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LIFE TABLE PARAMETERS OF THE PERFORMANCE OF *PROCTOLAE LAP S GIZAENSIS SP. NOV.* (ACARI: MESOSTIGMATA: MELIC HARIDAE) ON FIVE DIFFERENT DIETS

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ABSTRACT

The predatory mite *Proctolaelaps gizaensis* was found under palm trees and core of palm associated with mites, *Rhizoglyphus robini*, collembolan and other arthropods. The incubation period is affecting by different diets at 27±2°C and 65-70%, the shortest period was 1.61 days when reared on sugar cane + pollen +chitosan), and the duration of larval female increased than male. Statistical analysis showed significant effects on the duration of the total immature stages with different diets. At the same temperature, life cycle durated 11.32 days for female and 10.10 days for male reared on sugar cane, but when add pollen to sugar cane, this period was 12.56 days for female opposed to 11.6 days for male. The shortest period of life cycle was recorded when reared on (sugar cane + chitosan), whereas the longest period was recorded when the mite reared on (sugar cane +pollen+ chitosan). Fecundity was affected by rearing on different diets, the mean total number of deposited eggs 84.7, 88.9, 48 and 16.75 eggs when reared on (sugar cane), (sugar cane + pollen), (sugar cane + pollen +chitosan) and (sugar cane + chitosan) respectively. The most suitable diet was sugarcane with pollen followed by sugarcane at 27±2°C and 65-70% R.H. for increasing female fertility. The net reproductive rate (R_0) reached highest value when it reared on sugarcane+ pollen (35.076 females /female). However, the shortest value was obtained when it reared on Sugarcane+ Chitosan being 6.384 females /female. For the mean generation time (T), this period decreased with the add chitosan to (sugarcane) and (sugarcane+ pollen), it were averaged values 12.588 and 17.305. While, these values increased when reared on sugarcane (18.157) and sugarcane+ pollen (19.910).When the values of intrinsic rate of increase (r_m) were converted into finite rate of increase (λ), the population of *P. gizaensis* had the capacity decrease by 1.216, 1.195, 1.191 and 1.158 times/female/day when fed on obvious diets respectively. The highest capacity to increase was recorded on sugarcane. Time of population doubling varied according to different diets it decreased when reared on sugarcane (3.56 days). The growth reproductive rate (GRR) recorded high values in both (sugarcane) and (sugarcane +pollen) in respective (63.01) and (50.76). From the forgoing results, it could be concluded that, the most suitable diet for *P. gizaensis* was sugarcane with pollen followed by sugarcane at 27±2°C and 65-70% R.H. for increasing female fertility.

Keywords: *Proctolaelaps gizaensis* was, Biological control, Mass rearing, Alternative food source, *Rhynchophorus ferrugineus*

INTRODUCTION

Melicharids are known from soil, litter, plants (i.e., their flowers and fruits), rotten wood, stored products, seaweeds, colonies of fruit flies, on cockroaches, beetles, moths, ants, bees, bumblebees and their nests, birds, small mammals and their nests, corps, and excrements (De Leon, 1963; Westerboer, 1963; Treat and Niederman, 1967; Fain *et al.*, 1977; Karg, 1985, 1988a; Hanekom *et al.*, 1988; OConnor *et al.*, 1991; Halliday, 2001; Faraji, 2011; Mašán *et al.*, 2013),

Moraes *et al.* (2015) mentioned that, About 20% of the Melicharids have been described from soil, grasses, mosses or dead organic matter on the soil surface, while about 32 and 27% have been described from plant parts or in association with nectar feeding organisms.

Abo-Shnaf and Moraes (2016) mentioned that Melicharidae is one of the mesostigmatid families found in different habitats. Slightly over 200 world species have been considered to belong to this family, of which slightly over 130 have been assigned to *Proctolaelaps* Berlese. About 25 species of the genus *Proctolaelaps* are phoretic on beetles (cossonines, bark beetles, stag beetles, sap beetles, erotylids, scarab beetles, leaf beetles, silphids, and carabid beetles). These mites are especially common on subcortical beetles (Mašán, 1998; Ma *et al.*, 2003 and Gwiazdowicz, 2007).

Thirteen species of the genus *Proctolaelaps* have been reported from Russia: Maslov and Matusevich, 2008; Makarova, 2009, 2011, 2012; Khaustov *et al.*, 2016).

