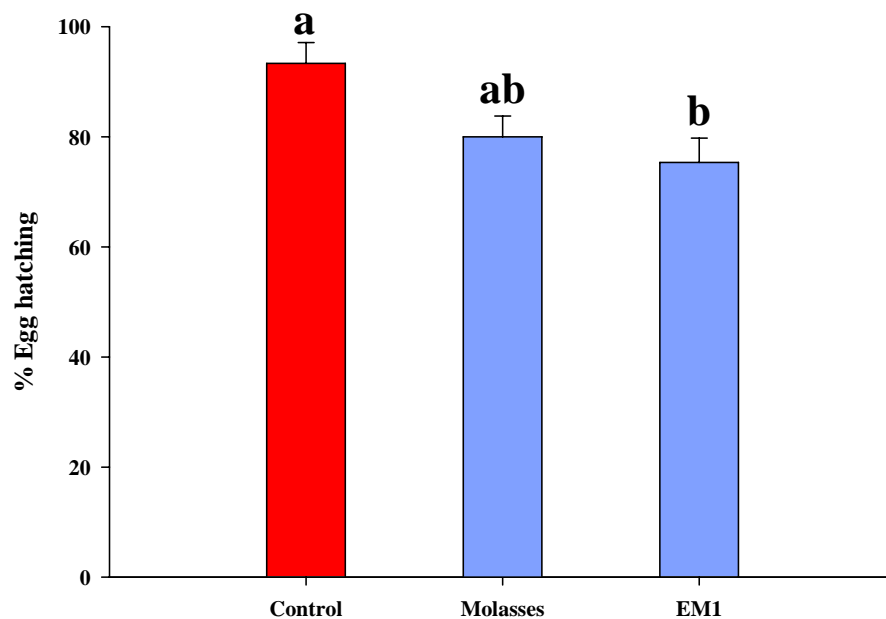
## 4.2 Effects of Effective Microorganism (EM<sup>TM</sup>) on the biology of *Xenylla* sp.

### 4.2.1 Effects of EM1 on the egg stage

The widely-used EM1 formula contains EM stock, molasses and water. Molasses and water were added in order to help increase the population of useful microorganisms. This is believed to promote the growth of crops.

Experiments were carried out to compare the influence of this EM1 formula on the biology of all three stages of development – egg, juvenile and adult – of this *Xenylla* sp. There were three treatment groups – EM1, molasses and water – for each developmental stage. The groups reared on substrate added with water served as the control. The parameter was the egg viability which was expressed as the percentage of hatching. The number of juveniles born was counted daily for the period of two weeks.

It was demonstrated that EM1 had effects on the percentage of hatching of *Xenylla* eggs. The eggs treated with EM1 hatched at the percentage significantly lower than the control ( $F = 5.404$ ; d.f. = 2, 12;  $p = 0.021$ ) whereas the hatching of eggs treated with molasses was not significantly different from the control ( $p > 0.05$ ). The highest percentage of the egg hatching were found in the control (93.33%) while the lowest values were recorded on EM1 (80.00%) (Fig. 4-26 and Table C-1 in Appendix C).

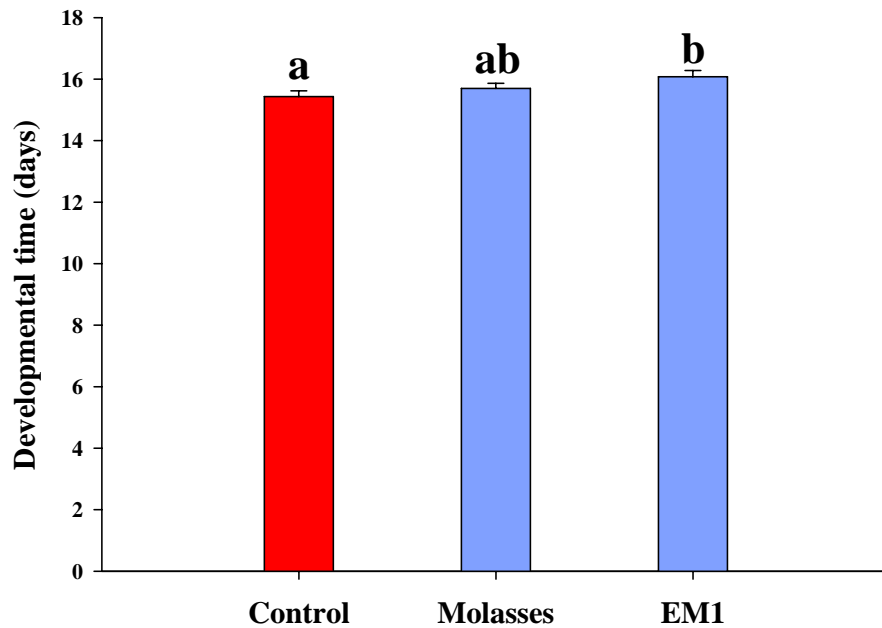


**Figure 4-26** The mean percentage of egg hatching of *Xenylla* sp. treated with water (control), molasses and EM1

Note = The same letters above bar indicate no significant differences,  $p > 0.05$ , Tukey's (HSD) test

#### 4.2.2 Effects of EM1 on the juvenile stage

The developmental time which is the time from hatching to the end of the juvenile stage was recorded (Table D-1 in Appendix D). It was found that the time spent from one stage of juvenile to the next was not significantly different in all treatments, but the overall period from juvenile to adult showed different result. The juveniles exposed to EM1 spent significantly longer time to emerge as adults ( $16.08 \pm 0.199$  days) than the control ( $F = 3.225$ ; d.f. = 2, 242;  $p = 0.041$ ). For those exposed to molasses, the time was not significantly different from the control ( $p > 0.05$ ).



**Figure 4-27** The mean developmental time of *Xenylla* sp. juvenile treated with water (control), molasses and EM1

Note = The same letters above bar indicate no significant differences,  $p > 0.05$ , Tukey's (HSD) test

#### 4.2.3 Effects of EM1 on the adult stage

The results demonstrated that EM1 and molasses caused the significant decrease of life expectancy when compared with the control ( $F = 7.023$ , d.f. = 2,  $p = 0.027$ ), while other parameters did not demonstrate any significant differences among control, molasses and EM1 (Table 4-7). However, the life expectancy of *Xenylla* sp. when reared in substrate with molasses and EM1 was not significantly different.

**Table 4-7** Life table statistics of *Xenylla* sp. treated with water, molasses and EM1

Parameter	Mean $\pm$ SE (n = 15)		
	Control	Molasses	EM1
*R <sub>0</sub>	17.08 <sup>a</sup> $\pm$ 5.29	25.23 <sup>a</sup> $\pm$ 6.33	18.62 <sup>a</sup> $\pm$ 3.42
$\lambda$	1.085 <sup>a</sup> $\pm$ 0.005	1.096 <sup>a</sup> $\pm$ 0.008	1.088 <sup>a</sup> $\pm$ 0.005
r <sub>c</sub>	0.074 <sup>a</sup> $\pm$ 0.005	0.084 <sup>a</sup> $\pm$ 0.007	0.078 <sup>a</sup> $\pm$ 0.004
r <sub>m</sub>	0.082 <sup>a</sup> $\pm$ 0.005	0.092 <sup>a</sup> $\pm$ 0.008	0.084 <sup>a</sup> $\pm$ 0.005
e <sub>x</sub>	45.81 <sup>a</sup> $\pm$ 1.84	39.63 <sup>b</sup> $\pm$ 1.08	39.92 <sup>b</sup> $\pm$ 0.801
T	33.52 <sup>a</sup> $\pm$ 1.47	34.16 <sup>a</sup> $\pm$ 0.54	34.46 <sup>a</sup> $\pm$ 0.34
T <sub>c</sub>	37.12 <sup>a</sup> $\pm$ 2.01	37.66 <sup>a</sup> $\pm$ 0.73	37.14 <sup>a</sup> $\pm$ 0.42
D	8.54 <sup>a</sup> $\pm$ 0.48	7.64 <sup>a</sup> $\pm$ 0.67	8.31 <sup>a</sup> $\pm$ 0.30

Note: R<sub>0</sub>: Net reproductive rate (females per female)

$\lambda$ : Finite rate of increase (females per female day<sup>-1</sup>)

r<sub>c</sub>: Capacity for increase (females per female day<sup>-1</sup>)

r<sub>m</sub>: Intrinsic rate of natural increase (females per female day<sup>-1</sup>)

e<sub>x</sub>: Life expectancy (days)

T: Generation time (days)

T<sub>c</sub>: Cohort generation time (days)

D: Doubling time (days)

The data were statistically analyzed using One-way ANOVA (p < 0.05).

The values with the same letter within a row are not significantly different at the 5% level judged by the Tukey's (HSD) test

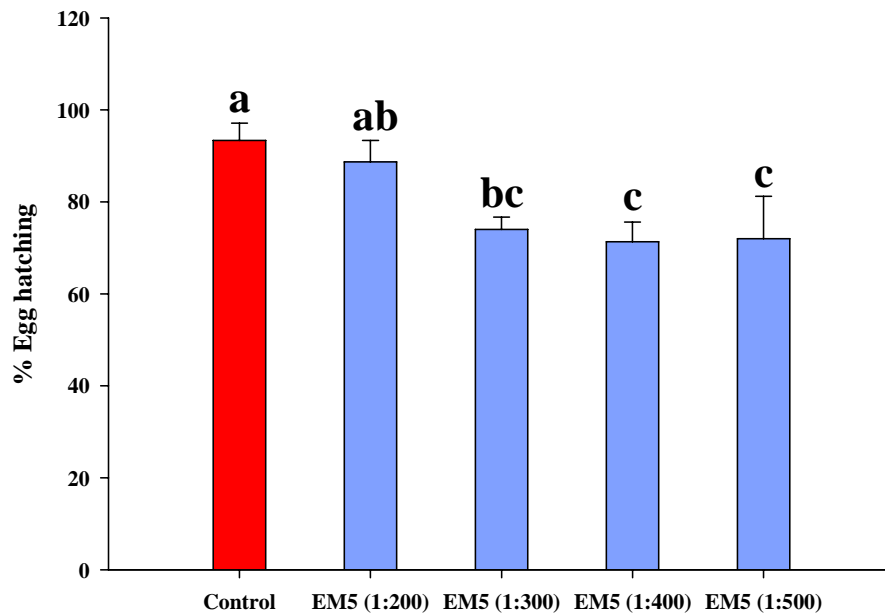
\* The data were statistically analyzed using Kruskal-wallis test (p < 0.05)

### **4.3 Effects of EM5 on the biology of *Xenylla* sp.**

EM5 is another EM formula widely used among agriculturists in Thailand. It is a specialized formula aimed at repelling insect pests. Consequently, vinegar and rice whiskey are added to the mixture of basic EM (EM stock + molasses + water) with varying concentrations of the two. The experiments were designed to evaluate the effects that the EM5 exposure might have on *Xenylla* biology.

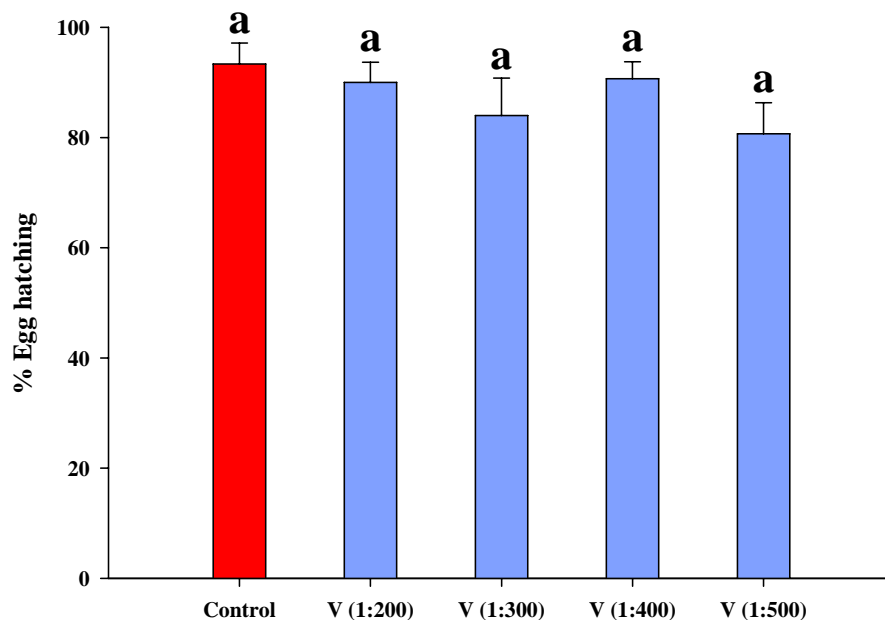
#### **4.3.1 Effects of EM5 on the egg stage**

The egg hatching percentages of *Xenylla* sp. treated with EM5 and rice whiskey were significantly lower than the control except EM5 at the concentration of 1:200 (Fig. 4-28) and rice whiskey at the concentration of 1:500 (Fig. 4-30) ( $F = 3.641$ ; d.f. = 4, 20;  $p = 0.022$  and  $\chi^2 = 16.94$ ; d.f. = 4;  $p = 0.002$ , respectively). The highest percentage were found in the control (93.33 %) while the lower concentrations (1:300, 1:400 and 1:500) of EM5 resulted in the significant reduction of the hatching percentage (Table C-2 in Appendix C). At the concentrations of 1:200, 1:300 and 1:400 of rice whiskey caused significant reduction and had the lowest hatching percentage when compared among control. In vinegar, It should be noted that all four concentrations did not cause significant reduction of hatching percentage when compared to the control (Fig. 4-29).



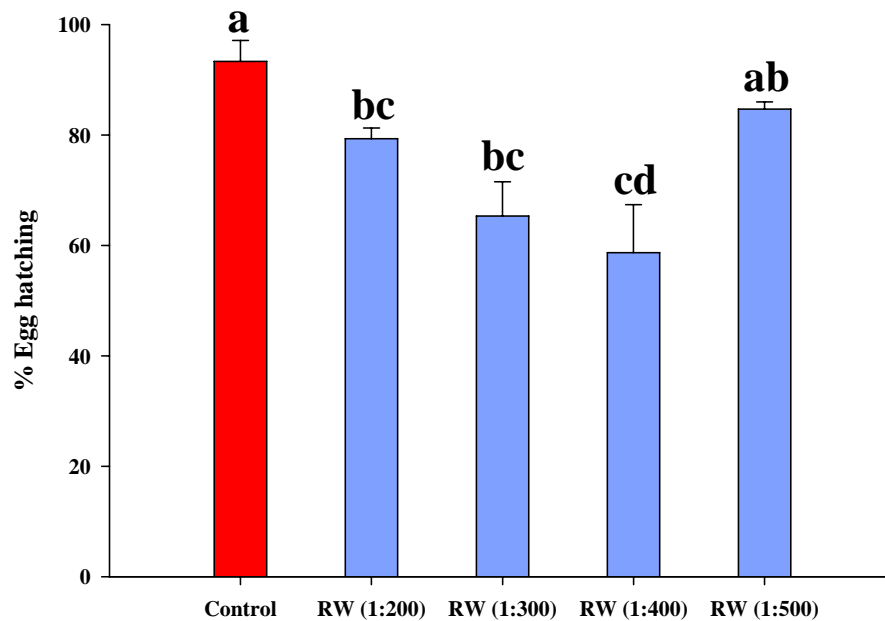
**Figure 4-28** The mean percentage of egg hatching of *Xenylla* sp. treated with different concentrations of EM5

Note = The same letters above bar indicate no significant differences,  $p > 0.05$ , Tukey's (HSD) test



**Figure 4-29** The mean percentage of egg hatching of *Xenylla* sp. treated with different concentrations of vinegar

Note = The same letters above bar indicate no significant differences,  $p > 0.05$ , Tukey's (HSD) test



**Figure 4-30** The mean percentage of egg hatching of *Xenylla* sp. treated with different concentrations of rice whiskey

Note = The same letters above bar indicate no significant differences,  $p > 0.05$ , Tukey's (HSD) test

#### 4.3.2 Effect of EM5 on the juvenile stage

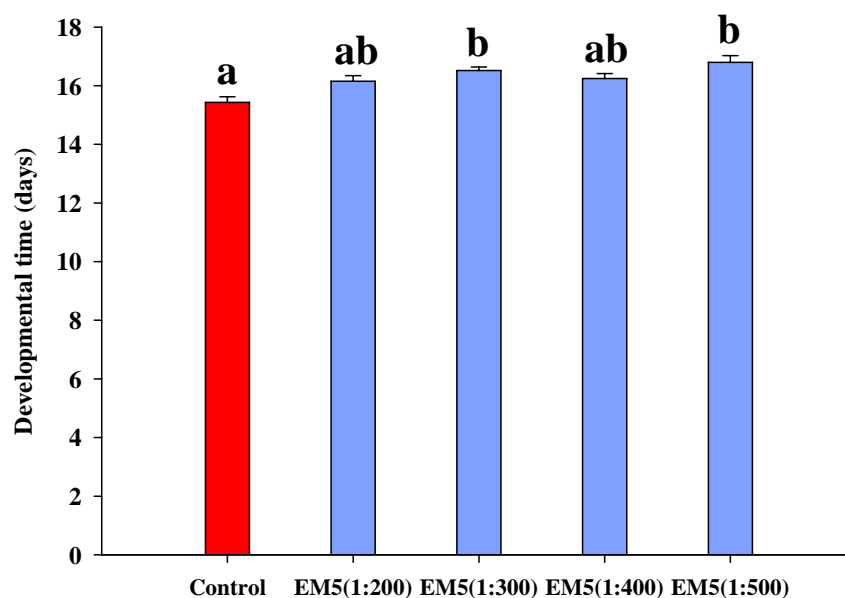
The experiments carried out demonstrated that some concentrations of EM5, vinegar and rice whiskey had effects on the developmental time of *Xenylla* sp. juvenile, but not consistently. Those treatments resulted in the delay of molting from one juvenile stage to the next when comparing to the control. The significant differences of developmental time of juvenile among control and different concentrations of EM5, vinegar and rice whiskey are shown in Table D-2 to D-4 in Appendix D.

In EM5 treatments, only the developmental time from the stage I – II, II – III, and III – IV were delayed (Table D-2 in Appendix D). The times from the stage IV - V and V - VI were not significantly affected. The concentration that caused the delay in the three periods was 1: 500 which is the most diluted one. At the highest concentration of 1:200, the delay was on the stage I - II only. The time from the last juvenile stage to become adult was longer than the control at 1:400 only. In total, the

significantly longer time was found when treated with 1:300 and 1:500 concentrations (Fig. 4-31).

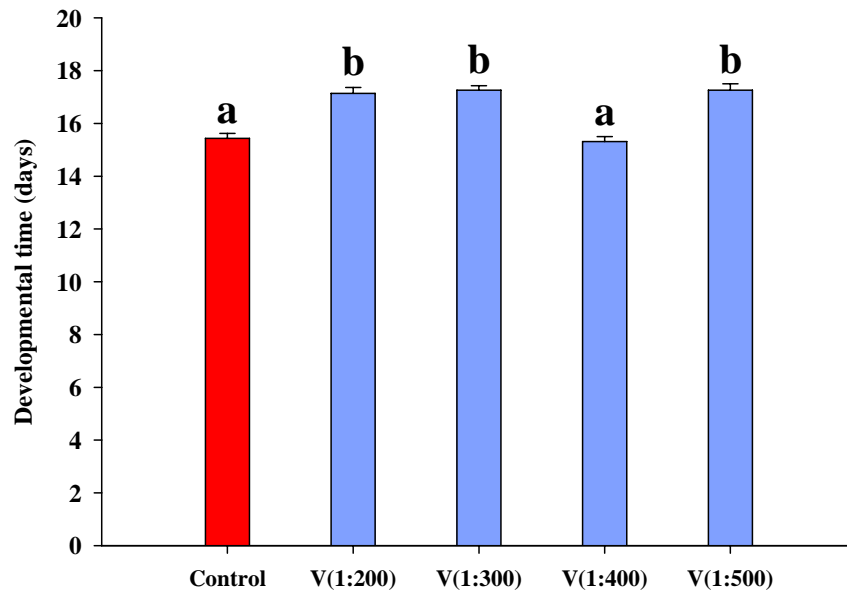
When tested with vinegar, it was observed that the concentration of 1:400 had no significant effects on any stages of juvenile including the total time (Table D-3 in Appendix D and Fig. 4-32). Other three concentrations caused the delayed developmental time from stage I to adult. Considering each interval separately, the delay was not consistent. The effects were as follows: 1:200 on stage II – III, IV – V and V – VI; 1:300 on stage I – II, II – III and III – IV; 1:500 on all intervals except stage II – III and V – VI.