Direct evidence of feeding behavior is only available for a few *Proctolaelaps* species; for most Melicharid mites it is generally considered to be predatory, with a minority of

species adapted to feeding on fungi, pollen, and nectar (Lindquist *et al.*, 2009). Feeding on nematodes is assumed for many species, but shown for only a very few (Halliday *et al.*, 1998; Andreev 1988). It has been demonstrated that an undescribed species of *Proctolaelaps* does feed on nematodes while on its phoront host, thus during transport and on nematodes that are transported as well (Krantz and Poinar, 2004); data on *Proctolaelaps* feeding on soil nematodes is lacking.

Predatory *Proctolaelaps* species may be facultative pollenophagous or phytosaprophagous (Krantz and Lindquist 1979). Varied feeding behaviour is confirmed in *Proctolaelaps* by some authors (Mathys and Tencalla, 1959; Nesbitt, 1951; Walter and Lindquist, 1989). They showed that the best-known species in the genus, *Proctolaelaps* is feeding and reproducing on a diet of various mites and fungal mycelia.

In Egypt, little has been reported about the Melicharids. Four new Melicharid species have been described from that country, all in *Proctolaelaps* (Nasr, 1978; Afifi *et al.*, 1984; Zaher, 1986; Ibrahim & Taha, 1989), while a species known from Europe was also reported from Egypt (Zaher, 1986). Three other species have been mentioned from Egypt, but unfortunately their adults were not characterised in the respective records (Nasr *et al.*, 1990; Ibrahim *et al.*, 1992; Taha & Mohmoud, 2007). The Egyptian *Proctolaelaps*, *Proctolaelaps gizaensis*, recorded from Egypt, collected and described, by Abo-Shnaf and Moraes (2016).

Chitosan (β -1,4-linked glucosamine) is a deacetylated derivative of chitin found in the composition of cell walls of many fungi (Bartnicki-Garcia, 1968). From data in previous reports, two biological roles can be ascribed to this compound. First, at defined concentrations, it presents antifungal properties as shown by its inhibitory action on the mycelia growth of a number of pathogenic fungi, including root pathogens, such as *Fusarium oxysporum* and *Pithium phanidermatum* (Leuba and Stossel, 1986; Benhamou, 1992; El-Ghaouth *et al.*, 1994; El-Hassni *et al.*, 2004) and also by its inhibitory effect on spore germination (Hadwiger and Beckman, 1980). Secondly, it acts as a potent elicitor, therefore enhancing plant resistance against pathogens (Benhamou and Thériault, 1992; El-Ghaouth *et al.*, 1994).

Guoping Li *et al.* (2009) during feeding on *Solanum dulcamara*, the gall mite *Eriophyes cladophthirus* perforated the walls of the epidermal cells; the epidermal cells reacted by forming callose around the feeding punctures. Within the first hour of mite activity important cytological changes occurred in the injured cells, especially in the nuclei—which enlarged and appeared optically empty. At the same time, affinity for specific fluorescent DNA-binding reagents was gradually lost.

Dembilio and Jacas (2013) mentioned that, *Steirnermema carpocapsae* in a chitosan formulation is highly effective against *Rhynchophorus ferrugineus* in the field. Different timings and product combinations were studied, and high efficacies were obtained in all cases. *Steirnermema carpocapsae* was applied on a monthly basis and therefore resulted more expensive and time consuming than chemical applications. However this invertebrate biological control agent could be most suitable for ornamental palms in public areas. The economic importance of mass rearing of this species to use this predator species in

a red palm weevil biological control program. The aims of this study were; (i) to compare embryonic development, adult longevity of *P. gizaensis* and different diets to reach the diet preference for mass rearing, (ii) to explain how the different artificial diets affected the life table parameters of *P. gizaensis*.

MATERIALS AND METHODS

1-Collect mites:

Extraction of mite collected from habitats of red palm weevil *Rhynchophorus ferrugineus* at El-wheat. Materials were carefully examined individually by using the dissecting microscope, and then the detected mites were removed gently with a fine brush or needle from different samples. Each sample was mixed carefully and put in petridishes were examined by using the dissecting microscope.

2-Source of food

The types of food used in the present study.

1. A piece of sugar cane.
2. A piece of sugar cane inoculated with pollen (palm tree).
3. A piece of sugar cane inoculated with pollen (palm tree) +chitosan.
4. A piece of sugar cane + chitosan
5. Chitosan is a sugar that is obtained from the hard outer skeleton of shellfish, including crab, lobster, and shrimp. It is used for medicine.

3-Specimens Identification

Adult individuals were firstly cleared in Nesbitt's solution then mounted in Hoyer's medium on glass slides and examined microscopically for identification. Slides were labeled with locality, stage, sex and date of mounting.

4- Identification and Description.

Identification and description of mite *Proctolaelaps gizaensis* were based mainly on those given by Abo-Shnaf and Moraes (2016)

5-Rearing procedure

Throughout the work of this investigation, one species of mesostigmatic mite; *Proctolaelaps gizaensis* was reared in plastic rings 2.8 cm. in diameter and 2.0 cm. in depth. They were filled up to 0.5 cm. with plaster of Paris and charcoal. Drops of water was added daily to maintain suitable relative humidity. For culturing mites; several adult females were placed in plastic rings supplied with food under investigation and kept at 27±2°C. For individuals rearing, newly deposited eggs were transferred singly to prepared ring. Each newly hatched larvae was supplied with a food, replaced before dry till reaching maturity. Mites were examined twice daily with the aid of stereomicroscope. Emerging females were copulated and kept for oviposition, Observation concerning all biological aspects were recorded during predator life span. Each rearing experiment was started with 20-25 newly hatched larvae. (A piece of sugar cane), (A piece of sugar cane inoculated with pollen palm tree), (A piece of sugar cane inoculated with pollen palm tree+chitosan), (A piece of sugar cane + chitosan) and (Chitosan) were used as diets.

6-Mass production unit

Mass production culture pieces of sugarcane were put in plastic unit (with length 43 cm, width 25 cm and height 9 cm) and put the mite each unit was put in incubator on 25°C.

7-Experimental procedure

After per elementary test we notice the piece of the sugarcane after cutting of a linear break in incubator on 25°C with using humidity 70% mites can live for 30 days. We put five pieces of sugarcane, on every piece put five male and five female from one species of mite we recorded the data every day. Typically it will take place in a closed environment such as incubator in laboratory and growth room when mass production by farmers *Proctolaelaps gizaensis* must be situated to a worm, dark and humid climate. Each piece of sugarcane examined and determined the stage of mites, behavior, and numbers of mites in fold on one cm in all units, also in the biological phenomena were observed one female and male such as Incubation period - Larval stage - protonymphal stage - Dutonymphal stage - Total immature stage - Life cycle - preoviposition - Oviposition - Fecundity – sex ration. These phenomena were very important to know how try the mass rearing to mass production in these mites that can be used by Egyptian farmers.

8-Procedure of SEM study

The live specimens from mite and chitosan were cleaned in several baths of distilled water to remove the debris from observation under the scanning electron microscope. They were then briefly submerged in distilled water near boiling point in order to force extension of appendages. Specimens were then fixed in 3.5% concentration of glutaraldehyde for 6 hours, dehydrated in ethyl alcohol, dried using the critical point procedure, individually affixed to stubs using double-sided sticky tape, and sputter coated with gold-palladium. Microscopy was performed with a (JEOL GM 4200) microscope. Scanning electron micrographs of the mites are presented. SEM study aims to objective fibers of chitosan on the different stage of *P. gizaensis*

9-Statistical analysis

Comparing means were calculated by SPSS®20.00. Statistics for each analysis are based on cases with no missing data for any variable in the analysis. Data were analysed by one-way analysis of variance (ANOVA) and comparisons were performed by Tukey and Student's test *T*-test, Life table parameters were calculated using a computer program based on Birch (1948) formulas: The age-specific survival (l_x), The age-specific fecundity (m_x)= born females/female, The net reproductive rate (R_0)= $\sum (l_x m_x)$, the intrinsic rate of increase (r_m)= $\sum (e^{-r_m} \times l_x m_x) = 1$, the mean generation time (T)= $\frac{\ln R_c}{r}$, the finite rate of increase (λ)= e^{r_m} , the doubling time (DT)= $\frac{\ln 2}{r}$ was calculated according to Mackauer (1983) and the Gross reproductive rate (GRR) = $\sum (m_x)$ according to Kairo and Murphy (1995). Variances and standard errors of the population parameters (r_m , R_0 , λ and T) analysed by SPSS®Tukey's uses harmonic mean sample size (n)= 14.

RESULTS

The experimental null hypothesis H_0 was performed. It suggested that there were no significant differences in *P. gizaensis* biological aspects and life table parameters when fed sugarcane, sugarcane + pollen, sugarcane + pollen + chitosan, sugarcane + chitosan, and chitosan diets. Although, alternative hypothesis H_1 suggested that *P. gizaensis* would definitely affected when fed these diets. A Kruskal-Wallis test conducted to determine either H_0 or H_1 is accepted. The test's results rejected the H_0 due to significant differences in *P. gizaensis* female (post-embryonic development, total female longevity, life span, and fecundity; $P=0.000$), and male (post-embryonic development; $P= 0.050$, total longevity; $P= 0.000$, life span; $P= 0.000$) at significant level of 95% (Figures 1 & 2).