The total developmental time (stage I of juvenile – adult) was significantly longer when exposed to all four concentrations of rice whiskey (Fig. 4-33). All six intervals were delayed according to some concentrations. The 1:200 affected IV – V, V – VI and VI – adult whereas 1:300 was on stage II – III and III – IV. The 1:400 affected early (stage I – II and II – III) while 1:500 had on the same intervals as 1:400 with the addition of stage III – IV (Table D-4 in Appendix D).



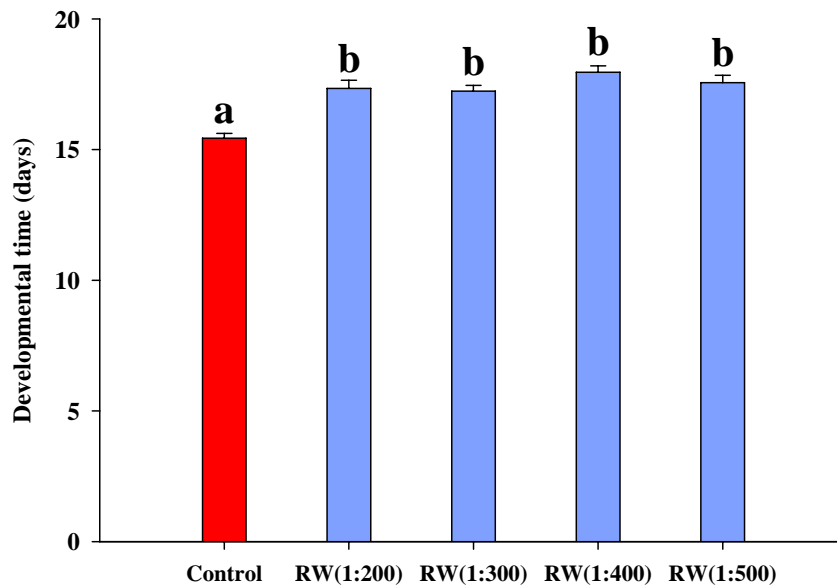
**Figure 4-31** The mean developmental time of *Xenylla* sp. juvenile treated with different concentrations of EM5

Note = The same letters above bar indicate no significant differences,  $p > 0.05$ , Tukey's (HSD) test



**Figure 4-32** The mean developmental time of *Xenylla* sp. juvenile treated with different concentrations of vinegar

Note = The same letters above bar indicate no significant differences,  $p > 0.05$ , Tukey's (HSD) test



**Figure 4-33** The mean developmental time of *Xenylla* sp. juvenile treated with different concentrations of rice whiskey

Note = The same letters above bar indicate no significant differences,  $p > 0.05$ , Tukey's (HSD) test

### 4.3.3 Effects of EM5 on the adult stage

The mean life table statistics of *Xenylla* sp. exposed to EM5, vinegar and rice whiskey are shown in Table 4-8 to 4-10. The experiments demonstrated that EM5 at the concentration of 1:400 and rice whiskey at the concentrations of 1:300 and 1:400 have influences on the life expectancy ( $e_x$ ) of *Xenylla* sp. adults whereas vinegar had effects on finite rate of increase ( $\lambda$ ), capacity for increase ( $r_c$ ), intrinsic rate of natural increase ( $r_m$ ) and doubling time (D) in varying degrees.

When compared to the control, vinegar at any concentrations did not affect on net reproductive rate ( $R_0$ ), life expectancy ( $e_x$ ), the generation time (T) and cohort generation time ( $T_c$ ). In contrast, both  $\lambda$  and  $r_m$  were significantly higher at the concentrations of 1:200, 1:300 and 1:500. The doubling time (D) for vinegar-treated was significantly shorter in the concentrations of 1:200, 1:300 and 1:500. The capacity of increase ( $r_c$ ) was significantly higher only at the concentration of 1:500.

Similar to EM5, rice whiskey demonstrated the negative effects on life expectancy which was shortest when compared among the three treatments, especially at the concentration of 1:300.

Among the three treatments, female *Xenylla* sp. treated with vinegar had the highest fecundity of 77.03 eggs per individual at 1:500 concentration, compared to 44.43 and 32.07 eggs per individual in EM5 (1:400) and RW (1:400), respectively.

**Table 4-8** Life table statistics of *Xenylla* sp. treated with EM5

Parameter	Mean $\pm$ SE				
	Control	EM5			
		1:200	1:300	1:400	1:500
*R <sub>0</sub>	17.08 <sup>a</sup> $\pm$ 5.29	15.75 <sup>a</sup> $\pm$ 2.52	21.09 <sup>a</sup> $\pm$ 3.86	21.35 <sup>a</sup> $\pm$ 5.78	15.35 <sup>a</sup> $\pm$ 2.23
$\lambda$	1.085 <sup>a</sup> $\pm$ 0.005	1.084 <sup>a</sup> $\pm$ 0.002	1.090 <sup>a</sup> $\pm$ 0.006	1.088 <sup>a</sup> $\pm$ 0.008	1.083 <sup>a</sup> $\pm$ 0.003
r <sub>c</sub>	0.074 <sup>a</sup> $\pm$ 0.005	0.074 <sup>a</sup> $\pm$ 0.004	0.077 <sup>a</sup> $\pm$ 0.005	0.075 <sup>a</sup> $\pm$ 0.006	0.074 <sup>a</sup> $\pm$ 0.002
r <sub>m</sub>	0.082 <sup>a</sup> $\pm$ 0.005	0.081 <sup>a</sup> $\pm$ 0.002	0.087 <sup>a</sup> $\pm$ 0.006	0.084 <sup>a</sup> $\pm$ 0.007	0.080 <sup>a</sup> $\pm$ 0.003
e <sub>x</sub>	45.81 <sup>a</sup> $\pm$ 1.84	41.52 <sup>a</sup> $\pm$ 3.15	35.30 <sup>a</sup> $\pm$ 1.05	32.88 <sup>b</sup> $\pm$ 0.86	35.51 <sup>a</sup> $\pm$ 0.61
T	33.52 <sup>a</sup> $\pm$ 1.47	33.83 <sup>a</sup> $\pm$ 1.53	34.84 <sup>a</sup> $\pm$ 0.31	35.05 <sup>a</sup> $\pm$ 0.92	33.81 <sup>a</sup> $\pm$ 0.71
T <sub>c</sub>	37.12 <sup>a</sup> $\pm$ 2.01	36.67 <sup>a</sup> $\pm$ 2.05	39.04 <sup>a</sup> $\pm$ 0.25	39.40 <sup>a</sup> $\pm$ 1.57	36.70 <sup>a</sup> $\pm$ 1.20
D	8.54 <sup>a</sup> $\pm$ 0.48	8.61 <sup>a</sup> $\pm$ 0.25	8.08 <sup>a</sup> $\pm$ 0.57	8.38 <sup>a</sup> $\pm$ 0.81	8.69 <sup>a</sup> $\pm$ 0.34

Note: R<sub>0</sub>: Net reproductive rate (females per female)

$\lambda$ : Finite rate of increase (females per female day<sup>-1</sup>)

r<sub>c</sub>: Capacity for increase (females per female day<sup>-1</sup>)

r<sub>m</sub>: Intrinsic rate of natural increase (females per female day<sup>-1</sup>)

e<sub>x</sub>: Life expectancy (days)

T: Generation time (days)

T<sub>c</sub>: Cohort generation time (days)

D: Doubling time (days)

The data were statistically analyzed using One-way ANOVA ( $p < 0.05$ ).

The values with the same letter within a row are not significantly different at the 5% level judged by the Tukey's (HSD) test

\* The data were statistically analyzed using Kruskal-wallis test ( $p < 0.05$ )

**Table 4-9** Life table statistics of *Xenylla* sp. treated with vinegar

Parameter	Mean $\pm$ SE				
	Control	Vinegar			
		1:200	1:300	1:400	1:500
*R <sub>0</sub>	17.08 <sup>a</sup> $\pm$ 5.29	39.33 <sup>a</sup> $\pm$ 9.38	48.97 <sup>a</sup> $\pm$ 9.33	21.41 <sup>a</sup> $\pm$ 2.52	44.41 <sup>a</sup> $\pm$ 6.13
$\lambda$	1.085 <sup>a</sup> $\pm$ 0.005	1.109 <sup>bc</sup> $\pm$ 0.007	1.109 <sup>bc</sup> $\pm$ 0.002	1.091 <sup>ab</sup> $\pm$ 0.004	1.112 <sup>c</sup> $\pm$ 0.002
r <sub>c</sub>	0.074 <sup>a</sup> $\pm$ 0.005	0.089 <sup>ab</sup> $\pm$ 0.003	0.086 <sup>ab</sup> $\pm$ 0.001	0.078 <sup>a</sup> $\pm$ 0.003	0.094 <sup>b</sup> $\pm$ 0.001
r <sub>m</sub>	0.082 <sup>a</sup> $\pm$ 0.005	0.103 <sup>bc</sup> $\pm$ 0.006	0.104 <sup>bc</sup> $\pm$ 0.002	0.087 <sup>ab</sup> $\pm$ 0.003	0.106 <sup>c</sup> $\pm$ 0.001
e <sub>x</sub>	45.81 <sup>a</sup> $\pm$ 1.84	46.00 <sup>a</sup> $\pm$ 2.17	48.67 <sup>a</sup> $\pm$ 2.63	44.87 <sup>a</sup> $\pm$ 1.46	41.06 <sup>a</sup> $\pm$ 2.62
T	33.52 <sup>a</sup> $\pm$ 1.47	34.89 <sup>a</sup> $\pm$ 0.42	37.08 <sup>a</sup> $\pm$ 0.99	34.96 <sup>a</sup> $\pm$ 0.63	35.41 <sup>a</sup> $\pm$ 0.99
T <sub>c</sub>	37.12 <sup>a</sup> $\pm$ 2.01	40.58 <sup>a</sup> $\pm$ 1.37	44.86 <sup>a</sup> $\pm$ 2.43	39.28 <sup>a</sup> $\pm$ 0.93	39.90 <sup>a</sup> $\pm$ 2.01
D	8.54 <sup>a</sup> $\pm$ 0.48	6.76 <sup>b</sup> $\pm$ 0.41	6.68 <sup>b</sup> $\pm$ 0.15	7.97 <sup>ab</sup> $\pm$ 0.32	6.51 <sup>b</sup> $\pm$ 0.09

Note: R<sub>0</sub>: Net reproductive rate (females per female)

$\lambda$ : Finite rate of increase (females per female day<sup>-1</sup>)

r<sub>c</sub>: Capacity for increase (females per female day<sup>-1</sup>)

r<sub>m</sub>: Intrinsic rate of natural increase (females per female day<sup>-1</sup>)

e<sub>x</sub>: Life expectancy (days)

T: Generation time (days)

T<sub>c</sub>: Cohort generation time (days)

D: Doubling time (days)

The data were statistically analyzed using One-way ANOVA ( $p < 0.05$ ).

The values with the same letter within a row are not significantly different at the 5% level judged by the Tukey's (HSD) test

\* The data were statistically analyzed using Kruskal-wallis test ( $p < 0.05$ )

**Table 4-10** Life table statistics of *Xenylla* sp. treated with rice whiskey

Parameter	Mean $\pm$ SE				
	Control	Rice whiskey			
		1:200	1:300	1:400	1:500
*R <sub>0</sub>	17.08 <sup>a</sup> $\pm$ 5.29	11.40 <sup>a</sup> $\pm$ 1.71	13.91 <sup>a</sup> $\pm$ 0.31	16.05 <sup>a</sup> $\pm$ 0.37	19.56 <sup>a</sup> $\pm$ 2.67
* $\lambda$	1.085 <sup>a</sup> $\pm$ 0.005	1.069 <sup>a</sup> $\pm$ 0.005	1.079 <sup>a</sup> $\pm$ 0.001	1.079 <sup>a</sup> $\pm$ 0.001	1.089 <sup>a</sup> $\pm$ 0.005
r <sub>c</sub>	0.074 <sup>ab</sup> $\pm$ 0.005	0.060 <sup>a</sup> $\pm$ 0.003	0.070 <sup>ab</sup> $\pm$ 0.001	0.068 <sup>ab</sup> $\pm$ 0.002	0.076 <sup>b</sup> $\pm$ 0.003
*r <sub>m</sub>	0.082 <sup>a</sup> $\pm$ 0.005	0.067 <sup>a</sup> $\pm$ 0.004	0.076 <sup>a</sup> $\pm$ 0.001	0.076 <sup>a</sup> $\pm$ 0.001	0.085 <sup>a</sup> $\pm$ 0.004
*e <sub>x</sub>	45.81 <sup>a</sup> $\pm$ 1.84	34.18 <sup>ab</sup> $\pm$ 0.54	30.06 <sup>b</sup> $\pm$ 0.87	30.18 <sup>b</sup> $\pm$ 0.83	38.91 <sup>ab</sup> $\pm$ 2.94
T	33.52 <sup>a</sup> $\pm$ 1.47	35.83 <sup>a</sup> $\pm$ 0.19	34.46 <sup>a</sup> $\pm$ 0.69	36.67 <sup>a</sup> $\pm$ 0.66	34.65 <sup>a</sup> $\pm$ 0.65
T <sub>c</sub>	37.12 <sup>a</sup> $\pm$ 2.01	40.00 <sup>a</sup> $\pm$ 0.98	37.80 <sup>a</sup> $\pm$ 0.96	40.98 <sup>a</sup> $\pm$ 1.30	38.86 <sup>a</sup> $\pm$ 1.11
D	8.54 <sup>a</sup> $\pm$ 0.48	10.41 <sup>a</sup> $\pm$ 0.72	9.07 <sup>a</sup> $\pm$ 0.15	9.16 <sup>a</sup> $\pm$ 0.13	8.17 <sup>a</sup> $\pm$ 0.44

Note: R<sub>0</sub>: Net reproductive rate (females per female)

$\lambda$ : Finite rate of increase (females per female day<sup>-1</sup>)

r<sub>c</sub>: Capacity for increase (females per female day<sup>-1</sup>)

r<sub>m</sub>: Intrinsic rate of natural increase (females per female day<sup>-1</sup>)

e<sub>x</sub>: Life expectancy (days)

T: Generation time (days)

T<sub>c</sub>: Cohort generation time (days)

D: Doubling time (days)

The data were statistically analyzed using One-way ANOVA ( $p < 0.05$ ).

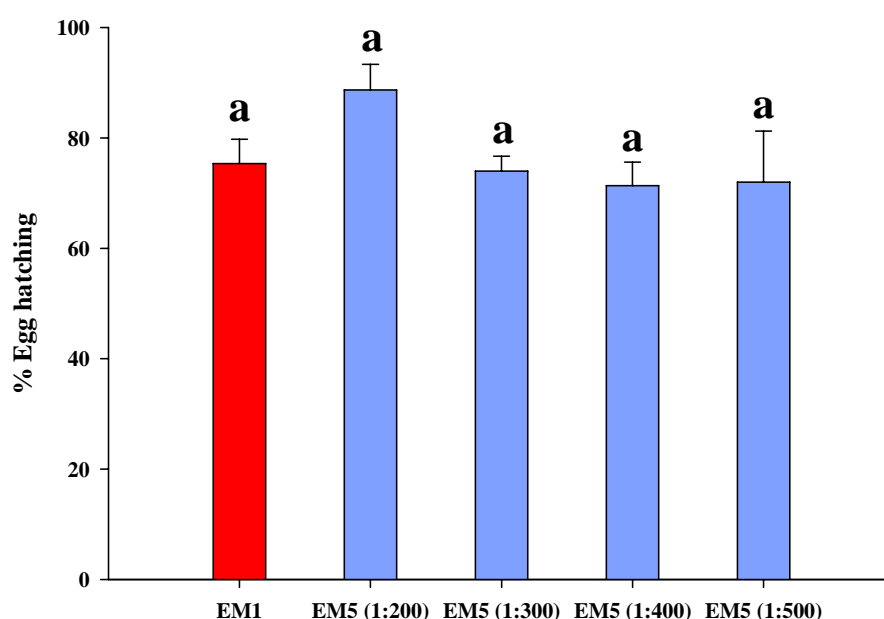
The values with the same letter within a row are not significantly different at the 5% level judged by the Tukey's (HSD) test

\* The data were statistically analyzed using Kruskal-wallis test ( $p < 0.05$ )

## 4.4 Comparison of effect of EM1 and EM5

### 4.4.1 Effects on the egg stage

The experiments carried out demonstrated that the effect of EM1 and EM5 were not significantly different on egg hatching ( $p > 0.05$ ). The highest percentage of the egg hatching was found in the EM5 (1:200) as 88.67% while the lowest value was recorded on EM5 (1:400) as 71.33% (Fig. 4-34 and Table C-3 in Appendix C).



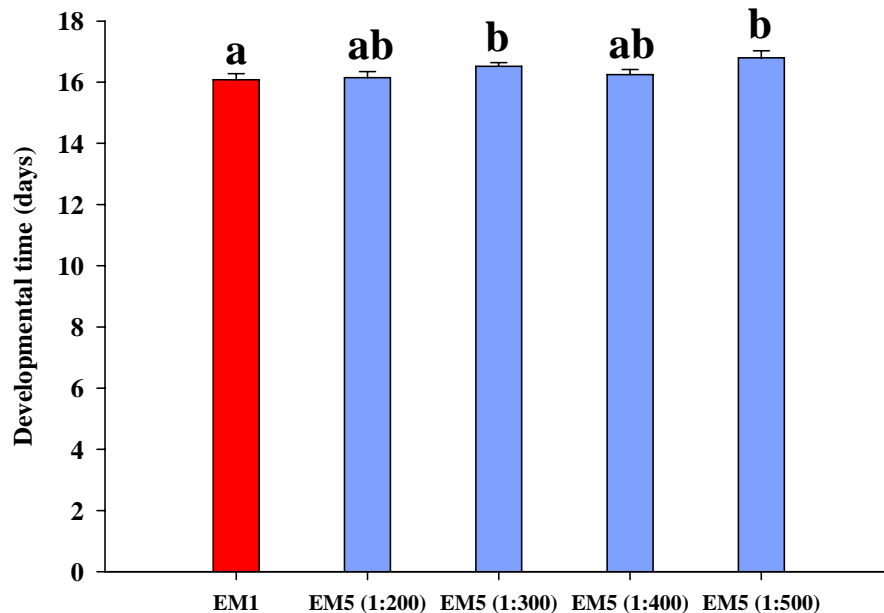
**Figure 4-34** Average percentage of egg hatching of *Xenylla* sp. treated with EM1 and EM5

Note = The same letters above bar are not significantly different,  $p > 0.05$ , Tukey's (HSD) test.

### 4.4.2 Effects on the juvenile stage

When compared the developmental time from juvenile to adult after the exposures to EM1 and EM5, the time of *Xenylla* sp. treated with EM5 at concentrations of 1:300 and 1:500 were significantly longer than the control (Fig. 4-35). For each stage, it was found that the times from stage IV – V and V – VI were not significantly different between the two treatments. For the other four periods as well as

the total developmental time, they were significantly longer than EM1 treatment (Table D-5 in Appendix D).



**Figure 4-35** Average developmental time of *Xenylla* sp. juvenile treated with EM1 and EM5

Note = The same letters above bar indicate no significant differences,  $p > 0.05$ , Tukey's (HSD) test

#### 4.4.3 Effects on the adult stage

In overall, the mean life table statistics of *Xenylla* sp. exposed to the treatments shown in Table 4-11 did not demonstrate any significant differences between EM1 and EM5 ( $p > 0.05$ ), except the life expectancy ( $F = 4.987$ , d.f. = 4,  $p = .018$ ). The shortest life expectancy was found when treated with EM5 at the concentration of 1:400. However, it should be noted that the highest net reproduction ( $R_0$ ) was found in EM5 and increased when the concentrations of EM5 decreased except at 1:500, while life expectancy ( $e_x$ ) was also the highest in EM5 and values decreased according to the reduction of concentrations.