1-Duration of developmental stages

At $27 \pm 2^\circ\text{C}$ and 65-70% R.H. Incubation period of *Proctolaelaps gizaensis* was recorded 2.64, 2.4, 1.61, 2.72 and 1.7 days, when adult female were fed on. (A piece of sugar cane), (A piece of sugar cane inoculated with pollen palm tree), (A piece of sugar cane inoculated with pollen palm tree +chitosan), (A piece of sugar cane + chitosan) and (chitosan) respectively. It is clear that, the incubation period is affected by different diets. In general, incubation period was shorter when adult female were fed on sugar cane +pollen palm tree +chitosan than other diets (Table1).

During experiment, data showed that, the percentage of hatching recorded high percent (92.8%) when adult female were fed on sugar cane, followed by (85.7%), (84.6%), (73.3%) and (30.8%) when adult female fed on obvious diets respectively. The duration of the larval were 3.31, 2.75, 3.36, 1.81 and 2.25 days in average for female and 2.5, 2.6, 2.8, 2.14 and 1.5 days in average for male, at the same temperature and RH and when feed on obvious diets respectively (Table 1). Significant effect was recorded for the effect of diets (sugar cane + chitosan) than other diets on the duration of the female larval stage ($F=8.276^*$, $P=0.00$), significant effect was recorded for the effect of diet (chitosan) on the duration of the larval male stage ($F=4.677^*$, $P=0.00$). Data showed that, the percentage of mortality in this stage reach to 50% when larva fed on chitosan only. Female protonymphal stage averaged 2.75, 3.33, 3.9, 2.11 and 2.66 days, male protonymphal, this period 2.6, 3.0, 3.9, 2.71 and 1.5 days when fed on the previously mentioned diets, respectively. Table 1 & 2. It is obvious that significant effects on the duration of the protonymphal stage were recorded for type of applied diets for female ($F=9.613^*$, $P=0.00$), and for male ($F=6.146^*$, $P=0.00$), especially between sugar (cane + chitosan) and other diets at the same temperature ($27 \pm 2^\circ\text{C}$). Data showed that, the percentage of mortality in this stage reach to 75% when larva fed on chitosan only. Statistical analysis proved that dutonymphal stage was affected by types of food and significant differed especially between sugar cane and other diets. Female dutonymphal stage averaged 2.62, 4.08, 4.3 and 3.44 days at $27 \pm 2^\circ\text{C}$ and 65-70% R.H. when fed on (A piece of sugar cane), (A piece of sugar cane inoculated with pollen palm tree), (A piece of sugar cane inoculated with pollen palm tree +chitosan), (A piece of sugar cane + chitosan) ($F=17.313^*$, $P=0.00$). Male duration averaged 2.5, 3.67, 3.3 and 2.85 days when reared on the above mentioned diets, respectively ($F=3.504^*$, $P=0.00$). Data

in table 1&2 showed that, deutonymphal stage do not reach to adult when fed on chitosan.

The shortest development duration and life cycle for female (10.08 ± 0.31) days, while the longest duration was recorded in (sugar cane + pollen +chitosan) (13.16 ± 0.38) days ($F=15.786^*$, $P=0.00$) Table 1. In case of male, life cycle recorded shortest period when fed on sugarcane (10.10 ± 0.4) days and the longest (11.6 ± 0.59) days when feed on sugarcane + pollen when feed on sugarcane ($F=2.149^*$, $P=0.00$) Table 2. Adult female longevity was significantly.

Affected by the different diets when it was short in sugar cane +chitosan (10.5 ± 0.65) days, moderate in (sugar cane + pollen +chitosan) (13.72 ± 0.19) days, the longest female longevity was (22.25 ± 0.92) days recorded in sugar cane ($F=29.680^*$, $P=0.00$). Female life span was significantly affected by different diets, the shortest when fed on sugar cane +chitosan (20.58 ± 0.64) days, and was the longest when fed (34.6 ± 0.58) days ($F=39.866^*$, $P=0.00$) Table 3. The shortest male longevity and life span recorded in sugar cane +chitosan (12.57 ± 0.57) and (22.69 ± 0.64) days respectively. While the longest longevity and life span recorded in (sugar cane + pollen) (18.25 ± 0.56) and (29.85 ± 0.88) days ($F=21.212^*$, $P=0.00$) and ($F=13.750^*$, $P=0.00$) respectively Table 4 and Figs. 1, 2.