**Table 4-11** Life table statistics of *Xenylla* sp. treated with EM1 and EM5

Parameter	Mean $\pm$ SE				
	EM	EM5			
		1:200	1:300	1:400	1:500
$R_0$	18.62 <sup>a</sup> $\pm$ 3.42	15.75 <sup>a</sup> $\pm$ 2.52	21.09 <sup>a</sup> $\pm$ 3.86	21.35 <sup>a</sup> $\pm$ 5.78	15.35 <sup>a</sup> $\pm$ 2.23
$\lambda$	1.088 <sup>a</sup> $\pm$ 0.005	1.084 <sup>a</sup> $\pm$ 0.002	1.090 <sup>a</sup> $\pm$ 0.006	1.088 <sup>a</sup> $\pm$ 0.008	1.083 <sup>a</sup> $\pm$ 0.003
$r_c$	0.078 <sup>a</sup> $\pm$ 0.004	0.074 <sup>a</sup> $\pm$ 0.004	0.077 <sup>a</sup> $\pm$ 0.005	0.075 <sup>a</sup> $\pm$ 0.006	0.074 <sup>a</sup> $\pm$ 0.002
$r_m$	0.084 <sup>a</sup> $\pm$ 0.005	0.081 <sup>a</sup> $\pm$ 0.002	0.087 <sup>a</sup> $\pm$ 0.006	0.084 <sup>a</sup> $\pm$ 0.007	0.080 <sup>a</sup> $\pm$ 0.003
$e_x$	39.92 <sup>a</sup> $\pm$ 0.801	41.52 <sup>ab</sup> $\pm$ 3.15	35.30 <sup>ab</sup> $\pm$ 1.05	32.88 <sup>b</sup> $\pm$ 0.86	35.51 <sup>ab</sup> $\pm$ 0.61
T	34.46 <sup>a</sup> $\pm$ 0.34	33.83 <sup>a</sup> $\pm$ 1.53	34.84 <sup>a</sup> $\pm$ 0.31	35.05 <sup>a</sup> $\pm$ 0.92	33.81 <sup>a</sup> $\pm$ 0.71
$T_c$	37.14 <sup>a</sup> $\pm$ 0.42	36.67 <sup>a</sup> $\pm$ 2.05	39.04 <sup>a</sup> $\pm$ 0.25	39.40 <sup>a</sup> $\pm$ 1.57	36.70 <sup>a</sup> $\pm$ 1.20
D	8.31 <sup>a</sup> $\pm$ 0.30	8.61 <sup>a</sup> $\pm$ 0.25	8.08 <sup>a</sup> $\pm$ 0.57	8.38 <sup>a</sup> $\pm$ 0.81	8.69 <sup>a</sup> $\pm$ 0.34

Note:  $R_0$ : Net reproductive rate (females per female)

$\lambda$ : Finite rate of increase (females per female day<sup>-1</sup>)

$r_c$ : Capacity for increase (females per female day<sup>-1</sup>)

$r_m$ : Intrinsic rate of natural increase (females per female day<sup>-1</sup>)

$e_x$ : Life expectancy (days)

T: Generation time (days)

$T_c$ : Cohort generation time (days)

D: Doubling time (days)

The data were statistically analyzed using One-way ANOVA ( $p < 0.05$ ).

The values with the same letter within a row are not significantly different at the 5% level judged by the Tukey's (HSD) test.

## CHAPTER V

### DISCUSSION

#### 5.1 Biology of *Xenylla* sp.

##### 5.1.1 Diagnostic characters

From the detailed examination under compound microscope of specimens used in this study, it was concluded that they belong to genus *Xenylla* by using mainly five characteristics: (1) the elongate body, (2) mandible with mandibular plate and apical teeth, (3) absent postantennal organ (PAO), (4) absent unguiculus and (5) short furcula. This was consistent with the key characteristics of genus *Xenylla* Tullberg, 1869 that was described by Thibaud et al. (2004). However, it was not possible to identify it down to the species level based on any available keys. There were so many different characteristics that were unique and distant itself from other species, even though they did share the features of the genus *Xenylla*. The characteristics in questions are, for example: (1) chaetotaxy, (2) the number of ocelli, (3) the number of setae on each antennal segment and (4) the body length and body color. When comparisons of morphology of *Xenylla* sp. in this study with species from Korea (Lee et al., 2005) and China (Wu & Yin, 2007) were made, some similarities were found to four *Xenylla* recorded from Korea - *X. convexopyga*, *X. longistriata*, *X. dotata* and *X. marina*. The similar structures were, for example, apical bulb and 4 sensilla on the fourth antennal segment, 4 apical teeth on mandible, 4+4 setae on ventral tube, 3+3 teeth of tenaculum, two clavate tenent hairs in each leg and two dorsal setae on dens. The differences from Korea and China species were in body color, size, number of ocelli, number of setae and chaetotaxy as shown in Table 5-1 and 5-2, respectively.

**Table 5-1** Comparisons of morphological characters of *Xenylla* sp. in this study with four species from Korea (Lee et al., 2005)

	<i>Xenylla</i> sp.	<i>X. convexopyga</i>	<i>X. longistriata</i>	<i>X. dotata</i>	<i>X. marina</i>
Color	yellow (female) yellowish gray(male)	blue black	blackish gray	blue gray to blackish	gray
Body length	710-1170	850-1100	1000-1400	680-1100	678-915
Eye	4+4	5+5	5+5	5+5	5+5
Number of setae					
Ant. I:II:III	6:11:17	7:13:19	7:12:18	7:12:18	7:12:16
Coxa	7	8	7	7	7
Trochanter	5	5	5	5	5
Femur	10	11	11	11	11
Tibiotarsus	19	18	18	18	18
Ratio					
Antenna: Head	0.75: 1	0.7: 1	0.9: 1	0.8: 1	0.7: 1
Ant. I:II:III:IV	1: 1.44: 1.63: 2.37	1: 1.3: 1.5: 2.1	1: 1.2 : 1.4: 2.1	1: 1.2: 1.3: 1.7	1: 1.8: 2.4: 3.1
Chaetotaxy					
Head (dorsal)	a <sub>0</sub> , c <sub>1</sub> , c <sub>2</sub> , p <sub>1</sub> , p <sub>2</sub>	a <sub>0</sub> , c <sub>1</sub> , c <sub>2</sub> , p <sub>1</sub> , p <sub>2</sub>	a <sub>0</sub> , c <sub>1</sub> , p <sub>1</sub> , p <sub>2</sub>	a <sub>0</sub> , c <sub>1</sub> , p <sub>1</sub> , p <sub>2</sub>	a <sub>0</sub> , c <sub>1</sub> , p <sub>1</sub> , p <sub>2</sub>
Head (ventral)	a <sub>1</sub> , m <sub>1</sub> , m <sub>3</sub> , p <sub>1</sub>	a <sub>1</sub> , m <sub>1</sub>	a <sub>1</sub> , m <sub>1</sub> , m <sub>3</sub> , p <sub>1</sub>	a <sub>1</sub> , m <sub>1</sub> , m <sub>3</sub> , p <sub>1</sub>	a <sub>1</sub> , m <sub>1</sub> , m <sub>3</sub> , p <sub>1</sub>
Th. II-III	no a <sub>3</sub> , m <sub>2</sub> , m <sub>3</sub>	no a <sub>3</sub> , m <sub>2</sub> , m <sub>3</sub>	no a <sub>3</sub> , m <sub>2</sub> , m <sub>4</sub>	no a <sub>3</sub> , m <sub>2</sub> , m <sub>4</sub>	no a <sub>3</sub> , m <sub>2</sub> , m <sub>4</sub>
Sensory setae on Abd. I-III	p <sub>5</sub>	p <sub>6</sub>	p <sub>6</sub>	p <sub>6</sub>	p <sub>6</sub>
Habitat	Thailand	Korea	Korea	Korea	Korea

Note: The letters in chaetotaxy indicate the positions of setae based on the system by Gama et al. (2001) and Lee et al. (2005)

**Table 5-2** Comparisons of morphological characters of *Xenylla* sp. in this study with two species from China (Wu & Yin, 2007)

	<i>Xenylla</i> sp.	<i>X. changlingensis</i>	<i>X. changchunensis</i>
Color	yellow (female) yellowish gray(male)	dark blue violet (in alcohol)	red-brown (in alcohol)
Body length	710-1170	up to 950	up to 810
Eye	4+4	5+5	5+5
Number of setae			
Ant. I:II:III	6:11:17	7: 12: -	7: 12: -
Coxa	7	-	-
Trochanter	5	-	-
Femur	10	-	-
Tibiotarsus	19	-	-
Ratio			
Antenna: Head	0.75: 1	-	-
Ant. I:II:III:IV	1: 1.44: 1.63: 2.37	-	-
Chaetotaxy			
Head (dorsal)	a <sub>0</sub> , c <sub>1</sub> , c <sub>2</sub> , p <sub>1</sub> , p <sub>2</sub>	a <sub>0</sub> , c <sub>1</sub> , p <sub>1</sub> , p <sub>2</sub>	a <sub>0</sub> , c <sub>2</sub> , p <sub>1</sub> , p <sub>2</sub>
Head (ventral)	a <sub>1</sub> , m <sub>1</sub> , m <sub>3</sub> , p <sub>1</sub>	a <sub>1</sub> , m <sub>1</sub> , m <sub>3</sub>	a <sub>1</sub> , m <sub>1</sub> , m <sub>3</sub> , p <sub>1</sub>
Th. II-III	no a <sub>3</sub> , m <sub>2</sub> , m <sub>3</sub>	no a <sub>3</sub> , m <sub>2</sub> , m <sub>3</sub>	no a <sub>3</sub> , m <sub>2</sub> , m <sub>3</sub>
Sensory setae on Abd. I-III	p <sub>5</sub>	p <sub>6</sub>	p <sub>6</sub>
Habitat	Thailand	China	China

Note: The letters in chaetotaxy indicate the positions of setae based on the system by Gama et al. (2001) and Lee et al. (2005)

The morphological variations among individuals in the same species do exist and should not be ignored. But in case of springtails, the clearest characters are the body color as well as the number and ratio of setae which usually serve as the diagnostic characters (Heckman, 2001).

The body color of this species was yellow or yellowish gray which was very distinct from the color of blackish or brown or blue of the species from Korea and China. It is known that in some species, the distribution of pigments is species-specific. Another big difference was found in the number of ocelli in each group. All other species had five whereas this species had four. Chaetotaxy is probably the most controversial taxonomic method but it is widely used and still acceptable among taxonomists. It is a difficult and tedious task that needs high accuracy and well-prepared slides. The differences in chaetotaxy in this study from the closest species from Korea and China were found especially on the antenna, femur, tibiotarsus and abdominal segments I – III. The determination of an unknown minute insect inhabiting in soil like this one is usually difficult as one may imagine. After closely observing a large number of the specimens, it is proposed here that this species is most likely a new species of *Xenylla* genus.

This *Xenylla* sp. was also compared to the species from Singapore which was identified by Yosii (1959) as *Xenylla similata*. The resemblance was in 4 characters: 4+4 ocelli, two setae on mucro, two latent hairs on tibiotarsus and a pair of anal spines on the abdominal segment. For chaetotaxy and body color, Yosii did not give detailed descriptions of *X. similata* as needed or enough to be able to make comparisons. However, the illustrated chaetotaxy of *X. similata* revealed that the setae on the Th. III and Abd. I at p3 and p4 were longer than the others. This differed from the studied species because it was the setae at p4 and p5 which were longer. In addition, the body color of *X. similata* was not mentioned in the report. Consequently, it could be stated that this studied species was similar to *X. similata* in most features but not conclusive to be identified as belonging to *X. similata*.

### 5.1.2 Life cycle

From the investigation in the laboratory, *Xenylla* sp. was an ametabolous insect and had three stages in its life cycle. The juvenile went through six ecdyses to become adult. This is similar to *X. welchi* as reported by Bandyopadhyaya and Choudhuri (2002). However, at 25-28 C, the comparisons of the developmental time (in days) of *Xenylla* sp. obtained in this study with *X. welchi* demonstrate that the life cycle was longer in the species studied than *X. welchi* (21.67 days vs. 15.5 days). In contrast, the life span of an individual of *X. welchi* was longer (83 days vs. 79.5 days). It should be noted that the egg size of *X. welchi* was much larger as shown in Table 5-3.

**Table 5-3** Comparisons of the biology between the reared *Xenylla* sp. and *Xenylla welchi* under laboratory conditions (25 – 28 °C)

	<i>Xenylla</i> sp.	<i>Xenylla welchi</i>
Egg size (mm)	0.120	0.210
Egg viability (%)	93.33	74
Developmental time (days)		
Embryonic stage	6-7	5
Postembryonic stage	12-16	10.5
Fecundity (eggs)	137.07	134-148
Number of juvenile stages	6	6
Life span (days)	79.5	83

When the survivorship curve was plotted, it closely resembled Type I which are basically found in mammals with few offspring and having parental care. However, it could be found in other groups of animals reared under ideal conditions in laboratory as well.

### 5.1.3 Sex determination

Adult males and females of most springtails are generally indistinct in their morphological characters. Studies on this aspect were rare as well. However, there were some species that exhibited sexual dimorphism. In most of such cases, sexes could be determined by using the combination of body length and body color. When

these two parameters were implemented for the species in this study, it was found that the body length became distinct into two groups at the age of eight days after hatch. Those with larger bodies gradually changed body color from white to bright yellow. It was most visible about two weeks after becoming adults. This group is the female that was confirmed by histological method. For body color of *Xenylla* sp. male, it was gray due to the increase of dark pigments around the body except on the inter-segmental portion and ventral side. It was also found that female reared over two months old became darkened due to the dark pigments covering the dorsal side of the body.

The histological study of *Xenylla* sp. was carried out in order to confirm the hypothesis: Female had bright yellow body color and larger body while body color of male was darker and body was shorter. Twenty specimens with dark body color and twenty with yellow body color were selected from the mass culture. Two or three specimens were embedded into each paraffin block before sectioning because they were very light in weight and easy to float to the surface, making it impossible to set the positions during embedding. Serial cross sections of the body were made. After staining the tissue sections, only a few slides of serial sections could be used to observe reproductive system. The position of the reproductive system and the position of specimens of most slides did not allow to clearly observe the system. Oocytes were found in the springtails with bright yellow body and the body length over 1 mm. The male reproductive system was not found due to the small size of testes. Dallai et al. (2000) observed under electron microscope and described the morphology of testes as cylindrical sacs having beak at apical region. The active spermatogenesis occurred into the apical and the middle region, and then formed and stored spermatids in the basal region of the gonad at the distal testes.

This is the first report on sexual dimorphism of a *Xenylla* species with the confirmation by histological method. In 1976, Sharma and Metz studied *Xenylla grisea* and found that male and female could be differentiated by the distribution of black pigmentation. Male *X. grisea* had small dark spots scattered densely on almost all body segments while female's spots were dense on the head.

The ovipository behavior of *Xenylla* sp. was similar to the observations of Bandyopadhyaya and Choudhuri (2002) who recorded the egg batches of *Xenylla welchi* as being laid in holes, exuviae and the side wall of the culture bottle. This

species did not lay eggs throughout its whole life span. Eggs laid were the highest at the early weeks (2<sup>nd</sup> and 3<sup>rd</sup> week) and gradually decreased later in life. This was consistent with studies by Bandyopadhyaya and Choudhuri (2002) on *Xenylla welchi* and Snider (1973) on *Folsomia candida*.

## 5.2 Effects of EM1 on the biology of *Xenylla* sp.

The demographic study applied for use in biological researches mainly involves toxicological approach. It is an evaluation of the lethal as well as sublethal effects of substances, *i.e.* insecticides, on animals. In this study, the effects of Effective Microorganisms (EM<sup>TM</sup>) were evaluated. So, the toxic effects were not expected since EM served as the substance that might promote the population of beneficial soil insects like springtails. They are the primary consumers of organic materials and fungal feeders. There were evidences that feeding of favorable fungi and the nitrogen content in their hyphae had the positive effects on the growth rate and fecundity of some springtails (Booth & Anderson, 1979; Chen et al., 1995; Klironomos et al., 1999).

The hypothesis of this experiment is that EM1 might have a significant role in promoting the growth of springtails (*Xenylla* sp. in this case) indirectly by adding more beneficial microorganisms including fungi in the soil. With increasing amount of fungal food, springtails could have a larger population and be more effective in the turnover of organic matters. This would finally have enhanced the soil fertility and the crop yields would have been increased.

So the experiments were designed to record the biological performances of springtails once exposed to EM1 in comparison with the control. The parameters are the egg viability, developmental time from juvenile to adult and the life table statistics of adults. About 1 cm<sup>3</sup> of EM1 were added on the substrate before test and three fresh drops were added once a week until the end of the experiment. This followed the practice by farmers. When EM1 was sprayed into the bottle, it was taken up by *Xenylla* sp. individuals through two possible routes: passing into the body of *Xenylla* sp. by the ventral tube which functioned about water and salt exchange (Hopkin, 1997) and through the indirect absorption of EM1 by way of feeding on baker's yeast.

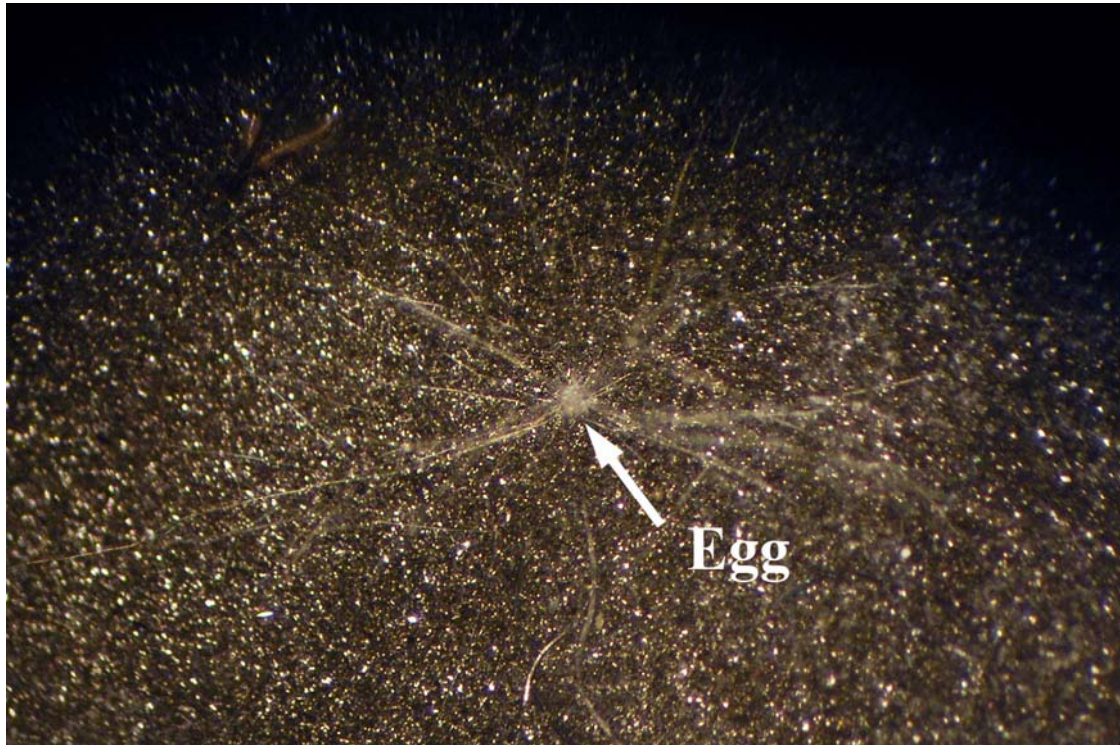
As a result, the percentage of egg hatching treated with EM1 was lower than the control and the developmental time from juvenile stage I to adult was longer than the control, while the life table parameters were not significantly different between the control and EM1, except life expectancy ( $e_x$ ). For molasses, there were no effects on egg and juvenile stages of *Xenylla* sp. So it appears that EM1 did not have positive effects on the reproduction ( $R_0$ ,  $\lambda$ ,  $r_c$ , and  $r_m$ ) and had the effect on the longevity ( $e_x$ ) of *Xenylla* sp. Instead, it even acted negatively in some aspects under laboratory conditions. Possible reasons:

For the treatment on egg stage, a rapid growth of fungi was found to cover the egg surfaces which led to the lower rate of egg hatching (Fig. 5-1). When EM were added into the substrate, they helped increase microorganisms including fungi, providing unsuitable conditions for eggs. In addition, fungal hyphae that covered egg might absorb water and nutrients from eggs resulting in the inhibition of egg hatching.