Fecundity of *Proctolaelaps gizaensis*

There were significant differences in the fecundity and sex ratio of *P. gizaensis* females according to feeding. The highest fecundity rate was recorded in Sugarcane + Pollen (88.9 ± 5.30 eggs/female and 4.72 ± 0.24 eggs/female/day), while the lowest fecundity rates recorded in Sugarcane + Chitosan (16.75 ± 0.97 eggs/female and 3.61 ± 0.52 eggs/female/day). Significant differences were reported in the fecundity rate between four artificial diets, at probability level 99%, the total number of eggs per a female ($F=42.078$, $P=0.000$), and the daily number of eggs deposited per female (eggs/♀/day) ($F=5.600$, $P=0.000$) (Table 5 & Figs. 1,2). The sex ratio of males and females among the progeny produced from rearing on different diets above mentioned. The present of female among the progeny reached to 61, 55, 50 and 52% at $27 \pm 2^\circ\text{C}$ and 65-70% R.H. respectively.

Life table parameters of *Proctolaelaps gizaensis*

Feeding on the four tested diets resulted in the highest value for *P. Gizaensis* fed Sugarcane of intrinsic rate of increase (r_m) was 0.195 ± 0.04 individuals/female/day; the net reproductive rate (R_0) was 34.329 ± 4.71 ; the finite rate of increase (λ) was 1.216 ± 0.28 , the shortest mean generation time (T) was 12.588 ± 2.59 days, the shortest doubling time (DT) was 3.56 ± 0.05 days and highest gross reproductive rate (GRR) was 63.01 ± 3.90 . The lowest value for *P. Gizaensis* fed (Sugarcane + Chitosan) intrinsic rate of increase (r_m) was 0.147 ± 0.07 individuals/ female/day; the net reproductive rate (R_0) was 6.384 ± 1.43 ; the finite rate of increase (λ) was 1.158 ± 0.67 , the longest mean generation time (T) was 12.588 ± 2.59 days, the highest doubling time (DT) was 4.71 ± 0.64 days and lowest gross reproductive rate (GRR) was 10.43 ± 1.50 (Table 6 & Figs. 1,2). The rate of survival (Lx) (percent of surviving females at the instant x) and the rate of female progeny per female (Mx) (number of female eggs lay per female per day) for *P. gizaensis* fed Sugarcane, Sugarcane + pollen, Sugarcane + pollen + Chitosan, Sugarcane + Chitosan and Chitosan in Figure (4).

DISCUSSION

The use of an artificial diet may represent a step toward more cost-effective rearing of generalist mites and other bio control agents (Abou-Awad *et al.*, 1992). Artificial diets can be an alternative to natural and factitious prey for the mass production of bio-control agents, reducing costs and facilitating automation of the production (Kennett and Hamai, 1980).

Our study revealed distinct effects of different artificial diets on bio ecological parameters of the predatory mite *P. gizaensis*. Our results show that *P. gizaensis* can feed and develop to the adult stage on all the tested diets (except on chitosan); however, pre-adult duration was different among tested diets. All immature stage when fed on chitosan could not complete their life cycle and all of them died before adulthood in the larval and protonymphal stages and also when exposed newly emerged of adults to chitosan as a food, it noticed that, the adults (females and males) were died without the females lay any eggs after 2 or 3 days a maximum and also the individuals changed to red mummies with transparent cuticle. Also, it was observed that, chitosan affected on sugarcane, because it absorb the humidity (sugar) from the sugarcane and change to sponge tissue. And also using chitosan with sugarcane increased the humidity inside the body of mite, so it increased in size.

Sugarcane reduced the duration of immature developmental time. This can be explained by deficiency of primary essential nutrients for growth and development in the basic artificial diet and this deficiency could be compensated by adding supplements (especially protein). The developmental time of *P. gizaensis* has been reported to be 12.56 ± 0.30 days and 88.9 ± 5.30 eggs /female on date palm pollens, this results agreement with (Khanamani *et al.*, 2017) which is mentioned that, pollens can be considered to be favorite food for the immature stages of *N. californicus* in mass-production system.