For the delayed developmental time of juvenile stages, it is not clearly understood. However, it might be due to the decrease of humidity and accumulation of secreted toxic substances and waste products from microorganisms added into the culture bottle. EM and springtails might take up a large amount of water for their metabolism. The relative humidity (RH) was reported to have effects on various species of collembolans in several papers. For example, Davies (1928) concluded that five epigeonistic species of springtails required 100 % RH for survival, Mayer (1957) reported that an euedaphic springtail (*Onychiurus armatus*) required 100% RH for its long-term survival and Ashraf (1971) also reported that euedaphic collembolan (group of soil-dweller species) were very susceptible to low humidity. In addition, researches by Thibaud (1968a, 1986b, 1970) demonstrated that both temperature and humidity played an important role in embryonic and post-embryonic development of Hypogastruridae and the highest post-embryonic survival of species investigated was from 98 – 100 % RH.

Water is not only essential for the activity of soil microorganisms but also essential for microbial activity (Sylvia et al., 2005). When EM were added into the bottle, it did not only compete for water to be used in metabolic activity, but also caused the decrease in humidity in the bottle. As a result of the decrease of humidity or % RH, it caused the delay of molting of juvenile in each stage. So the total

developmental time of *Xenylla* sp. from juvenile stage I to adult when exposed to EM were longer than the control.



**Figure 5-1** Hyphae on an *Xenylla* sp. egg

In terms of toxic substances or waste products excreted from microorganisms, although the substrate had charcoal that absorbed any harmful volatiles from the plaster or metabolites from the cultured animals (Booth, 1983), it had limitation for absorption due to area and quality of charcoal. EM added into the bottle may be the cause of the higher rate of metabolic waste production than the rate of absorption. Finally, their wastes had negative effects on the growth of animals. This result was in agreement with the observation of springtails in culture stock. The rate of ecdysis and oviposition of springtails reared on old substrate was usually lower than those reared on the fresh culture substrate. Booth (1983) reported that the higher the molting rate, egg laying rate and clutch size of egg of *Folsomia candida*, the higher the proportion of charcoal in the culture substrate. This indicates that the waste products in the substrate were an important factor on the growth and fecundity of springtails.

In adult stage, the effects of EM on the longevity and fecundity of *Xenylla* sp. by using demography were studied. The life expectancy of *Xenylla* sp. treated with EM1 and molasses were significantly shorter than the control ( $p < 0.05$ ) whereas other parameters were not significantly different. Considering the higher fecundity (even though not significant) when treated with EM1, it could be seen that shorter life span was compensated for higher egg production as stated by Addison and Parkinson (1978), Chen et al. (1995) and Klironomos et al. (1992). In overall, at least it indicates non-toxic environment for *Xenylla* sp. But *Xenylla* sp. was reported to be somewhat specific to some kind of food in order to growth well. Preferred foods especially fungal diet generally resulted in higher egg production and survival (Addison & Parkinson, 1978; Chen et al., 1995; Klironomos et al., 1992). However, it is known that the quality of fungi as food varies with the N content of their hyphae (Booth & Anderson, 1979, Verhoef et al., 1988). Other studies have shown that food quality varies with fungal species and that the quality of food is an important driving factor for population dynamics in collembolan (Chen et al., 1995; Joose & Testerink, 1977; Walsh & Bolger, 1990). It should be also mentioned that the study by Jiwajinda et al. (1996) showed that EM did not affect the mortality of *Plutella xylostella* and the contents of N, P and K in EM did not quantify to use as fertilizer as 0.035, 0.11 and 0.6 %w/w, respectively.

As a result, EM did not benefit or do any harmful effects on this *Xenylla* species. Any positive effects EM might have on better performances of the crops could be from other factors than through the activities of this *Xenylla* sp.

### **5.3 Effects of EM5 on the biology of *Xenylla* sp.**

The tests for effects of EM5 on biology of *Xenylla* sp. followed the same procedures as the EM test in 5.2 with the addition of treatments using vinegar and rice whiskey at varying concentrations. These experiments were designed to test the effects of EM5 in comparison with vinegar and rice whiskey which are the substances added in order to repel insects.

When compared among EM5, vinegar, rice whiskey and the control, vinegar probably played a key role in having positive effects on *Xenylla* sp. Even though the

egg viability was not significantly different from the control with water, it outperformed EM5 and rice whiskey. The pH was as low as 4.48 (vinegar 1:200) and was 5.26 at the highest (vinegar 1:500) (Appendix A). This caused the acidic condition of the substrate. In other works done on other insects, the proportion of hatched first instar larvae increased with increasing acidity (Heliövaara et al., 1992). The acidity could cause increase of egg hatching and mortality of larval stages (Gunnarsson & Johnsson, 1989; Heliövaara et al., 1992; Ke et al., 2002). However, acidification did not affect the total number of mites and collembolans, but liming decreased both groups (Hågvar, 1984; Hågvar & Amundsen, 1981; Hågvar & Kjöndal, 1981). In addition, Wolters (1989) reported that Collembola and Protura reached their highest density in the most acidic soil while Diplura only occurred in the soil with the higher pH value. This was in agreement with the study by Sumanothum (2007) who found collembolans and mites at the soil with pH 5.5 – 6 at Salaya campus, Mahidol University.

In juvenile and adult stages, the experiments demonstrated that vinegar and rice whiskey at some concentrations delayed the developmental time in juvenile as well as longevity and fecundity in adults, except for EM5 that had effects only in juvenile stages. When considered the developmental time in each stage of juveniles, it was found that the juvenile stages I to III were sensitive to both acidic and alkaline conditions while in stage VI, *Xenylla* sp. was more sensitive to high pH than to low pH.

For the fecundity of *Xenylla* sp., at low pH,  $R_0$  or the average production of female offspring per female during her lifetime, was higher than the control and was the highest value when compared among three treatments. This result was consistent with the experiments by Hutson (1978) who reported the fecundity of *Tullbergia krausbaueri* and *Isotoma notabilis* to be the highest at pH 5.2. The high reproduction of *Xenylla* sp. may result from the feeding on high quality food of acidophilic fungi in EM5. Microorganisms generally cannot tolerate extreme pH value. Under highly alkaline or acidic conditions, some microbial cell components may be hydrolyzed or enzymes may be denatured. Fungi and yeasts exist over a wide pH range but are more tolerant to acidic soil than are other microorganisms (Atlas & Bartha, 1946). When vinegar was added, acidophilic fungi and yeasts could rapidly grow and serve as food

for collembolans. As a result, *Xenylla* sp. reared in acidic condition have higher reproduction when compared with the control.

It could be stated that the two ingredients in EM5 were the factors affecting *Xenylla* sp. in different ways. It was vinegar which had effects on the higher population of *Xenylla* sp. and higher percentage of egg hatching and higher fecundity in adult stage. On the other hand, it caused the longer developmental time of juvenile. Rice whiskey had negative effects on all stages and lowered the population of *Xenylla* sp.

#### **5.4 Comparisons of effects of EM1 and EM5 on the biology of *Xenylla* sp.**

When comparisons were made between EM and EM5, the percentage of egg hatching was not significantly different in all concentrations. In juvenile stages, three concentrations -1:200, 1:300 and 1:500- caused the delayed developmental time from juvenile stage I to adult. However, the effect of EM5 was not consistent and mainly found during early stages of juvenile (I to III). For life table statistics, EM5 caused significantly shorter life expectancy at the concentration at 1:400. Data indicate no significant differences between EM and EM5 on the effects on *Xenylla* species.

Based on overall performances, EM and EM5 did not support the hypothesis stating the possible positive effects on *Xenylla* sp. studied under laboratory conditions. Field studies might result in different ways due to other environmental factors, *i.e.* predators, soil type, light, temperature, humidity, plant types and other soil microorganisms.

For example, in term of predators, microorganisms in EM1 may have positive or negative effects on the growth of predator population. The number of predators in the field had influences on the reproduction of collembolans because they may feed on eggs and kill juveniles and adults of collembolans. Whereas the population of other soil microorganisms may increase due to the preferred fungi in EM1 and, in turn, help increase the organic matter and preferred food of collembolans. This might lead to the increase of collembolan population.

## CHAPTER VI

### CONCLUSIONS AND RECOMMENDATIONS

#### Conclusions

1. The diagnostic characters of *Xenylla* sp. are the presence of 4+4 eyes, 4 apical teeth on mandible, 4+4 setae on ventral tube, 3+3 teeth of tenaculum, 2 clavate tenent hairs in each leg and 2 dorsal setae on dens. The furcula are short and postantennal organs (PAO) and unguiculus are absent. Chaetotaxy was used for identification as well.
2. The life history of *Xenylla* consists of three developmental stages- egg, juvenile and adult- which took the average of 21.67 days to complete at 25 – 28 °C.
3. This species exhibited sexual dimorphism. The sexes could be differentiated by the combination of body size and color. Females were visibly larger in body size and the body color was bright yellow whereas males at the same age had darker body due to the distribution of pigments.
4. Eggs were first laid between 12-16 days after hatch and the fecundity averaged 137.07 eggs.
5. The life table statistics comprised net reproductive rate ( $R_0$ ): 17.08; finite rate of increase ( $\lambda$ ): 1.085; capacity of increase ( $r_c$ ): 0.0739; intrinsic rate of natural increase ( $r_m$ ): 0.0817; life expectancy ( $e_x$ ): 45.814; generation time (T): 33.52; cohort generation time ( $T_c$ ): 37.12 and doubling time (D): 8.54 days.
6. Survivorship curve of *Xenylla* was of Type I due to low mortality rate in early age and high mortality rate in older organisms.
7. EM1, EM5 and rice whiskey had negative effects on all developmental stages of *Xenylla* sp. by reducing the percentage of egg hatching and life expectancy in adults and delaying the developmental time of juvenile stage.
8. Vinegar had no effects on egg viability but helped promote the fecundity of *Xenylla* sp.

9. The effects of EM1 and EM5 were not significantly different on the biology of *Xenylla* sp.

### **Recommendations**

Experiments in the laboratory and under field conditions are recommended.

In the laboratory:

#### 1. Study of sexual differentiation

This study clearly demonstrates that sexual dimorphism exists even in a minute insect such as *Xenylla* sp. A handful of published papers were on this aspect. There may be no differences between male and female of a species at all or there are some different characters between sexes that are visible only when the adults are older. Newly-emerged or young adults may not show the sexual morphological differences like in the case of the *Xenylla* sp. studied here. It is a tedious work and needs strong attention to follow the adult life to the end. It is strongly believed here that sexual dimorphism may be overlooked and may not be closely observed in many springtail species. For any entomologists who are interested in the general biology of springtails, sex differentiation of the specimens in stock culture is a good choice of topic.

#### 2. Study of effects of EM<sup>TM</sup> on other species or families of springtails or other soil animals

Even though the life expectancy ( $e_x$ ) of the studied *Xenylla* sp. treated with EM was significantly shorter than the control, the results may be different when tested with other species or families of springtails. This might be due, in part, to their preference of fungi in the EM. The fungi in the EM may not be the ones favored by the studied *Xenylla* sp. In contrast, other springtail species may prefer it and, thus, respond positively.

Field studies:

To compare and confirm the results from the experiments under ideal conditions of the laboratory, field studies are necessary. Field conditions provide abiotic and biotic factors that could have influences on the population of *Xenylla* sp. Abiotic factors include temperature, soil type, humidity, amount of nutrients (N, P, K etc) and

organic matter. Biotic factors are other organisms that, in nature are natural enemies or predators and other soil animals that may benefit or harmful to the survival and biology of *Xenylla* sp.

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## **APPENDIX**

**Appendix A: pH in each treatments**

Treatment	pH
H <sub>2</sub> O	7.82
EM	7.6
Molasses	7.12
EM5(1:200)	6.52
EM5(1:300)	6.78
EM5(1:400)	7.01
EM5(1:500)	7.01
V(1:200)	4.48
V(1:300)	4.89
V(1:400)	5.18
V(1:500)	5.26
RW(1:200)	7.71
RW(1:300)	7.77
RW(1:400)	7.82
RW(1:500)	7.77

## **Appendix B: Preparation of chemicals for staining and mounting slides (Humason, 1972)**

### **Harris hematoxylin**

Hematoxylin crystals	5	g
Absolute ethyl alcohol	50	ml
Ammonium or potassium alum	100	g
Distilled water	1000	ml
Mercuric oxide red	2.5	g

#### **Preparation**

1. Dissolve Hematoxylin crystals in absolute ethyl alcohol.
2. Dissolve alum in distilled water with heat.
3. Mix solution of (1) and (2).
4. Boil the mix solution with stirring rod for approximately one minute.
5. Add mercuric oxide red and then boil the mix solution again.
6. Boil until color changed to dark purple.
7. Wait to cool and keep in the bottle.

### **Eosin**

Eosin Y (water soluble)	1	g
Distilled water	20	ml
95 % ethyl alcohol	80	ml

#### **Preparation**

1. Mix Eosin Y in distilled water.
2. Add 95 % ethyl alcohol and stir until solution was homogeneous.

Note: Before use, it must be mixed with 80 % ethyl alcohol in the ratio of 1: 3 by volume.

**Hoyer's mounting media**

Gum Arabic	30	g
Choral hydrate	200	g
Glycerine	16	ml
Distilled water	50	ml

**Preparation**

1. Dissolve gum arabic in distilled water.
2. Boil and add choral hydrate.
3. Mix with glycerine.

**Appendix C: The mean percentage of egg hatching of *Xenylla* sp.**

**Table C-1** The mean percentage of egg hatching of *Xenylla* sp. exposed to water (control), molasses and EM1

<b>Treatment</b>	<b>% Egg Hatching</b>
Water (Control)	93.33 <sup>a</sup> ± 3.80
Molasses	80.00 <sup>ab</sup> ± 3.80
EM1	75.33 <sup>b</sup> ± 4.42

Note: The data were statistically analyzed using One-way ANOVA ( $p < 0.05$ )

The values with the same letter within a column are not significantly different at the 5% level judged by the Tukey's (HSD) test

**Table C-2** The mean percentage of egg hatching of *Xenylla* sp. exposed to different treatments and concentrations.

Treatment	Mean $\pm$ SE (days)				
	Concentration				
	1:200	1:300	1:400	1:500	Control
<b>EM5</b>	88.67 <sup>ab</sup> $\pm$ 4.67	74.00 <sup>bc</sup> $\pm$ 2.67	71.33 <sup>c</sup> $\pm$ 4.29	72.00 <sup>c</sup> $\pm$ 9.22	93.33 <sup>a</sup> $\pm$ 3.8
<b>Vinegar</b>	90.00 <sup>a</sup> $\pm$ 3.65	84.00 <sup>a</sup> $\pm$ 6.78	90.67 <sup>a</sup> $\pm$ 3.06	80.67 <sup>a</sup> $\pm$ 5.62	93.33 <sup>a</sup> $\pm$ 3.8
<b>Rice whiskey</b>	79.33 <sup>bc</sup> $\pm$ 1.94	65.33 <sup>bc</sup> $\pm$ 6.20	58.67 <sup>cd</sup> $\pm$ 8.73	84.67 <sup>ab</sup> $\pm$ 1.33	93.33 <sup>a</sup> $\pm$ 3.8

Note: The data were statistically analyzed using One-way ANOVA ( $p < 0.05$ ).

The values with the same letter within a row are not significantly different at the 5% level judged by the Tukey's (HSD) test.

**Table C-3** Comparison of mean percentage of egg hatching of *Xenylla* treated with EM1 and EM5

Treatment	% Egg hatching
EM1	75.33 <sup>a</sup> $\pm$ 4.422
EM5 (1:200)	88.67 <sup>a</sup> $\pm$ 4.667
EM5 (1:300)	74.00 <sup>a</sup> $\pm$ 2.667
EM5 (1:400)	71.33 <sup>a</sup> $\pm$ 4.295
EM5 (1:500)	72.00 <sup>a</sup> $\pm$ 9.226

Note: The data were statistically analyzed using One-way ANOVA ( $p < 0.05$ ).

The values with the same letter within a column are not significantly different at the 5% level judged by the Tukey's (HSD) test

### Appendix D: The mean developmental time of *Xenylla* juvenile

**Table D-1** The mean developmental time of *Xenylla* sp. juvenile treated with water (control), molasses and EM1

Juvenile stage	Mean $\pm$ SE (days)		
	Treatment		
	Control	Molasses	EM1
n	90	90	90
I – II	4.45 <sup>a</sup> $\pm$ 0.091	4.42 <sup>a</sup> $\pm$ 0.106	4.41 <sup>a</sup> $\pm$ 0.081
II – III	2.14 <sup>a</sup> $\pm$ 0.058	2.13 <sup>a</sup> $\pm$ 0.044	2.15 <sup>a</sup> $\pm$ 0.052
III – IV	2.10 <sup>a</sup> $\pm$ 0.052	2.25 <sup>a</sup> $\pm$ 0.053	2.26 <sup>a</sup> $\pm$ 0.064
IV – V	2.21 <sup>a</sup> $\pm$ 0.063	2.15 <sup>a</sup> $\pm$ 0.046	2.35 <sup>a</sup> $\pm$ 0.069
V – VI	2.28 <sup>a</sup> $\pm$ 0.077	2.45 <sup>a</sup> $\pm$ 0.080	2.46 <sup>a</sup> $\pm$ 0.086
VI – Adult	2.26 <sup>a</sup> $\pm$ 0.083	2.28 <sup>a</sup> $\pm$ 0.092	2.45 <sup>a</sup> $\pm$ 0.117
<b>Total</b>	15.43 <sup>a</sup> $\pm$ 0.189	15.70 <sup>ab</sup> $\pm$ 0.164	16.08 <sup>b</sup> $\pm$ 0.199

Note: The data were statistically analyzed using One-way ANOVA ( $p < 0.05$ ).

The values with the same letter within a row are not significantly different at the 5% level judged by the Tukey's (HSD) test.

**Table D-2** The mean developmental time of *Xenylla* sp. juvenile treated with different concentrations of EM5

Juvenile stage	Mean $\pm$ SE (days)				
	Treatment				
	EM5				
	Control	1:200	1:300	1:400	1:500
n	90	90	90	90	90
I – II	4.45 <sup>a</sup> $\pm$ 0.091	4.99 <sup>c</sup> $\pm$ 0.098	4.98 <sup>c</sup> $\pm$ 0.080	4.33 <sup>a</sup> $\pm$ 0.068	4.79 <sup>bc</sup> $\pm$ 0.102
II – III	2.14 <sup>a</sup> $\pm$ 0.058	2.09 <sup>a</sup> $\pm$ 0.067	2.32 <sup>ab</sup> $\pm$ 0.065	2.33 <sup>ab</sup> $\pm$ 0.069	2.46 <sup>b</sup> $\pm$ 0.073
III – IV	2.10 <sup>a</sup> $\pm$ 0.052	2.08 <sup>a</sup> $\pm$ 0.060	2.59 <sup>c</sup> $\pm$ 0.061	2.24 <sup>ab</sup> $\pm$ 0.068	2.42 <sup>bc</sup> $\pm$ 0.069
IV – V	2.21 <sup>a</sup> $\pm$ 0.063	2.30 <sup>a</sup> $\pm$ 0.068	2.26 <sup>a</sup> $\pm$ 0.058	2.34 <sup>a</sup> $\pm$ 0.074	2.31 <sup>a</sup> $\pm$ 0.075
V – VI	2.28 <sup>a</sup> $\pm$ 0.077	2.48 <sup>a</sup> $\pm$ 0.085	2.36 <sup>a</sup> $\pm$ 0.068	2.36 <sup>a</sup> $\pm$ 0.090	2.59 <sup>a</sup> $\pm$ 0.095
VI – Adult	2.26 <sup>a</sup> $\pm$ 0.083	2.19 <sup>a</sup> $\pm$ 0.084	2.02 <sup>a</sup> $\pm$ 0.074	2.62 <sup>b</sup> $\pm$ 0.091	2.25 <sup>a</sup> $\pm$ 0.100
<b>Total</b>	15.43 <sup>a</sup> $\pm$ 0.189	16.15 <sup>ab</sup> $\pm$ 0.196	16.52 <sup>b</sup> $\pm$ 0.119	16.25 <sup>ab</sup> $\pm$ 0.166	16.80 <sup>b</sup> $\pm$ 0.227

Note: The data were statistically analyzed using One-way ANOVA ( $p < 0.05$ ).

The values with the same letter within a row are not significantly different at the 5% level judged by the Tukey's (HSD) test.

**Table D-3** The mean developmental time of *Xenylla* sp. juvenile treated with different concentrations of vinegar

Juvenile stage	Mean $\pm$ SE (days)				
	Treatment				
	Vinegar				
	Control	1:200	1:300	1:400	1:500
n	90	90	90	90	90
I – II	4.45 <sup>a</sup> $\pm$ 0.091	4.66 <sup>ab</sup> $\pm$ 0.095	5.40 <sup>c</sup> $\pm$ 0.116	4.45 <sup>a</sup> $\pm$ 0.094	5.04 <sup>bc</sup> $\pm$ 0.130
II – III	2.14 <sup>ab</sup> $\pm$ 0.058	2.44 <sup>cd</sup> $\pm$ 0.082	2.56 <sup>d</sup> $\pm$ 0.087	1.88 <sup>a</sup> $\pm$ 0.073	2.18 <sup>bc</sup> $\pm$ 0.051
III – IV	2.10 <sup>a</sup> $\pm$ 0.052	2.25 <sup>ab</sup> $\pm$ 0.062	2.61 <sup>c</sup> $\pm$ 0.078	2.19 <sup>ab</sup> $\pm$ 0.067	2.39 <sup>bc</sup> $\pm$ 0.071
IV – V	2.21 <sup>a</sup> $\pm$ 0.063	2.53 <sup>b</sup> $\pm$ 0.084	2.25 <sup>ab</sup> $\pm$ 0.083	2.30 <sup>ab</sup> $\pm$ 0.079	2.54 <sup>b</sup> $\pm$ 0.081
V – VI	2.28 <sup>a</sup> $\pm$ 0.077	2.70 <sup>b</sup> $\pm$ 0.095	2.41 <sup>ab</sup> $\pm$ 0.082	2.35 <sup>a</sup> $\pm$ 0.088	2.54 <sup>ab</sup> $\pm$ 0.083
VI – Adult	2.26 <sup>ab</sup> $\pm$ 0.083	2.52 <sup>bc</sup> $\pm$ 0.092	2.00 <sup>a</sup> $\pm$ 0.073	2.14 <sup>a</sup> $\pm$ 0.086	2.62 <sup>c</sup> $\pm$ 0.108
<b>Total</b>	15.43 <sup>a</sup> $\pm$ 0.189	17.14 <sup>b</sup> $\pm$ 0.223	17.26 <sup>b</sup> $\pm$ 0.163	15.31 <sup>a</sup> $\pm$ 0.191	17.26 <sup>b</sup> $\pm$ 0.238

Note: The data were statistically analyzed using One-way ANOVA ( $p < 0.05$ ).

The values with the same letter within a row are not significantly different at the 5% level judged by the Tukey's (HSD) test.

**Table D-4** The mean of developmental time of *Xenylla* sp. juvenile treated with different concentrations of rice whiskey

Juvenile stage	Mean $\pm$ SE (days)				
	Treatment				
	Rice whiskey				
	Control	1:200	1:300	1:400	1:500
n	90	90	90	90	90
I – II	4.45 <sup>a</sup> $\pm$ 0.091	4.40 <sup>a</sup> $\pm$ 0.078	5.03 <sup>ab</sup> $\pm$ 0.189	5.78 <sup>b</sup> $\pm$ 0.220	5.50 <sup>b</sup> $\pm$ 0.240
II – III	2.14 <sup>a</sup> $\pm$ 0.058	2.30 <sup>ab</sup> $\pm$ 0.104	2.73 <sup>bc</sup> $\pm$ 0.106	3.01 <sup>c</sup> $\pm$ 0.149	2.96 <sup>c</sup> $\pm$ 0.178
III – IV	2.10 <sup>a</sup> $\pm$ 0.052	2.23 <sup>ab</sup> $\pm$ 0.069	2.65 <sup>c</sup> $\pm$ 0.082	2.42 <sup>abc</sup> $\pm$ 0.125	2.44 <sup>bc</sup> $\pm$ 0.097
IV – V	2.21 <sup>a</sup> $\pm$ 0.063	2.81 <sup>b</sup> $\pm$ 0.092	2.36 <sup>a</sup> $\pm$ 0.071	2.13 <sup>a</sup> $\pm$ 0.051	2.21 <sup>a</sup> $\pm$ 0.064
V – VI	2.28 <sup>a</sup> $\pm$ 0.077	2.94 <sup>b</sup> $\pm$ 0.114	2.22 <sup>a</sup> $\pm$ 0.097	2.36 <sup>a</sup> $\pm$ 0.072	2.19 <sup>a</sup> $\pm$ 0.063
VI – Adult	2.26 <sup>a</sup> $\pm$ 0.083	2.72 <sup>b</sup> $\pm$ 0.125	2.35 <sup>a</sup> $\pm$ 0.078	2.36 <sup>a</sup> $\pm$ 0.083	2.27 <sup>a</sup> $\pm$ 0.076
<b>Total</b>	15.43 <sup>a</sup> $\pm$ 0.189	17.34 <sup>b</sup> $\pm$ 0.308	17.24 <sup>b</sup> $\pm$ 0.217	17.96 <sup>b</sup> $\pm$ 0.245	17.56 <sup>b</sup> $\pm$ 0.284

Note: The data were statistically analyzed using One-way ANOVA (  $p < 0.05$ ).

The values with the same letter within a row are not significantly different at the 5% level judged by the Tukey's (HSD) test.

**Table D-5** Average of developmental time of *Xenylla* sp. juvenile treated with EM1 and EM5

Juvenile stage	Mean $\pm$ SE (days)				
	Treatment				
	EM1	EM5			
		1:200	1:300	1:400	1:500
n	90	90	90	90	90
I – II	4.42 <sup>ab</sup> $\pm$ 0.106	4.99 <sup>c</sup> $\pm$ 0.098	4.98 <sup>c</sup> $\pm$ 0.080	4.33 <sup>a</sup> $\pm$ 0.068	4.79 <sup>bc</sup> $\pm$ 0.102
II – III	2.15 <sup>a</sup> $\pm$ 0.052	2.09 <sup>a</sup> $\pm$ 0.067	2.32 <sup>ab</sup> $\pm$ 0.065	2.33 <sup>ab</sup> $\pm$ 0.069	2.46 <sup>b</sup> $\pm$ 0.073
III – IV	2.26 <sup>ab</sup> $\pm$ 0.064	2.08 <sup>a</sup> $\pm$ 0.060	2.59 <sup>c</sup> $\pm$ 0.061	2.24 <sup>ab</sup> $\pm$ 0.068	2.42 <sup>bc</sup> $\pm$ 0.069
IV – V	2.35 <sup>a</sup> $\pm$ 0.069	2.30 <sup>a</sup> $\pm$ 0.068	2.26 <sup>a</sup> $\pm$ 0.058	2.34 <sup>a</sup> $\pm$ 0.074	2.31 <sup>a</sup> $\pm$ 0.075
V – VI	2.46 <sup>a</sup> $\pm$ 0.086	2.48 <sup>a</sup> $\pm$ 0.085	2.36 <sup>a</sup> $\pm$ 0.068	2.36 <sup>a</sup> $\pm$ 0.090	2.59 <sup>a</sup> $\pm$ 0.095
VI – Adult	2.45 <sup>bc</sup> $\pm$ 0.117	2.19 <sup>ab</sup> $\pm$ 0.084	2.02 <sup>a</sup> $\pm$ 0.074	2.62 <sup>c</sup> $\pm$ 0.091	2.25 <sup>ab</sup> $\pm$ 0.100
<b>Total</b>	16.08 <sup>a</sup> $\pm$ 0.199	16.15 <sup>ab</sup> $\pm$ 0.196	16.52 <sup>b</sup> $\pm$ 0.119	16.25 <sup>ab</sup> $\pm$ 0.166	16.80 <sup>b</sup> $\pm$ 0.227

Note: The data were statistically analyzed using One-way ANOVA ( $p < 0.05$ ).

The values with the same letter within a row are not significantly different at the 5% level judged by the Tukey's (HSD) test

## Appendix E: Data analysis of one-way ANOVA and Kruskal Wallis test

**Table E-1** Comparisons of effects of EM1 and molasses on egg stage with the control

Note: (1) = control, (2) = EM1, (3) = molasses

### Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
HATCH 1	.253	5	.200*	.854	5	.207
2	.282	5	.200*	.897	5	.391
3	.184	5	.200*	.944	5	.692

\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

### Test of Homogeneity of Variances

HATCH

Levene Statistic	df1	df2	Sig.
.280	2	12	.760

### ANOVA

HATCH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	872.593	2	436.296	5.404	.021
Within Groups	968.889	12	80.741		
Total	1841.481	14			

### Multiple Comparisons

Dependent Variable: HATCH

Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	18.00000*	5.682983	.021	2.838576	33.161424
	3	13.333333	5.682983	.087	-1.828091	28.494757
2	1	-18.00000*	5.682983	.021	-33.161424	-2.838576
	3	-4.666667	5.682983	.698	-19.828091	10.494757
3	1	-13.333333	5.682983	.087	-28.494757	1.828091
	2	4.666667	5.682983	.698	-10.494757	19.828091

\*. The mean difference is significant at the .05 level.

**Table E-2** Comparisons of effects of EM5 on egg stage with the control

Note: (1) = control

(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
HATCH 1	.253	5	.200*	.854	5	.207
2	.273	5	.200*	.931	5	.603
3	.243	5	.200*	.894	5	.377
4	.245	5	.200*	.931	5	.601
5	.198	5	.200*	.982	5	.944

\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

**Test of Homogeneity of Variances**

HATCH

Levene Statistic	df1	df2	Sig.
1.365	4	20	.281

**ANOVA**

HATCH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2139.556	4	534.889	3.641	.022
Within Groups	2937.778	20	146.889		
Total	5077.333	24			

**Multiple Comparisons**

Dependent Variable: HATCH

Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	4.666667	7.665217	.972	-18.270535	27.603868
	3	19.333333	7.665217	.125	-3.603868	42.270535
	4	22.000000	7.665217	.064	-.937201	44.937201
	5	21.333333	7.665217	.076	-1.603868	44.270535
2	1	-4.666667	7.665217	.972	-27.603868	18.270535
	3	14.666667	7.665217	.343	-8.270535	37.603868
	4	17.333333	7.665217	.199	-5.603868	40.270535
	5	16.666667	7.665217	.229	-6.270535	39.603868
3	1	-19.333333	7.665217	.125	-42.270535	3.603868
	2	-14.666667	7.665217	.343	-37.603868	8.270535
	4	2.666667	7.665217	.997	-20.270535	25.603868
	5	2.000000	7.665217	.999	-20.937201	24.937201
4	1	-22.000000	7.665217	.064	-44.937201	.937201
	2	-17.333333	7.665217	.199	-40.270535	5.603868
	3	-2.666667	7.665217	.997	-25.603868	20.270535
	5	-.666667	7.665217	1.000	-23.603868	22.270535
5	1	-21.333333	7.665217	.076	-44.270535	1.603868
	2	-16.666667	7.665217	.229	-39.603868	6.270535
	3	-2.000000	7.665217	.999	-24.937201	20.937201
	4	.666667	7.665217	1.000	-22.270535	23.603868

**Table E-3** Comparisons of effects of vinegar on egg stage with the control

Note: (1) = control

(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
HATCH 1	.253	5	.200*	.854	5	.207
2	.300	5	.161	.833	5	.146
3	.222	5	.200*	.889	5	.350
4	.261	5	.200*	.862	5	.236
5	.243	5	.200*	.864	5	.244

\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

**Test of Homogeneity of Variances**

HATCH

Levene Statistic	df1	df2	Sig.
2.145	4	20	.113

**ANOVA**

HATCH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	544.889	4	136.222	1.188	.346
Within Groups	2293.333	20	114.667		
Total	2838.222	24			

**Table E-4** Comparisons of effects of rice whiskey on egg stage with the control

Note: (1) = control

(2), (3), (4), (5) = concentrations of whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
HATCH 1	.253	5	.200*	.854	5	.207
2	.221	5	.200*	.902	5	.421
3	.318	5	.109	.837	5	.158
4	.192	5	.200*	.981	5	.938
5	.349	5	.046	.771	5	.046

\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

**Test Statistics<sup>a,b</sup>**

	HATCH
Chi-Square	16.943
df	4
Asymp. Sig.	.002

a. Kruskal Wallis Test

b. Grouping Variable: TREAT

**Table E-5** Comparisons of effects of EM1 and EM5 on egg stage

Note: (1) = EM1

(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
HATCH 1	.282	5	.200*	.897	5	.391
2	.273	5	.200*	.931	5	.603
3	.243	5	.200*	.894	5	.377
4	.245	5	.200*	.931	5	.601
5	.198	5	.200*	.982	5	.944

\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

**Test of Homogeneity of Variances**

HATCH

Levene Statistic	df1	df2	Sig.
1.325	4	20	.295

**ANOVA**

HATCH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1011.556	4	252.889	1.664	.198
Within Groups	3040.000	20	152.000		
Total	4051.556	24			

**Table E-6** Comparisons of effects of EM1 and molasses on juvenile stage with the control

Stage I - II

**Test of Homogeneity of Variances**

DAY

Levene Statistic	df1	df2	Sig.
1.758	2	246	.175

**ANOVA**

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.122	2	.061	.087	.917
Within Groups	172.754	246	.702		
Total	172.876	248			

Stage II – III

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
.737	2	246	.479

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.027	2	.013	.060	.941
Within Groups	54.054	246	.220		
Total	54.080	248			

Stage III – IV

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
4.005	2	246	.019

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.356	2	.678	2.559	.079
Within Groups	65.198	246	.265		
Total	66.554	248			

Multiple Comparisons

Dependent Variable: DAY

Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.16	.082	.137	-.36	.03
	3	-.15	.074	.123	-.33	.03
2	1	.16	.082	.137	-.03	.36
	3	.01	.083	.999	-.19	.21
3	1	.15	.074	.123	-.03	.33
	2	-.01	.083	.999	-.21	.19

Note: (1) = control, (2) = EM1, (3) = molasses

Stage IV – V

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
10.295	2	246	.000

**ANOVA**

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.612	2	.806	2.701	.069
Within Groups	73.408	246	.298		
Total	75.020	248			

**Post Hoc**

**Multiple Comparisons**

Dependent Variable: DAY

Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.14	.093	.379	-.36	.09
	3	.06	.078	.859	-.13	.24
2	1	.14	.093	.379	-.09	.36
	3	.19	.083	.067	-.01	.39
3	1	-.06	.078	.859	-.24	.13
	2	-.19	.083	.067	-.39	.01

Note: (1) = control, (2) = EM1, (3) = molasses

**Stage V – VI**

**Test of Homogeneity of Variances**

DAY

Levene Statistic	df1	df2	Sig.
1.030	2	244	.359

**ANOVA**

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.755	2	.878	1.606	.203
Within Groups	133.362	244	.547		
Total	135.117	246			

**Stage VI - Adult**

**Test of Homogeneity of Variances**

DAY

Levene Statistic	df1	df2	Sig.
.444	2	242	.642

**ANOVA**

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.678	2	.839	1.055	.350
Within Groups	192.542	242	.796		
Total	194.220	244			

Stage I to adult

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
.398	2	242	.672

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.944	2	8.972	3.225	.041
Within Groups	673.338	242	2.782		
Total	691.282	244			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY

Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.66*	.261	.033	-1.28	-.04
	3	-.27	.262	.558	-.89	.35
2	1	.66*	.261	.033	.04	1.28
	3	.39	.260	.293	-.22	1.00
3	1	.27	.262	.558	-.35	.89
	2	-.39	.260	.293	-1.00	.22

\*. The mean difference is significant at the .05 level.

Note: (1) = control, (2) = EM1, (3) = molasses

**Table E-7** Comparisons of effects of EM5 on juvenile stage with the control

Stage I – II

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
5.354	4	409	.000

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28.790	4	7.197	11.037	.000
Within Groups	266.708	409	.652		
Total	295.498	413			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-.5548*	.13323	.001	-.9327	-.1770
	3.00	-.5079*	.11852	.000	-.8442	-.1717
	4.00	.1024	.11649	.991	-.2283	.4330
	5.00	-.3143	.13354	.179	-.6931	.0645
2.00	1.00	.5548*	.13323	.001	.1770	.9327
	3.00	.0469	.12537	1.000	-.3087	.4026
	4.00	.6572*	.12346	.000	.3068	1.0076
	5.00	.2405	.13966	.590	-.1554	.6365
3.00	1.00	.5079*	.11852	.000	.1717	.8442
	2.00	-.0469	.12537	1.000	-.4026	.3087
	4.00	.6103*	.10741	.000	.3056	.9149
	5.00	.1936	.12570	.731	-.1631	.5503
4.00	1.00	-.1024	.11649	.991	-.4330	.2283
	2.00	-.6572*	.12346	.000	-1.0076	-.3068
	3.00	-.6103*	.10741	.000	-.9149	-.3056
	5.00	-.4167*	.12379	.010	-.7681	-.0652
5.00	1.00	.3143	.13354	.179	-.0645	.6931
	2.00	-.2405	.13966	.590	-.6365	.1554
	3.00	-.1936	.12570	.731	-.5503	.1631
	4.00	.4167*	.12379	.010	.0652	.7681

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Stage II – III

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
7.541	4	409	.000

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.542	4	1.885	5.099	.001
Within Groups	151.231	409	.370		
Total	158.773	413			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.04	.089	1.000	-.21	.29
	3	-.18	.087	.319	-.43	.07
	4	-.19	.090	.284	-.45	.06
	5	-.32*	.093	.007	-.59	-.06
2	1	-.04	.089	1.000	-.29	.21
	3	-.22	.094	.163	-.49	.04
	4	-.24	.097	.144	-.51	.04
	5	-.36*	.100	.003	-.65	-.08
3	1	.18	.087	.319	-.07	.43
	2	.22	.094	.163	-.04	.49
	4	-.01	.095	1.000	-.28	.26
	5	-.14	.098	.809	-.42	.14
4	1	.19	.090	.284	-.06	.45
	2	.24	.097	.144	-.04	.51
	3	.01	.095	1.000	-.26	.28
	5	-.13	.101	.892	-.41	.16
5	1	.32*	.093	.007	.06	.59
	2	.36*	.100	.003	.08	.65
	3	.14	.098	.809	-.14	.42
	4	.13	.101	.892	-.16	.41

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Stage III – IV

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
7.016	4	409	.000

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.895	4	3.974	12.416	.000
Within Groups	130.900	409	.320		
Total	146.795	413			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.02	.079	1.000	-.21	.24
	3	-.49*	.080	.000	-.72	-.26
	4	-.14	.085	.638	-.38	.10
	5	-.32*	.086	.002	-.57	-.08
2	1	-.02	.079	1.000	-.24	.21
	3	-.51*	.086	.000	-.75	-.26
	4	-.16	.091	.565	-.42	.10
	5	-.34*	.091	.003	-.60	-.08
3	1	.49*	.080	.000	.26	.72
	2	.51*	.086	.000	.26	.75
	4	.35*	.091	.002	.09	.61
	5	.17	.092	.520	-.09	.43
4	1	.14	.085	.638	-.10	.38
	2	.16	.091	.565	-.10	.42
	3	-.35*	.091	.002	-.61	-.09
	5	-.18	.097	.472	-.46	.09
5	1	.32*	.086	.002	.08	.57
	2	.34*	.091	.003	.08	.60
	3	-.17	.092	.520	-.43	.09
	4	.18	.097	.472	-.09	.46