Katarzyna *et al.* (2015) recorded that, Pollen contains 22.7% of protein on average, including 10,4% of essential amino acids such as methionine, lysine, threonine, histidine, leucine, isoleucine, valine, phenylalanine, and tryptophan-carbohydrates occur in the pollen in the amount of 30,8% on average. sugars, mainly fructose and glucose, are present in this product in about 25,7% and all of them are very important to good development for immature stages and fecundity. According to the latest National Data, the average content of main ingredients in the air-dried pollen (at the temperature 40°C) amounts to such values as follows: proteins, 32,8%, including essential amino acids, 11,5%, and reducing sugars, 40,7%, including sucrose, 3,7%, lipids, 12,8%, vitamin C, 0,19%, β -carotene, 0,07%, and bio elements, 4,0%.

Differences in survival, developmental and reproductive characteristics were reflected in the life table parameters especially in the r value. The highest r value in our study were observed when *P. gizaensis* was fed with the diets Sugarcane + Pollen (35.076 ± 3.00) and Sugarcane (34.329 ± 4.71). These growth reproductive reported (50.76 ± 6.56) and (63.01 ± 3.90) respectively. However, these values were lower than those reported for *P. gizaensis* when fed on Sugarcane + Chitosan (10.43 ± 1.50). It is cleared that, pollen with sugarcane are more suitable diets for mass-rearing of *P. gizaensis* because it increased the total average of deposited

eggs and daily rate of their deposition. Fecundity was also affected by the type of food, being higher when female fed on sugarcane, compared to (48 and 16.8 eggs) when fed on food contain with chitosan.

In addition, interesting results were obtained with the alternative prey *Petrobia harti* (Ewing) and pollens of *Carpobrotus edulis* (L.) and *Scrophularia peregrina* L. (Ragusa et al., 2009).

CONCLUSION

The most suitable diet for *P. gizaensis* was sugarcane with pollen followed by sugarcane at 27±2°C and 65-70% R.H. for increasing female fertility.

However, further experiments are needed to develop a suitable artificial diet for providing higher reproductive performance for Melicharid mites. The diets should be as simple as possible but support a good population growth rate.

Table 1 : Post-embryonic developmental duration (days) of *Proctolaelaps gizaensis* females (mean ± SE), fed five diets; Sugarcane, sugarcane + pollen, sugarcane + pollen + chitosan, sugarcane + chitosan, and chitosan, under laboratory conditions 27±2°C and 65-70% R.H.

	Sugarcane	Sugarcane + Pollen	Sugarcane + Pollen + Chitosan	Sugarcane + Chitosan	Chitosan	F-test
N	14	12	11	11	8	
Egg	2.64±0.22 ^b	2.40±0.18 ^c	1.61±0.20 ^e	2.72±0.14 ^a	1.7±0.18 ^d	6.247*
Larva	3.31±0.24 ^b	2.75±0.25 ^c	3.36±0.20 ^a	1.81±0.18 ^c	2.25±0.25 ^d	8.276*
Protonymph	2.75±0.22 ^c	3.33±0.19 ^b	3.90±0.28 ^a	2.11±0.11 ^c	2.66±0.33 ^d	9.613*
Deutonymph	2.62±0.14 ^d	4.08±0.26 ^b	4.30±0.14 ^a	3.44±0.18 ^c	-----	17.313*
life cycle	11.32±0.45 ^c	12.56±0.30 ^b	13.16±0.38 ^a	10.08±0.31 ^d	-----	15.786*

Means within rows, followed by the same letter are not significantly different (Tukey).

(*) significant at $P \leq 0.05$

Table 2: Post-embryonic developmental duration (days) of *Proctolaelaps gizaensis* males (mean ± SE), fed five diets; Sugarcane, sugarcane + pollen, sugarcane + pollen + chitosan, sugarcane + chitosan, and chitosan, under laboratory conditions 27±2°C and 65-70% R.H

	Sugarcane	Sugarcane + Pollen	Sugarcane + Pollen + Chitosan	Sugarcane + Chitosan	Chitosan	F-test
N	10	10	10	10	8	
Egg	2.5±0.22 ^a	2.4±0.18 ^c	1.6±0.16 ^e	2.42±0.29 ^b	1.71±0.18 ^d	4.311*
Larva	2.5±0.17 ^c	2.6±0.18 ^b	2.8±0.20 ^a	2.14±0.14 ^d	1.5±0.29 ^c	4.677*
Protonymph	2.6±0.16 ^d	3.0±0.27 ^b	3.9±0.31 ^a	2.71±0.29 ^c	1.5±0.50 ^c	6.146*
Deutonymph	2.5±0.22 ^d	3.67±0.31 ^a	3.3±0.34 ^b	2.85±0.26 ^c	-----	3.504*
life cycle	10.10±0.4 ^c	11.60±0.59 ^a	11.60±0.47 ^a	10.12±0.45 ^b	-----	2.149*

Means within rows, followed by the same letter are not significantly different (Tukey).