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Stage IV – V

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
2.420	4	409	.048

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.862	4	.216	.567	.687
Within Groups	155.505	409	.380		
Total	156.367	413			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.09	.093	.977	-.36	.17
	3	-.05	.086	1.000	-.29	.19
	4	-.13	.097	.850	-.41	.14
	5	-.10	.097	.965	-.38	.17
2	1	.09	.093	.977	-.17	.36
	3	.04	.090	1.000	-.21	.30
	4	-.04	.101	1.000	-.33	.25
	5	-.01	.101	1.000	-.30	.28
3	1	.05	.086	1.000	-.19	.29
	2	-.04	.090	1.000	-.30	.21
	4	-.08	.094	.991	-.35	.18
	5	-.05	.095	1.000	-.32	.21
4	1	.13	.097	.850	-.14	.41
	2	.04	.101	1.000	-.25	.33
	3	.08	.094	.991	-.18	.35
	5	.03	.105	1.000	-.27	.33
5	1	.10	.097	.965	-.17	.38
	2	.01	.101	1.000	-.28	.30
	3	.05	.095	1.000	-.21	.32
	4	-.03	.105	1.000	-.33	.27

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Stage V – VI

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
1.648	4	399	.161

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.703	4	1.176	2.083	.082
Within Groups	225.265	399	.565		
Total	229.968	403			

Stage VI – Adult

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
4.320	4	397	.002

**ANOVA**

DAY					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.051	4	3.763	6.238	.000
Within Groups	239.468	397	.603		
Total	254.520	401			

**Post Hoc**

**Multiple Comparisons**

Dependent Variable: DAY

Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.07	.118	1.000	-.26	.41
	3	.24	.111	.292	-.08	.55
	4	-.36*	.123	.037	-.71	-.01
	5	.01	.130	1.000	-.36	.38
2	1	-.07	.118	1.000	-.41	.26
	3	.17	.112	.775	-.15	.48
	4	-.43*	.123	.006	-.78	-.08
	5	-.06	.130	1.000	-.43	.31
3	1	-.24	.111	.292	-.55	.08
	2	-.17	.112	.775	-.48	.15
	4	-.60*	.117	.000	-.93	-.27
	5	-.23	.125	.522	-.58	.13
4	1	.36*	.123	.037	.01	.71
	2	.43*	.123	.006	.08	.78
	3	.60*	.117	.000	.27	.93
	5	.37	.135	.061	-.01	.76
5	1	-.01	.130	1.000	-.38	.36
	2	.06	.130	1.000	-.31	.43
	3	.23	.125	.522	-.13	.58
	4	-.37	.135	.061	-.76	.01

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**Stage I to adult**

**Test of Homogeneity of Variances**

DAY			
Levene Statistic	df1	df2	Sig.
4.107	4	397	.003

**ANOVA**

DAY					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	85.073	4	21.268	7.863	.000
Within Groups	1073.872	397	2.705		
Total	1158.945	401			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-.7298	.27252	.078	-1.5027	.0432
	3.00	-1.0935*	.22343	.000	-1.7290	-.4580
	4.00	-.8218*	.25116	.013	-1.5347	-.1088
	5.00	-1.3750*	.29563	.000	-2.2142	-.5358
2.00	1.00	.7298	.27252	.078	-.0432	1.5027
	3.00	-.3638	.22981	.700	-1.0172	.2897
	4.00	-.0920	.25686	1.000	-.8209	.6369
	5.00	-.6452	.30048	.284	-1.4978	.2074
3.00	1.00	1.0935*	.22343	.000	.4580	1.7290
	2.00	.3638	.22981	.700	-.2897	1.0172
	4.00	.2718	.20402	.865	-.3081	.8517
	5.00	-.2815	.25679	.957	-1.0132	.4503
4.00	1.00	.8218*	.25116	.013	.1088	1.5347
	2.00	.0920	.25686	1.000	-.6369	.8209
	3.00	-.2718	.20402	.865	-.8517	.3081
	5.00	-.5532	.28125	.403	-1.3524	.2459
5.00	1.00	1.3750*	.29563	.000	.5358	2.2142
	2.00	.6452	.30048	.284	-.2074	1.4978
	3.00	.2815	.25679	.957	-.4503	1.0132
	4.00	.5532	.28125	.403	-.2459	1.3524

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**Table E-8** Comparisons of effects of vinegar on juvenile stage with the control  
Stage I – II

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
.701	4	410	.592

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	58.329	4	14.582	15.923	.000
Within Groups	375.468	410	.916		
Total	433.798	414			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-.1852	.15037	.733	-.5972	.2268
	3.00	-.9480*	.14902	.000	-1.3563	-.5397
	4.00	.0163	.14902	1.000	-.3920	.4246
	5.00	-.5903*	.14859	.001	-.9974	-.1831
2.00	1.00	.1852	.15037	.733	-.2268	.5972
	3.00	-.7628*	.14902	.000	-1.1711	-.3545
	4.00	.2015	.14902	.659	-.2068	.6098
	5.00	-.4051	.14859	.052	-.8122	.0021
3.00	1.00	.9480*	.14902	.000	.5397	1.3563
	2.00	.7628*	.14902	.000	.3545	1.1711
	4.00	.9643*	.14766	.000	.5597	1.3689
	5.00	.3577	.14723	.110	-.0457	.7611
4.00	1.00	-.0163	.14902	1.000	-.4246	.3920
	2.00	-.2015	.14902	.659	-.6098	.2068
	3.00	-.9643*	.14766	.000	-1.3689	-.5597
	5.00	-.6066*	.14723	.000	-1.0100	-.2032
5.00	1.00	.5903*	.14859	.001	.1831	.9974
	2.00	.4051	.14859	.052	-.0021	.8122
	3.00	-.3577	.14723	.110	-.7611	.0457
	4.00	.6066*	.14723	.000	.2032	1.0100

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

Stage II – III

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
12.395	4	410	.000

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24.012	4	6.003	14.196	.000
Within Groups	173.371	410	.423		
Total	197.383	414			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.31*	.101	.025	-.59	-.02
	3	-.42*	.104	.001	-.72	-.13
	4	.25	.093	.065	-.01	.52
	5	-.04	.077	1.000	-.26	.18
2	1	.31*	.101	.025	.02	.59
	3	-.12	.120	.983	-.45	.22
	4	.56*	.110	.000	.25	.88
	5	.27	.097	.062	-.01	.54
3	1	.42*	.104	.001	.13	.72
	2	.12	.120	.983	-.22	.45
	4	.68*	.113	.000	.36	1.00
	5	.38*	.101	.002	.10	.67
4	1	-.25	.093	.065	-.52	.01
	2	-.56*	.110	.000	-.88	-.25
	3	-.68*	.113	.000	-1.00	-.36
	5	-.30*	.089	.011	-.55	-.04
5	1	.04	.077	1.000	-.18	.26
	2	-.27	.097	.062	-.54	.01
	3	-.38*	.101	.002	-.67	-.10
	4	.30*	.089	.011	.04	.55

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

Stage III – IV

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
7.350	4	410	.000

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.073	4	3.268	8.848	.000
Within Groups	151.448	410	.369		
Total	164.520	414			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.15	.081	.502	-.38	.08
	3	-.51*	.093	.000	-.77	-.24
	4	-.09	.084	.959	-.33	.15
	5	-.29*	.088	.012	-.54	-.04
2	1	.15	.081	.502	-.08	.38
	3	-.36*	.099	.004	-.64	-.08
	4	.06	.091	.999	-.20	.31
	5	-.14	.095	.764	-.41	.13
3	1	.51*	.093	.000	.24	.77
	2	.36*	.099	.004	.08	.64
	4	.42*	.102	.001	.13	.71
	5	.22	.105	.326	-.08	.52
4	1	-.09	.084	.959	-.15	.33
	2	-.06	.091	.999	-.31	.20
	3	-.42*	.102	.001	-.71	-.13
	5	-.20	.098	.359	-.47	.08
5	1	.29*	.088	.012	.04	.54
	2	.14	.095	.764	-.13	.41
	3	-.22	.105	.326	-.52	.08
	4	.20	.098	.359	-.08	.47

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

Stage IV – V

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
2.643	4	410	.033

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.307	4	2.077	4.054	.003
Within Groups	210.020	410	.512		
Total	218.328	414			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.32*	.105	.026	-.62	-.02
	3	-.04	.104	1.000	-.33	.25
	4	-.09	.101	.992	-.37	.20
	5	-.33*	.103	.015	-.62	-.04
2	1	.32*	.105	.026	.02	.62
	3	.28	.118	.170	-.05	.62
	4	.23	.116	.367	-.09	.56
	5	-.01	.117	1.000	-.34	.32
3	1	.04	.104	1.000	-.25	.33
	2	-.28	.118	.170	-.62	.05
	4	-.05	.114	1.000	-.37	.28
	5	-.29	.116	.122	-.62	.04
4	1	.09	.101	.992	-.20	.37
	2	-.23	.116	.367	-.56	.09
	3	.05	.114	1.000	-.28	.37
	5	-.24	.113	.282	-.56	.08
5	1	.33*	.103	.015	.04	.62
	2	.01	.117	1.000	-.32	.34
	3	.29	.116	.122	-.04	.62
	4	.24	.113	.282	-.08	.56

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

Stage V – VI

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
.273	4	405	.895

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.329	4	2.332	3.897	.004
Within Groups	242.381	405	.598		
Total	251.710	409			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
 Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.43*	.122	.004	-.76	-.09
	3	-.13	.121	.801	-.47	.20
	4	-.07	.121	.973	-.41	.26
	5	-.27	.121	.180	-.60	.06
2	1	.43*	.122	.004	.09	.76
	3	.29	.121	.108	-.04	.63
	4	.35*	.121	.029	.02	.69
	5	.16	.121	.668	-.17	.49
3	1	.13	.121	.801	-.20	.47
	2	-.29	.121	.108	-.63	.04
	4	.06	.120	.987	-.27	.39
	5	-.13	.120	.805	-.46	.20
4	1	.07	.121	.973	-.26	.41
	2	-.35*	.121	.029	-.69	-.02
	3	-.06	.120	.987	-.39	.27
	5	-.19	.120	.495	-.52	.14
5	1	.27	.121	.180	-.06	.60
	2	-.16	.121	.668	-.49	.17
	3	.13	.120	.805	-.20	.46
	4	.19	.120	.495	-.14	.52

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

Stage VI - Adult

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
4.283	4	398	.002

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21.236	4	5.309	8.299	.000
Within Groups	254.610	398	.640		
Total	275.846	402			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY

Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.26	.124	.328	-.61	.09
	3	.26	.111	.173	-.05	.58
	4	.12	.119	.979	-.22	.46
	5	-.35	.136	.095	-.74	.03
2	1	.26	.124	.328	-.09	.61
	3	.52*	.117	.000	.19	.85
	4	.37*	.126	.033	.02	.73
	5	-.10	.141	.999	-.50	.30
3	1	-.26	.111	.173	-.58	.05
	2	-.52*	.117	.000	-.85	-.19
	4	-.14	.113	.891	-.47	.18
	5	-.62*	.130	.000	-.99	-.25
4	1	-.12	.119	.979	-.46	.22
	2	-.37*	.126	.033	-.73	-.02
	3	.14	.113	.891	-.18	.47
	5	-.47*	.138	.008	-.86	-.08
5	1	.35	.136	.095	-.03	.74
	2	.10	.141	.999	-.30	.50
	3	.62*	.130	.000	.25	.99
	4	.47*	.138	.008	.08	.86

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

Stage I to adult

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
3.224	4	398	.013

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	334.505	4	83.626	25.370	.000
Within Groups	1311.917	398	3.296		
Total	1646.422	402			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-1.7142*	.29200	.000	-2.5431	-.8854
	3.00	-1.8375*	.24938	.000	-2.5453	-1.1297
	4.00	.1117	.26852	1.000	-.6500	.8734
	5.00	-1.8343*	.30348	.000	-2.6958	-.9727
2.00	1.00	1.7142*	.29200	.000	.8854	2.5431
	3.00	-.1233	.27582	1.000	-.9069	.6604
	4.00	1.8260*	.29324	.000	.9938	2.6582
	5.00	-.1200	.32555	1.000	-1.0438	.8037
3.00	1.00	1.8375*	.24938	.000	1.1297	2.5453
	2.00	.1233	.27582	1.000	-.6604	.9069
	4.00	1.9492*	.25084	.000	1.2375	2.6609
	5.00	.0032	.28795	1.000	-.8151	.8215
4.00	1.00	-.1117	.26852	1.000	-.8734	.6500
	2.00	-1.8260*	.29324	.000	-2.6582	-.9938
	3.00	-1.9492*	.25084	.000	-2.6609	-1.2375
	5.00	-1.9460*	.30468	.000	-2.8108	-1.0812
5.00	1.00	1.8343*	.30348	.000	.9727	2.6958
	2.00	.1200	.32555	1.000	-.8037	1.0438
	3.00	-.0032	.28795	1.000	-.8215	.8151
	4.00	1.9460*	.30468	.000	1.0812	2.8108

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

**Table E-9** Comparisons of effects of rice whiskey on juvenile stage with the control Stage I – II

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
22.491	4	377	.000

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	116.513	4	29.128	13.311	.000
Within Groups	824.974	377	2.188		
Total	941.487	381			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	.0458	.11535	1.000	-.2817	.3734
	3.00	-.5562	.20337	.070	-1.1365	.0241
	4.00	-1.3064*	.23380	.000	-1.9749	-.6379
	5.00	-1.1032*	.24351	.000	-1.8000	-.4064
2.00	1.00	-.0458	.11535	1.000	-.3734	.2817
	3.00	-.6020*	.19643	.028	-1.1638	-.0403
	4.00	-1.3522*	.22778	.000	-2.0048	-.6996
	5.00	-1.1490*	.23774	.000	-1.8306	-.4674
3.00	1.00	.5562	.20337	.070	-.0241	1.1365
	2.00	.6020*	.19643	.028	.0403	1.1638
	4.00	-.7502	.28274	.084	-1.5532	.0529
	5.00	-.5470	.29082	.466	-1.3733	.2793
4.00	1.00	1.3064*	.23380	.000	.6379	1.9749
	2.00	1.3522*	.22778	.000	.6996	2.0048
	3.00	.7502	.28274	.084	-.0529	1.5532
	5.00	.2032	.31285	.999	-.6853	1.0916
5.00	1.00	1.1032*	.24351	.000	.4064	1.8000
	2.00	1.1490*	.23774	.000	.4674	1.8306
	3.00	.5470	.29082	.466	-.2793	1.3733
	4.00	-.2032	.31285	.999	-1.0916	.6853

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

Stage II – III

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
17.360	4	377	.000

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	47.702	4	11.925	10.080	.000
Within Groups	446.016	377	1.183		
Total	493.717	381			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY

Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.17	.119	.833	-.51	.17
	3	-.59*	.120	.000	-.93	-.25
	4	-.88*	.160	.000	-1.34	-.42
	5	-.82*	.187	.000	-1.36	-.29
2	1	.17	.119	.833	-.17	.51
	3	-.43*	.149	.046	-.85	.00
	4	-.71*	.182	.001	-1.23	-.19
	5	-.66*	.206	.018	-1.25	-.07
3	1	.59*	.120	.000	.25	.93
	2	.43*	.149	.046	.00	.85
	4	-.29	.183	.714	-.81	.23
	5	-.23	.207	.948	-.82	.36
4	1	.88*	.160	.000	.42	1.34
	2	.71*	.182	.001	.19	1.23
	3	.29	.183	.714	-.23	.81
	5	.05	.232	1.000	-.61	.71
5	1	.82*	.187	.000	.29	1.36
	2	.66*	.206	.018	.07	1.25
	3	.23	.207	.948	-.36	.82
	4	-.05	.232	1.000	-.71	.61

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

Stage III – IV

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
9.292	4	377	.000

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.901	4	3.475	5.881	.000
Within Groups	222.790	377	.591		
Total	236.691	381			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.13	.086	.719	-.38	.11
	3	-.55*	.097	.000	-.83	-.27
	4	-.32	.135	.169	-.71	.06
	5	-.34*	.110	.024	-.66	-.03
2	1	.13	.086	.719	-.11	.38
	3	-.42*	.107	.002	-.72	-.11
	4	-.19	.142	.870	-.59	.22
	5	-.21	.119	.579	-.55	.13
3	1	.55*	.097	.000	.27	.83
	2	.42*	.107	.002	.11	.72
	4	.23	.149	.739	-.20	.65
	5	.21	.127	.652	-.15	.57
4	1	.32	.135	.169	-.06	.71
	2	.19	.142	.870	-.22	.59
	3	-.23	.149	.739	-.65	.20
	5	-.02	.158	1.000	-.47	.43
5	1	.34*	.110	.024	.03	.66
	2	.21	.119	.579	-.13	.55
	3	-.21	.127	.652	-.57	.15
	4	.02	.158	1.000	-.43	.47

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

Stage IV – V

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
5.947	4	376	.000

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21.524	4	5.381	14.899	.000
Within Groups	135.799	376	.361		
Total	157.323	380			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.60*	.111	.000	-.91	-.28
	3	-.15	.095	.672	-.42	.12
	4	.08	.081	.981	-.15	.31
	5	.00	.089	1.000	-.26	.25
2	1	.60*	.111	.000	.28	.91
	3	.44*	.116	.002	.11	.77
	4	.67*	.105	.000	.37	.97
	5	.59*	.112	.000	.27	.91
3	1	.15	.095	.672	-.12	.42
	2	-.44*	.116	.002	-.77	-.11
	4	.23	.088	.086	-.02	.48
	5	.15	.096	.709	-.12	.42
4	1	-.08	.081	.981	-.31	.15
	2	-.67*	.105	.000	-.97	-.37
	3	-.23	.088	.086	-.48	.02
	5	-.08	.082	.976	-.31	.15
5	1	.00	.089	1.000	-.25	.26
	2	-.59*	.112	.000	-.91	-.27
	3	-.15	.096	.709	-.42	.12
	4	.08	.082	.976	-.15	.31

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

Stage V – VI

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
1.409	4	369	.230

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26.606	4	6.652	12.168	.000
Within Groups	201.717	369	.547		
Total	228.324	373			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
 Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.67*	.122	.000	-1.00	-.33
	3	.05	.118	.991	-.27	.38
	4	-.08	.118	.961	-.40	.24
	5	.09	.119	.952	-.24	.41
2	1	.67*	.122	.000	.33	1.00
	3	.72*	.124	.000	.38	1.06
	4	.59*	.124	.000	.25	.92
	5	.75*	.125	.000	.41	1.09
3	1	-.05	.118	.991	-.38	.27
	2	-.72*	.124	.000	-1.06	-.38
	4	-.13	.120	.793	-.46	.19
	5	.03	.120	.999	-.30	.36
4	1	.08	.118	.961	-.24	.40
	2	-.59*	.124	.000	-.92	-.25
	3	.13	.120	.793	-.19	.46
	5	.17	.121	.644	-.16	.50
5	1	-.09	.119	.952	-.41	.24
	2	-.75*	.125	.000	-1.09	-.41
	3	-.03	.120	.999	-.36	.30
	4	-.17	.121	.644	-.50	.16

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

Stage VI - Adult

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
1.863	4	358	.116

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.646	4	2.412	4.184	.003
Within Groups	206.359	358	.576		
Total	216.006	362			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
 Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.46*	.127	.003	-.81	-.11
	3	-.09	.122	.950	-.42	.25
	4	-.10	.122	.919	-.44	.23
	5	-.01	.124	1.000	-.35	.33
2	1	.46*	.127	.003	.11	.81
	3	.37*	.129	.034	.02	.73
	4	.36*	.129	.046	.00	.71
	5	.45*	.131	.006	.09	.81
3	1	.09	.122	.950	-.25	.42
	2	-.37*	.129	.034	-.73	-.02
	4	-.01	.125	1.000	-.36	.33
	5	.08	.127	.970	-.27	.43
4	1	.10	.122	.919	-.23	.44
	2	-.36*	.129	.046	-.71	.00
	3	.01	.125	1.000	-.33	.36
	5	.09	.127	.947	-.25	.44
5	1	.01	.124	1.000	-.33	.35
	2	-.45*	.131	.006	-.81	-.09
	3	-.08	.127	.970	-.43	.27
	4	-.09	.127	.947	-.44	.25