(*) significant at $P \leq 0.05$

Table 3 : Adult female longevity and life span (mean ± SE) of *Proctolaelaps gizaensis* fed five diets; Sugarcane, sugarcane + pollen, sugarcane + pollen + chitosan, sugarcane + chitosan, and chitosan, under laboratory conditions 27±2°C and 65-70% R.H.

	Sugarcane	Sugarcane + Pollen	Sugarcane + Pollen + Chitosan	Sugarcane + Chitosan	F-test
N	11	10	11	9	
Pre-ovi position	2.64±0.15 ^b	1.3±0.15 ^c	1.18±0.12 ^d	2.75±0.36 ^a	15.477*
Ovi-position	18.9±1.1 ^a	18.8±0.41 ^b	9.63±0.24 ^c	5.12±0.54 ^d	39.770*
Post-ovi position	1.4±0.16 ^d	1.6±0.22 ^c	2.90±0.28 ^a	2.62±0.53 ^b	6.267*
Longevity	22.25±0.92 ^a	21.7±0.53 ^b	13.72±0.19 ^c	10.5±0.65 ^d	29.680*
Life span	33.57±0.92 ^b	34.6±0.58 ^a	26.88±0.39 ^c	20.58±0.64 ^d	39.866*

Means within rows, followed by the same letter are not significantly different (Tukey).

(*) significant at $P \leq 0.05$

Table 4 : Adult male longevity and life span (Mean ± SE) of *Proctolaelaps gizaensis* fed five diets; Sugarcane, sugarcane + pollen, sugarcane + pollen + chitosan, and sugarcane + chitosan under laboratory conditions 27±2°C and 65-70% R.H.

	Sugarcane	Sugarcane + Pollen	Sugarcane + Pollen + Chitosan	Sugarcane + Chitosan	F-test
N (replicates)	10	10	10	8	
Longevity	17.4±0.91 ^b	18.25±0.56 ^a	12.7±0.30 ^c	12.57±0.57 ^d	21.212*
Life span	27.5±1.08 ^b	29.85±0.88 ^a	24.3±0.53 ^c	22.69±0.64 ^d	13.750*

Means within rows, followed by the same letter are not significantly different (Tukey).

(*) significant at $P \leq 0.05$

Table 5 : Hatchability percentage, fecundity and sex ratio of *Proctolaelaps gizaensis* females fed five diets; Sugarcane, sugarcane + pollen, sugarcane + pollen + chitosan, and sugarcane + chitosan under laboratory conditions 27±2°C. and 65-70% R.H.

	Sugarcane	Sugarcane + Pollen	Sugarcane + Pollen + Chitosan	Sugarcane + Chitosan	F-test
N (replicates)	10	10	11	8	
Total fecundity	84.7±6.19 ^b	88.9±5.30 ^a	48.0±2.47 ^c	16.75±0.97 ^d	42.078 [*]
Daily fecundity	4.44±0.37 ^c	4.72±0.24 ^b	4.99±0.26 ^a	3.61±0.52 ^d	5.600 [*]
Egg hatchability	92.8%	85.7%	84.6%	73.3% [^]	
sex ratio	61.00 %	55.00 %	50.00 %	52.00%	

Means within rows, followed by the same letter are not significantly different (Tukey).

(*) significant at $P \leq 0.05$

Table 6 : Demographic parameters (± SE) of *Proctolaelaps gizaensis* females fed five diets; Sugarcane, sugarcane + pollen, sugarcane + pollen + chitosan, and sugarcane + chitosan under laboratory conditions 27±2°C. and 65-70% R.H.

	Sugarcane	Sugarcane+ pollen	Sugarcane+ pollen+Chitosan	Sugarcane + Chitosan
R_0	34.329 ± 4.71 b	35.076 ± 3.00 a	20.650 ± 3.31 c	6.384 ± 1.43 d
T	18.157 ± 3.24 b	19.910 ± 3.84 a	17.3058 ± 2.50 c	12.588 ± 2.59 d
r_m	0.195 ± 0.04 a	0.180 ± 0.06 b	0.175 ± 0.05 c	0.147 ± 0.07 d
λ	1.216 ± 0.28 d	1.195 ± 0.64 a	1.191 ± 0.19 b	1.158 ± 0.67 c
GRR	63.01 ± 3.90 a	50.76 ± 6.56 b	25.25 ± 1.20 c	10.43 ± 1.50 d
D(T)	3.56 ± 0.05 d	3.88 ± 0.22 c	3.96 ± 0.86 b	4.71 ± 0.64 a

Means within rows, followed by the same letter are not significantly different (Tukey, $P \leq 0.05$).