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

Stage I to adult

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
.661	4	358	.620

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	298.538	4	74.634	16.827	.000
Within Groups	1587.875	358	4.435		
Total	1886.413	362			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-1.91*	.352	.000	-2.88	-.95
	3	-1.82*	.340	.000	-2.75	-.89
	4	-2.53*	.340	.000	-3.47	-1.60
	5	-2.13*	.345	.000	-3.08	-1.19
2	1	1.91*	.352	.000	.95	2.88
	3	.10	.358	.999	-.89	1.08
	4	-.62	.358	.414	-1.60	.36
	5	-.22	.363	.975	-1.21	.78
3	1	1.82*	.340	.000	.89	2.75
	2	-.10	.358	.999	-1.08	.89
	4	-.72	.346	.236	-1.67	.23
	5	-.31	.351	.899	-1.28	.65
4	1	2.53*	.340	.000	1.60	3.47
	2	.62	.358	.414	-.36	1.60
	3	.72	.346	.236	-.23	1.67
	5	.40	.351	.782	-.56	1.37
5	1	2.13*	.345	.000	1.19	3.08
	2	.22	.363	.975	-.78	1.21
	3	.31	.351	.899	-.65	1.28
	4	-.40	.351	.782	-1.37	.56

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

**Table E-6** Comparisons of effects of EM1 and EM5 on juvenile stage

Stage I – II

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
5.247	4	412	.000

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	30.864	4	7.716	10.923	.000
Within Groups	291.026	412	.706		
Total	321.890	416			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-.5950*	.14378	.001	-1.0026	-.1873
	3.00	-.5480*	.13026	.000	-.9178	-.1783
	4.00	.0622	.12842	1.000	-.3025	.4269
	5.00	-.3544	.14406	.138	-.7629	.0541
2.00	1.00	.5950*	.14378	.001	.1873	1.0026
	3.00	.0469	.12537	1.000	-.3087	.4026
	4.00	.6572*	.12346	.000	.3068	1.0076
	5.00	.2405	.13966	.590	-.1554	.6365
3.00	1.00	.5480*	.13026	.000	.1783	.9178
	2.00	-.0469	.12537	1.000	-.4026	.3087
	4.00	.6103*	.10741	.000	.3056	.9149
	5.00	.1936	.12570	.731	-.1631	.5503
4.00	1.00	-.0622	.12842	1.000	-.4269	.3025
	2.00	-.6572*	.12346	.000	-1.0076	-.3068
	3.00	-.6103*	.10741	.000	-.9149	-.3056
	5.00	-.4167*	.12379	.010	-.7681	-.0652
5.00	1.00	.3544	.14406	.138	-.0541	.7629
	2.00	-.2405	.13966	.590	-.6365	.1554
	3.00	-.1936	.12570	.731	-.5503	.1631
	4.00	.4167*	.12379	.010	.0652	.7681

\*. The mean difference is significant at the .05 level.

Note: (1) = EM1

(2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

Stage II – III

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
8.077	4	412	.000

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.206	4	1.801	4.991	.001
Within Groups	148.713	412	.361		
Total	155.918	416			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.06	.085	.998	-.18	.30
	3	-.16	.084	.415	-.40	.07
	4	-.17	.087	.370	-.42	.07
	5	-.30*	.090	.010	-.56	-.05
2	1	-.06	.085	.998	-.30	.18
	3	-.22	.094	.163	-.49	.04
	4	-.24	.097	.144	-.51	.04
	5	-.36*	.100	.003	-.65	-.08
3	1	.16	.084	.415	-.07	.40
	2	.22	.094	.163	-.04	.49
	4	-.01	.095	1.000	-.28	.26
	5	-.14	.098	.809	-.42	.14
4	1	.17	.087	.370	-.07	.42
	2	.24	.097	.144	-.04	.51
	3	.01	.095	1.000	-.26	.28
	5	-.13	.101	.892	-.41	.16
5	1	.30*	.090	.010	.05	.56
	2	.36*	.100	.003	.08	.65
	3	.14	.098	.809	-.14	.42
	4	.13	.101	.892	-.16	.41

\*. The mean difference is significant at the .05 level.

Note: (1) = EM1

(2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

Stage III – IV

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
3.803	4	412	.005

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.652	4	3.163	9.182	.000
Within Groups	141.928	412	.344		
Total	154.580	416			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY  
Dunnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.18	.088	.337	-.07	.43
	3	-.33*	.088	.003	-.58	-.08
	4	.02	.093	1.000	-.24	.29
	5	-.16	.094	.605	-.43	.11
2	1	-.18	.088	.337	-.43	.07
	3	-.51*	.086	.000	-.75	-.26
	4	-.16	.091	.565	-.42	.10
	5	-.34*	.091	.003	-.60	-.08
3	1	.33*	.088	.003	.08	.58
	2	.51*	.086	.000	.26	.75
	4	.35*	.091	.002	.09	.61
	5	.17	.092	.520	-.09	.43
4	1	-.02	.093	1.000	-.29	.24
	2	.16	.091	.565	-.10	.42
	3	-.35*	.091	.002	-.61	-.09
	5	-.18	.097	.472	-.46	.09
5	1	.16	.094	.605	-.11	.43
	2	.34*	.091	.003	.08	.60
	3	-.17	.092	.520	-.43	.09
	4	.18	.097	.472	-.09	.46

\*. The mean difference is significant at the .05 level.

Note: (1) = EM1

(2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

Stage IV – V

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
1.905	4	412	.109

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.411	4	.103	.260	.904
Within Groups	163.061	412	.396		
Total	163.472	416			

Stage V – VI

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
1.364	4	402	.246

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.800	4	.700	1.183	.318
Within Groups	237.917	402	.592		
Total	240.717	406			

Stage VI – Adult

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
3.505	4	400	.008

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.158	4	4.289	5.947	.000
Within Groups	288.487	400	.721		
Total	305.644	404			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY

Dunnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.26	.144	.546	-.15	.66
	3	.42*	.138	.027	.03	.81
	4	-.18	.148	.924	-.60	.24
	5	.20	.153	.893	-.24	.63
2	1	-.26	.144	.546	-.66	.15
	3	.17	.112	.775	-.15	.48
	4	-.43*	.123	.006	-.78	-.08
	5	-.06	.130	1.000	-.43	.31
3	1	-.42*	.138	.027	-.81	-.03
	2	-.17	.112	.775	-.48	.15
	4	-.60*	.117	.000	-.93	-.27
	5	-.23	.125	.522	-.58	.13
4	1	.18	.148	.924	-.24	.60
	2	.43*	.123	.006	.08	.78
	3	.60*	.117	.000	.27	.93
	5	.37	.135	.061	-.01	.76
5	1	-.20	.153	.893	-.63	.24
	2	.06	.130	1.000	-.31	.43
	3	.23	.125	.522	-.13	.58
	4	-.37	.135	.061	-.76	.01

\*. The mean difference is significant at the .05 level.

Note: (1) = EM1  
 (2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

Stage I to adult

Test of Homogeneity of Variances

DAY

Levene Statistic	df1	df2	Sig.
3.603	4	400	.007

ANOVA

DAY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28.355	4	7.089	2.539	.040
Within Groups	1116.732	400	2.792		
Total	1145.086	404			

Post Hoc

Multiple Comparisons

Dependent Variable: DAY

Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-.0704	.27932	1.000	-.8625	.7216
	3.00	-.4342	.23167	.472	-1.0931	.2247
	4.00	-.1624	.25852	.999	-.8961	.5713
	5.00	-.7157	.30190	.173	-1.5723	.1410
2.00	1.00	.0704	.27932	1.000	-.7216	.8625
	3.00	-.3638	.22981	.700	-1.0172	.2897
	4.00	-.0920	.25686	1.000	-.8209	.6369
	5.00	-.6452	.30048	.284	-1.4978	.2074
3.00	1.00	.4342	.23167	.472	-.2247	1.0931
	2.00	.3638	.22981	.700	-.2897	1.0172
	4.00	.2718	.20402	.865	-.3081	.8517
	5.00	-.2815	.25679	.957	-1.0132	.4503
4.00	1.00	.1624	.25852	.999	-.5713	.8961
	2.00	.0920	.25686	1.000	-.6369	.8209
	3.00	-.2718	.20402	.865	-.8517	.3081
	5.00	-.5532	.28125	.403	-1.3524	.2459
5.00	1.00	.7157	.30190	.173	-.1410	1.5723
	2.00	.6452	.30048	.284	-.2074	1.4978
	3.00	.2815	.25679	.957	-.4503	1.0132
	4.00	.5532	.28125	.403	-.2459	1.3524

Note: (1) = EM1  
 (2), (3), (4), (5) = concentrations of rice whiskey at 1:200, 1:300, 1:400 and 1:500, respectively

**Table E-10** Comparisons of effects of EM1 and molasses on adult stage with the control  
Net reproductive rate

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RO 1	.378	3	.	.767	3	.037
2	.257	3	.	.961	3	.620
3	.377	3	.	.770	3	.045

a. Lilliefors Significance Correction

Note: (1) = control, (2) = EM1, (3) = molasses

**Test Statistics<sup>a,b</sup>**

	NETRE
Chi-Square	2.222
df	2
Asymp. Sig.	.329

a. Kruskal Wallis Test

b. Grouping Variable: TREAT

Finite rate of increase

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
LAMBDA 1	.337	3	.	.854	3	.251
2	.239	3	.	.975	3	.698
3	.313	3	.	.894	3	.368

a. Lilliefors Significance Correction

Note: (1) = control, (2) = EM1, (3) = molasses

**Test of Homogeneity of Variances**

LAMBDA

Levene Statistic	df1	df2	Sig.
.551	2	6	.603

**ANOVA**

LAMBDA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	2	.000	.844	.475
Within Groups	.001	6	.000		
Total	.001	8			

Capacity for increase

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RC 1	.182	3	.	.999	3	.936
2	.235	3	.	.978	3	.714
3	.311	3	.	.897	3	.375

a. Lilliefors Significance Correction

Note: (1) = control, (2) = EM1, (3) = molasses

**Test of Homogeneity of Variances**

RC

Levene Statistic	df1	df2	Sig.
.363	2	6	.710

**ANOVA**

RC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	2	.000	.798	.493
Within Groups	.001	6	.000		
Total	.001	8			

Intrinsic rate of natural increase

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RM 1	.337	3	.	.854	3	.251
2	.241	3	.	.974	3	.691
3	.313	3	.	.894	3	.366

a. Lilliefors Significance Correction

Note: (1) = control, (2) = EM1, (3) = molasses

**Test of Homogeneity of Variances**

RM

Levene Statistic	df1	df2	Sig.
.549	2	6	.604

**ANOVA**

RM

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	2	.000	.838	.478
Within Groups	.001	6	.000		
Total	.001	8			

Life expectancy

Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
EX 1	.233	3	.	.979	3	.722
2	.219	3	.	.987	3	.783
3	.374	3	.	.776	3	.059

a. Lilliefors Significance Correction

Note: (1) = control, (2) = EM1, (3) = molasses

Test of Homogeneity of Variances

EX

Levene Statistic	df1	df2	Sig.
1.069	2	6	.401

ANOVA

EX

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	73.054	2	36.527	7.023	.027
Within Groups	31.208	6	5.201		
Total	104.262	8			

Post Hoc

Multiple Comparisons

Dependent Variable: EX

Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	6.179633*	1.862147	.037	.466054	11.893212
	3	5.898000*	1.862147	.044	.184421	11.611579
2	1	-6.179633*	1.862147	.037	-11.893212	-.466054
	3	-.281633	1.862147	.987	-5.995212	5.431946
3	1	-5.898000*	1.862147	.044	-11.611579	-.184421
	2	.281633	1.862147	.987	-5.431946	5.995212

\*. The mean difference is significant at the .05 level.

Note: (1) = control, (2) = EM1, (3) = molasses

Generation time

Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
T 1	.285	3	.	.932	3	.497
2	.201	3	.	.994	3	.858
3	.276	3	.	.943	3	.539

a. Lilliefors Significance Correction

Note: (1) = control, (2) = EM1, (3) = molasses

**Test of Homogeneity of Variances**

T

Levene Statistic	df1	df2	Sig.
3.974	2	6	.080

**ANOVA**

T

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.386	2	.693	.270	.772
Within Groups	15.411	6	2.568		
Total	16.796	8			

**Cohort generation time**

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
TC 1	.254	3	.	.963	3	.631
2	.193	3	.	.997	3	.889
3	.347	3	.	.836	3	.204

a. Lilliefors Significance Correction

Note: (1) = control, (2) = EM1, (3) = molasses

**Test of Homogeneity of Variances**

TC

Levene Statistic	df1	df2	Sig.
3.391	2	6	.103

**ANOVA**

TC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.553	2	.276	.058	.944
Within Groups	28.579	6	4.763		
Total	29.132	8			

**Doubling time**

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
D 1	.328	3	.	.871	3	.297
2	.272	3	.	.946	3	.554
3	.300	3	.	.913	3	.429

a. Lilliefors Significance Correction

Note: (1) = control, (2) = EM1, (3) = molasses

**Test of Homogeneity of Variances**

D

Levene Statistic	df1	df2	Sig.
.423	2	6	.673

**ANOVA**

D

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.314	2	.657	.733	.519
Within Groups	5.379	6	.896		
Total	6.693	8			

**Table E-11** Comparisons of effects of EM5 on adult stage with the control Net reproductive rate

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RO 1	.378	3	.	.767	3	.037
2	.204	3	.	.993	3	.843
3	.177	3	.	1.000	3	.963
4	.306	3	.	.904	3	.400
5	.265	3	.	.954	3	.586

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Finite rate of increase

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
LAMBDA 1	.337	3	.	.854	3	.251
2	.237	3	.	.976	3	.704
3	.244	3	.	.971	3	.676
4	.358	3	.	.812	3	.144
5	.219	3	.	.987	3	.780

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**Test of Homogeneity of Variances**

LAMBDA

Levene Statistic	df1	df2	Sig.
1.952	4	10	.178

**ANOVA**

LAMBDA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	4	.000	.296	.874
Within Groups	.001	10	.000		
Total	.001	14			

Capacity for increase

Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RC 1	.182	3	.	.999	3	.936
2	.260	3	.	.958	3	.605
3	.237	3	.	.977	3	.706
4	.365	3	.	.797	3	.107
5	.310	3	.	.899	3	.382

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Test of Homogeneity of Variances

RC

Levene Statistic	df1	df2	Sig.
1.475	4	10	.281

ANOVA

RC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	4	.000	.111	.976
Within Groups	.001	10	.000		
Total	.001	14			

Intrinsic rate of natural increase

Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RM 1	.337	3	.	.854	3	.251
2	.236	3	.	.977	3	.709
3	.246	3	.	.970	3	.669
4	.359	3	.	.811	3	.140
5	.221	3	.	.986	3	.772

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Test of Homogeneity of Variances

RM

Levene Statistic	df1	df2	Sig.
1.968	4	10	.176

**ANOVA**

RM

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	4	.000	.292	.877
Within Groups	.001	10	.000		
Total	.001	14			

## Life expectancy

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
EX 1	.233	3	.	.979	3	.722
2	.366	3	.	.796	3	.105
3	.177	3	.	1.000	3	.971
4	.296	3	.	.918	3	.445
5	.227	3	.	.983	3	.749

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**ANOVA**

EX

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	338.813	4	84.703	9.088	.002
Within Groups	93.207	10	9.321		
Total	432.020	14			

## Post Hoc

**Multiple Comparisons**

Dependent Variable: EX

Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	4.296433	3.648427	.882	-15.817244	24.410110
	3	10.511800	2.119866	.069	-1.308687	22.332287
	4	12.934933*	2.034544	.044	.586660	25.283207
	5	10.306367	1.941705	.091	-3.160862	23.773596
2	1	-4.296433	3.648427	.882	-24.410110	15.817244
	3	6.215367	3.319368	.596	-16.810674	29.241407
	4	8.638500	3.265538	.372	-15.367005	32.644005
	5	6.009933	3.208518	.604	-19.288677	31.308544
3	1	-10.511800	2.119866	.069	-22.332287	1.308687
	2	-6.215367	3.319368	.596	-29.241407	16.810674
	4	2.423133	1.358880	.607	-4.258335	9.104601
	5	-.205433	1.215485	1.000	-6.905033	6.494167
4	1	-12.934933*	2.034544	.044	-25.283207	-.586660
	2	-8.638500	3.265538	.372	-32.644005	15.367005
	3	-2.423133	1.358880	.607	-9.104601	4.258335
	5	-2.628567	1.059688	.347	-8.050986	2.793852
5	1	-10.306367	1.941705	.091	-23.773596	3.160862
	2	-6.009933	3.208518	.604	-31.308544	19.288677
	3	.205433	1.215485	1.000	-6.494167	6.905033
	4	2.628567	1.059688	.347	-2.793852	8.050986

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Generation time

Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
T 1	.285	3	.	.932	3	.497
2	.282	3	.	.935	3	.509
3	.201	3	.	.994	3	.858
4	.268	3	.	.951	3	.572
5	.366	3	.	.796	3	.106

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Test of Homogeneity of Variances

T

Levene Statistic	df1	df2	Sig.
2.209	4	10	.141

ANOVA

T

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.654	4	1.413	.395	.808
Within Groups	35.755	10	3.576		
Total	41.409	14			

Cohort generation time

Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
TC 1	.254	3	.	.963	3	.631
2	.281	3	.	.936	3	.512
3	.178	3	.	.999	3	.952
4	.289	3	.	.927	3	.476
5	.280	3	.	.938	3	.518

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Test of Homogeneity of Variances

TC

Levene Statistic	df1	df2	Sig.
2.035	4	10	.165

**ANOVA**

TC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21.122	4	5.281	.721	.597
Within Groups	73.273	10	7.327		
Total	94.395	14			

**Doubling time****Tests of Normality**

D	TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
	1	.328	3	.	.871	3	.297
	2	.248	3	.	.969	3	.661
	3	.271	3	.	.948	3	.560
	4	.365	3	.	.797	3	.106
	5	.237	3	.	.976	3	.704

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**Test of Homogeneity of Variances**

D			
Levene Statistic	df1	df2	Sig.
2.173	4	10	.146

**ANOVA**

D					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.698	4	.175	.208	.928
Within Groups	8.395	10	.840		
Total	9.094	14			

**Table E-11** Comparisons of effects of vinegar on adult stage with the control

Net reproductive rate

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RO 1	.378	3	.	.767	3	.037
2	.175	3	.	1.000	3	.991
3	.269	3	.	.950	3	.568
4	.346	3	.	.837	3	.205
5	.360	3	.	.808	3	.133

a. Lilliefors Significance Correction

**Test Statistics<sup>a,b</sup>**

	RO
Chi-Square	8.567
df	4
Asymp. Sig.	.073

a. Kruskal Wallis Test

b. Grouping Variable: TREAT

Finite rate of increase

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
LAMBDA 1	.337	3	.	.854	3	.251
2	.226	3	.	.984	3	.754
3	.188	3	.	.998	3	.911
4	.177	3	.	1.000	3	.967
5	.199	3	.	.995	3	.865

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)

(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

**Test of Homogeneity of Variances**

LAMBDA

Levene Statistic	df1	df2	Sig.
1.621	4	10	.244

**ANOVA**

LAMBDA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	4	.000	7.872	.004
Within Groups	.001	10	.000		
Total	.002	14			

Post Hoc

Multiple Comparisons

Dependent Variable: LAMBDA  
Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.023733*	.0062302	.022	-.044237	-.003229
	3	-.024333*	.0062302	.019	-.044837	-.003829
	4	-.006000	.0062302	.865	-.026504	.014504
	5	-.027233*	.0062302	.010	-.047737	-.006729
2	1	.023733*	.0062302	.022	.003229	.044237
	3	-.000600	.0062302	1.000	-.021104	.019904
	4	.017733	.0062302	.099	-.002771	.038237
	5	-.003500	.0062302	.978	-.024004	.017004
3	1	.024333*	.0062302	.019	.003829	.044837
	2	.000600	.0062302	1.000	-.019904	.021104
	4	.018333	.0062302	.086	-.002171	.038837
	5	-.002900	.0062302	.989	-.023404	.017604
4	1	.006000	.0062302	.865	-.014504	.026504
	2	-.017733	.0062302	.099	-.038237	.002771
	3	-.018333	.0062302	.086	-.038837	.002171
	5	-.021233*	.0062302	.042	-.041737	-.000729
5	1	.027233*	.0062302	.010	.006729	.047737
	2	.003500	.0062302	.978	-.017004	.024004
	3	.002900	.0062302	.989	-.017604	.023404
	4	.021233*	.0062302	.042	.000729	.041737

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

Capacity for increase

Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RC	1	.182	3	.999	3	.936
	2	.177	3	1.000	3	.972
	3	.238	3	.976	3	.702
	4	.225	3	.984	3	.758
	5	.336	3	.857	3	.259

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

Test of Homogeneity of Variances

RC			
Levene Statistic	df1	df2	Sig.
1.346	4	10	.319

**ANOVA**

RC					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	4	.000	6.900	.006
Within Groups	.000	10	.000		
Total	.001	14			

**Post Hoc**

**Multiple Comparisons**

Dependent Variable: RC

Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.014767	.0045135	.052	-.029621	.000088
	3	-.012067	.0045135	.129	-.026921	.002788
	4	-.003733	.0045135	.916	-.018588	.011121
	5	-.020733*	.0045135	.007	-.035588	-.005879
2	1	.014767	.0045135	.052	-.000088	.029621
	3	.002700	.0045135	.972	-.012154	.017554
	4	.011033	.0045135	.180	-.003821	.025888
	5	-.005967	.0045135	.685	-.020821	.008888
3	1	.012067	.0045135	.129	-.002788	.026921
	2	-.002700	.0045135	.972	-.017554	.012154
	4	.008333	.0045135	.401	-.006521	.023188
	5	-.008667	.0045135	.367	-.023521	.006188
4	1	.003733	.0045135	.916	-.011121	.018588
	2	-.011033	.0045135	.180	-.025888	.003821
	3	-.008333	.0045135	.401	-.023188	.006521
	5	-.017000*	.0045135	.024	-.031854	-.002146
5	1	.020733*	.0045135	.007	.005879	.035588
	2	.005967	.0045135	.685	-.008888	.020821
	3	.008667	.0045135	.367	-.006188	.023521
	4	.017000*	.0045135	.024	.002146	.031854

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

**Intrinsic rate of natural increase**

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RM 1	.337	3	.	.854	3	.251
2	.227	3	.	.983	3	.749
3	.190	3	.	.997	3	.902
4	.177	3	.	1.000	3	.973
5	.203	3	.	.994	3	.849

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

## Test of Homogeneity of Variances

RM

Levene Statistic	df1	df2	Sig.
1.635	4	10	.241

## ANOVA

RM

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	4	.000	7.854	.004
Within Groups	.000	10	.000		
Total	.002	14			

## Post Hoc

## Multiple Comparisons

Dependent Variable: RM

Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.021600*	.0056721	.022	-.040267	-.002933
	3	-.022167*	.0056721	.019	-.040834	-.003499
	4	-.005500	.0056721	.863	-.024167	.013167
	5	-.024767*	.0056721	.010	-.043434	-.006099
2	1	.021600*	.0056721	.022	.002933	.040267
	3	-.000567	.0056721	1.000	-.019234	.018101
	4	.016100	.0056721	.100	-.002567	.034767
3	1	.022167*	.0056721	.019	.003499	.040834
	2	.000567	.0056721	1.000	-.018101	.019234
	4	.016667	.0056721	.086	-.002001	.035334
4	1	-.005500	.0056721	.863	-.013167	.024167
	2	-.016100	.0056721	.100	-.034767	.002567
	3	-.016667	.0056721	.086	-.035334	.002001
5	1	-.019267*	.0056721	.042	-.037934	-.000599
	2	.024767*	.0056721	.010	.006099	.043434
	3	.003167	.0056721	.978	-.015501	.021834
5	4	.002600	.0056721	.990	-.016067	.021267
	5	.019267*	.0056721	.042	.000599	.037934

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

### Life expectancy

#### Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
EX 1	.233	3	.	.979	3	.722
2	.352	3	.	.825	3	.176
3	.328	3	.	.871	3	.298
4	.319	3	.	.885	3	.340
5	.325	3	.	.876	3	.312

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

#### Test of Homogeneity of Variances

EX

Levene Statistic	df1	df2	Sig.
.690	4	10	.615

#### ANOVA

EX

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	90.659	4	22.665	1.572	.256
Within Groups	144.145	10	14.415		
Total	234.804	14			

### Generation time

#### Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
T 1	.285	3	.	.932	3	.497
2	.239	3	.	.975	3	.697
3	.295	3	.	.920	3	.453
4	.351	3	.	.828	3	.182
5	.335	3	.	.858	3	.263

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

#### Test of Homogeneity of Variances

T

Levene Statistic	df1	df2	Sig.
1.833	4	10	.199

**ANOVA**

T

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.708	4	4.927	1.743	.217
Within Groups	28.262	10	2.826		
Total	47.970	14			

**Cohort generation time****Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
TC 1	.254	3	.	.963	3	.631
2	.309	3	.	.900	3	.385
3	.277	3	.	.941	3	.533
4	.303	3	.	.909	3	.416
5	.301	3	.	.912	3	.424

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

**Test of Homogeneity of Variances**

TC

Levene Statistic	df1	df2	Sig.
.969	4	10	.466

**ANOVA**

TC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	96.470	4	24.117	2.402	.119
Within Groups	100.401	10	10.040		
Total	196.871	14			

**Doubling time****Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
D 1	.328	3	.	.871	3	.297
2	.251	3	.	.966	3	.647
3	.200	3	.	.995	3	.861
4	.179	3	.	.999	3	.951
5	.209	3	.	.991	3	.823

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

**Test of Homogeneity of Variances**

D

Levene Statistic	df1	df2	Sig.
2.296	4	10	.131

**ANOVA**

D

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.875	4	2.469	7.735	.004
Within Groups	3.192	10	.319		
Total	13.067	14			

**Post Hoc**

**Multiple Comparisons**

Dependent Variable: D

Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	1.782333*	.4612701	.021	.264256	3.300411
	3	1.861033*	.4612701	.016	.342956	3.379111
	4	.566433	.4612701	.737	-.951644	2.084511
	5	2.028000*	.4612701	.009	.509922	3.546078
2	1	-1.782333*	.4612701	.021	-3.300411	-.264256
	3	.078700	.4612701	1.000	-1.439378	1.596778
	4	-1.215900	.4612701	.136	-2.733978	.302178
	5	.245667	.4612701	.982	-1.272411	1.763744
3	1	-1.861033*	.4612701	.016	-3.379111	-.342956
	2	-.078700	.4612701	1.000	-1.596778	1.439378
	4	-1.294600	.4612701	.105	-2.812678	.223478
	5	.166967	.4612701	.996	-1.351111	1.685044
4	1	-.566433	.4612701	.737	-2.084511	.951644
	2	1.215900	.4612701	.136	-.302178	2.733978
	3	1.294600	.4612701	.105	-.223478	2.812678
	5	1.461567	.4612701	.060	-.056511	2.979644
5	1	-2.028000*	.4612701	.009	-3.546078	-.509922
	2	-.245667	.4612701	.982	-1.763744	1.272411
	3	-.166967	.4612701	.996	-1.685044	1.351111
	4	-1.461567	.4612701	.060	-2.979644	.056511

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

**Table E-12** Comparisons of effects of rice whiskey on adult stage with the control  
Net reproductive rate

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RO 1	.378	3	.	.767	3	.037
2	.365	3	.	.796	3	.106
3	.191	3	.	.997	3	.899
4	.178	3	.	.999	3	.951
5	.237	3	.	.976	3	.706

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

**Test Statistics<sup>a,b</sup>**

	RO
Chi-Square	7.433
df	4
Asymp. Sig.	.115

a. Kruskal Wallis Test

b. Grouping Variable: TREAT

**Finite rate of increase**

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
LAMBDA 1	.337	3	.	.854	3	.251
2	.385	3	.	.750	3	.000
3	.295	3	.	.920	3	.452
4	.320	3	.	.883	3	.332
5	.287	3	.	.929	3	.485

a. Lilliefors Significance Correction

**Test Statistics<sup>a,b</sup>**

	LAMBDA
Chi-Square	8.957
df	4
Asymp. Sig.	.062

a. Kruskal Wallis Test

b. Grouping Variable: TREAT

Capacity for increase

Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RC 1	.182	3	.	.999	3	.936
2	.289	3	.	.927	3	.478
3	.188	3	.	.998	3	.912
4	.313	3	.	.895	3	.369
5	.354	3	.	.822	3	.167

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
1.053	4	10	.428

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	4	.000	4.001	.034
Within Groups	.000	10	.000		
Total	.001	14			

Post Hoc

Multiple Comparisons

Dependent Variable: RC

Tukey HSD

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.013833	.0043864	.062	-.000603	.028269
	3	.004200	.0043864	.868	-.010236	.018636
	4	.006100	.0043864	.646	-.008336	.020536
	5	-.002133	.0043864	.987	-.016569	.012303
2	1	-.013833	.0043864	.062	-.028269	.000603
	3	-.009633	.0043864	.256	-.024069	.004803
	4	-.007733	.0043864	.442	-.022169	.006703
	5	-.015967*	.0043864	.029	-.030403	-.001531
3	1	-.004200	.0043864	.868	-.018636	.010236
	2	.009633	.0043864	.256	-.004803	.024069
	4	.001900	.0043864	.992	-.012536	.016336
	5	-.006333	.0043864	.616	-.020769	.008103
4	1	-.006100	.0043864	.646	-.020536	.008336
	2	.007733	.0043864	.442	-.006703	.022169
	3	-.001900	.0043864	.992	-.016336	.012536
	5	-.008233	.0043864	.387	-.022669	.006203
5	1	.002133	.0043864	.987	-.012303	.016569
	2	.015967*	.0043864	.029	.001531	.030403
	3	.006333	.0043864	.616	-.008103	.020769
	4	.008233	.0043864	.387	-.006203	.022669

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

**Intrinsic rate of natural increase**

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RM 1	.337	3	.	.854	3	.251
2	.385	3	.	.750	3	.000
3	.287	3	.	.930	3	.488
4	.326	3	.	.874	3	.307
5	.286	3	.	.931	3	.491

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

**Test Statistics<sup>a,b</sup>**

	RM
Chi-Square	8.957
df	4
Asymp. Sig.	.062

a. Kruskal Wallis Test

b. Grouping Variable: TREAT

**Life expectancy**

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
EX 1	.233	3	.	.979	3	.722
2	.188	3	.	.998	3	.913
3	.280	3	.	.938	3	.519
4	.175	3	.	1.000	3	1.000
5	.382	3	.	.757	3	.014

a. Lilliefors Significance Correction

**Test Statistics<sup>a,b</sup>**

	EX
Chi-Square	12.567
df	4
Asymp. Sig.	.014

a. Kruskal Wallis Test

b. Grouping Variable: TREAT

Post Hoc

Multiple Comparisons

Dependent Variable: EX  
Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	11.635033	1.918727	.074	-2.237800	25.507867
	3	15.755767*	2.036314	.026	3.422467	28.089066
	4	15.632833*	2.019633	.028	3.150363	28.115303
	5	6.908233	3.466504	.524	-11.661144	25.477610
2	1	-11.635033	1.918727	.074	-25.507867	2.237800
	3	4.120733	1.020511	.113	-1.376852	9.618319
	4	3.997800	.9868066	.108	-1.220550	9.216150
	5	-4.726800	2.985217	.714	-28.486895	19.033295
3	1	-15.755767*	2.036314	.026	-28.089066	-3.422467
	2	-4.120733	1.020511	.113	-9.618319	1.376852
	4	-.122933	1.199519	1.000	-5.907908	5.662042
	5	-8.847533	3.062120	.319	-30.898137	13.203070
4	1	-15.632833*	2.019633	.028	-28.115303	-3.150363
	2	-3.997800	.9868066	.108	-9.216150	1.220550
	3	.122933	1.199519	1.000	-5.662042	5.907908
	5	-8.724600	3.051053	.327	-30.988079	13.538879
5	1	-6.908233	3.466504	.524	-25.477610	11.661144
	2	4.726800	2.985217	.714	-19.033295	28.486895
	3	8.847533	3.062120	.319	-13.203070	30.898137
	4	8.724600	3.051053	.327	-13.538879	30.988079

\*. The mean difference is significant at the .05 level.

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

Generation time

Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
T 1	.285	3	.	.932	3	.497
2	.184	3	.	.999	3	.928
3	.230	3	.	.981	3	.734
4	.210	3	.	.991	3	.820
5	.267	3	.	.951	3	.574

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
(2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
2.677	4	10	.094

**ANOVA**

T

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18.207	4	4.552	2.153	.148
Within Groups	21.139	10	2.114		
Total	39.345	14			

**Cohort generation time****Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
TC 1	.254	3	.	.963	3	.631
2	.239	3	.	.975	3	.697
3	.308	3	.	.902	3	.392
4	.357	3	.	.815	3	.150
5	.216	3	.	.989	3	.795

a. Lilliefors Significance Correction

Note: (1) = control (H<sub>2</sub>O)  
 (2), (3), (4), (5) = concentrations of vinegar at 1:200, 1:300, 1:400 and 1:500, respectively

**Test of Homogeneity of Variances**

TC

Levene Statistic	df1	df2	Sig.
.901	4	10	.499

**ANOVA**

TC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29.795	4	7.449	1.401	.302
Within Groups	53.167	10	5.317		
Total	82.962	14			

**Doubling time****Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
D 1	.328	3	.	.871	3	.297
2	.385	3	.	.750	3	.000
3	.292	3	.	.924	3	.466
4	.328	3	.	.870	3	.295
5	.301	3	.	.912	3	.424

a. Lilliefors Significance Correction

**Test Statistics<sup>a,b</sup>**

	D
Chi-Square	8.957
df	4
Asymp. Sig.	.062

a. Kruskal Wallis Test

b. Grouping Variable: TREAT

**Table E-14** Comparisons of effects of EM1 and EM5 on adult stage  
Net reproductive rate

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RO 1	.377	3	.	.770	3	.045
2	.204	3	.	.993	3	.843
3	.177	3	.	1.000	3	.963
4	.306	3	.	.904	3	.400
5	.265	3	.	.954	3	.586

a. Lilliefors Significance Correction

Note: (1) = EM1  
(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**Test Statistics<sup>a,b</sup>**

	RO
Chi-Square	1.367
df	4
Asymp. Sig.	.850

a. Kruskal Wallis Test

b. Grouping Variable: TREAT

**Finite rate of increase**

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
LAMBDA 1	.313	3	.	.894	3	.368
2	.237	3	.	.976	3	.704
3	.244	3	.	.971	3	.676
4	.358	3	.	.812	3	.144
5	.219	3	.	.987	3	.780

a. Lilliefors Significance Correction

**Test of Homogeneity of Variances**

LAMBDA

Levene Statistic	df1	df2	Sig.
1.911	4	10	.185

**ANOVA**

LAMBDA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	4	.000	.296	.874
Within Groups	.001	10	.000		
Total	.001	14			

Capacity for increase

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RC 1	.311	3	.	.897	3	.375
2	.260	3	.	.958	3	.605
3	.237	3	.	.977	3	.706
4	.365	3	.	.797	3	.107
5	.310	3	.	.899	3	.382

a. Lilliefors Significance Correction

Note: (1) = EM1  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**Test of Homogeneity of Variances**

RC

Levene Statistic	df1	df2	Sig.
1.981	4	10	.173

**ANOVA**

RC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	4	.000	.204	.930
Within Groups	.000	10	.000		
Total	.001	14			

Intrinsic rate of natural increase

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RM 1	.313	3	.	.894	3	.366
2	.236	3	.	.977	3	.709
3	.246	3	.	.970	3	.669
4	.359	3	.	.811	3	.140
5	.221	3	.	.986	3	.772

a. Lilliefors Significance Correction

Note: (1) = EM1  
 (2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**Test of Homogeneity of Variances**

RM

Levene Statistic	df1	df2	Sig.
1.927	4	10	.182

**ANOVA**

RM

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	4	.000	.291	.877
Within Groups	.001	10	.000		
Total	.001	14			

Life expectancy

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
EX 1	.374	3	.	.776	3	.059
2	.366	3	.	.796	3	.105
3	.177	3	.	1.000	3	.971
4	.296	3	.	.918	3	.445
5	.227	3	.	.983	3	.749

a. Lilliefors Significance Correction

Note: (1) = EM1

(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**Test of Homogeneity of Variances**

EX

Levene Statistic	df1	df2	Sig.
5.717	4	10	.012

**ANOVA**

EX

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	153.001	4	38.250	4.987	.018
Within Groups	76.696	10	7.670		
Total	229.697	14			

Post Hoc

Multiple Comparisons

Dependent Variable: EX  
Dunnnett T3

(I) TREAT	(J) TREAT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-1.601567	3.249490	.999	-25.941862	22.738729
	3	4.613800	1.319847	.147	-1.995125	11.222725
	4	7.036933*	1.177936	.024	1.345056	12.728810
	5	4.408367	1.009148	.077	-.639326	9.456060
2	1	1.601567	3.249490	.999	-22.738729	25.941862
	3	6.215367	3.319368	.596	-16.810674	29.241407
	4	8.638500	3.265538	.372	-15.367005	32.644005
	5	6.009933	3.208518	.604	-19.288677	31.308544
3	1	-4.613800	1.319847	.147	-11.222725	1.995125
	2	-6.215367	3.319368	.596	-29.241407	16.810674
	4	2.423133	1.358880	.607	-4.258335	9.104601
	5	-.205433	1.215485	1.000	-6.905033	6.494167
4	1	-7.036933*	1.177936	.024	-12.728810	-1.345056
	2	-8.638500	3.265538	.372	-32.644005	15.367005
	3	-2.423133	1.358880	.607	-9.104601	4.258335
	5	-2.628567	1.059688	.347	-8.050986	2.793852
5	1	-4.408367	1.009148	.077	-9.456060	.639326
	2	-6.009933	3.208518	.604	-31.308544	19.288677
	3	.205433	1.215485	1.000	-6.494167	6.905033
	4	2.628567	1.059688	.347	-2.793852	8.050986

\*. The mean difference is significant at the .05 level.

Note: (1) = EM1  
(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Generation time

Tests of Normality

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
T 1	.276	3	.	.943	3	.539
2	.282	3	.	.935	3	.509
3	.201	3	.	.994	3	.858
4	.268	3	.	.951	3	.572
5	.366	3	.	.796	3	.106

a. Lilliefors Significance Correction

Note: (1) = EM1  
(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
3.123	4	10	.066

**ANOVA**

T

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.856	4	.964	.411	.797
Within Groups	23.484	10	2.348		
Total	27.340	14			

**Cohort generation time**

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
TC 1	.347	3	.	.836	3	.204
2	.281	3	.	.936	3	.512
3	.178	3	.	.999	3	.952
4	.289	3	.	.927	3	.476
5	.280	3	.	.938	3	.518

a. Lilliefors Significance Correction

Note: (1) = EM1

(2), (3), (4), (5) = concentrations of EM5 at 1:200, 1:300, 1:400 and 1:500, respectively

**Test of Homogeneity of Variances**

TC

Levene Statistic	df1	df2	Sig.
3.397	4	10	.053

**ANOVA**

TC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21.018	4	5.254	1.051	.429
Within Groups	49.999	10	5.000		
Total	71.017	14			

**Doubling time**

**Tests of Normality**

TREAT	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
D 1	.300	3	.	.913	3	.429
2	.248	3	.	.969	3	.661
3	.271	3	.	.948	3	.560
4	.365	3	.	.797	3	.106
5	.237	3	.	.976	3	.704

a. Lilliefors Significance Correction

**Test of Homogeneity of Variances**

D

Levene Statistic	df1	df2	Sig.
2.159	4	10	.148

**ANOVA**

D

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.718	4	.180	.217	.923
Within Groups	8.275	10	.827		
Total	8.993	14			

## **BIOGRAPHY**

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