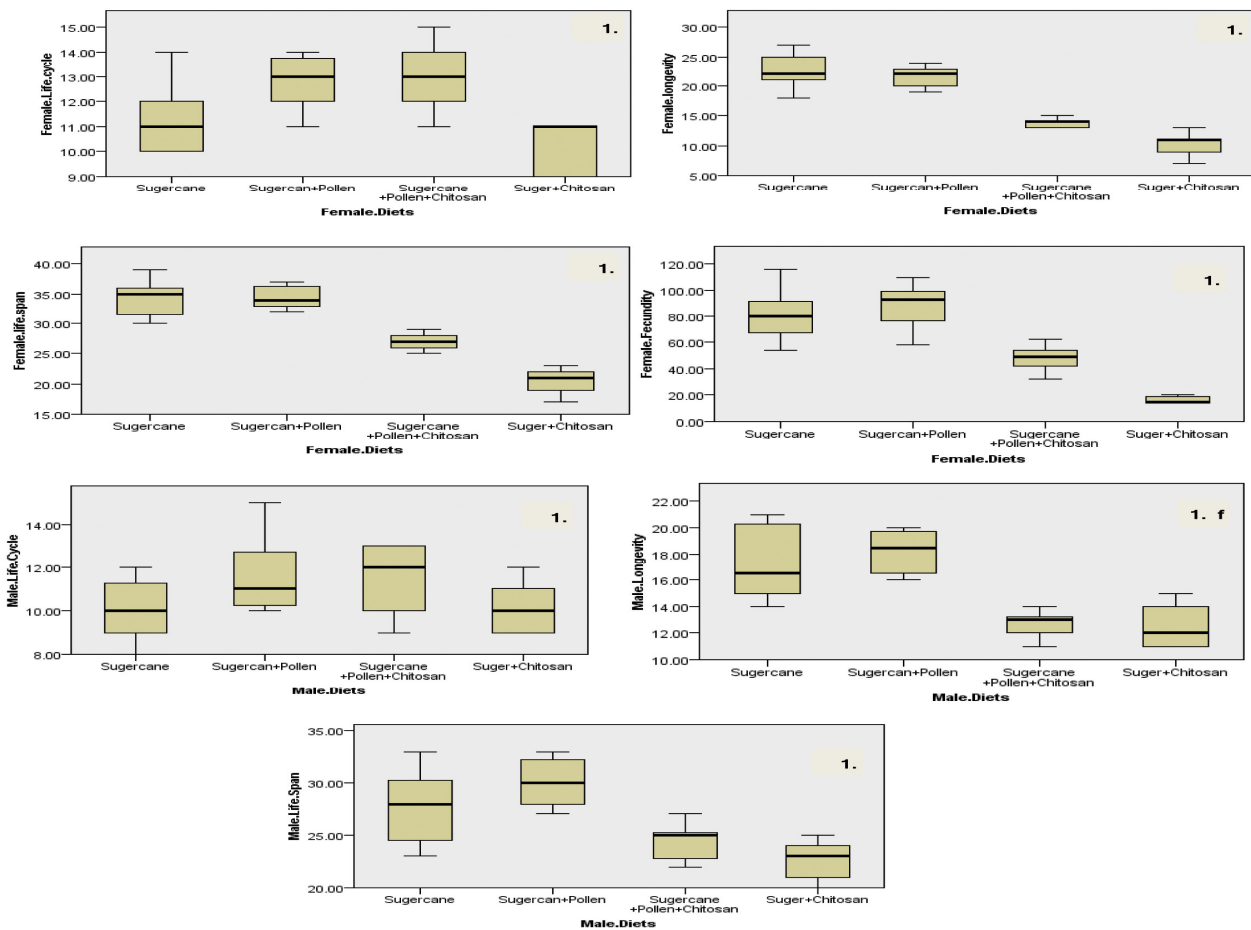


Fig. 1 : Independent samples (Kruskal-Wallis) test, a hypothesis test of five different diets (Sugarcane, Sugarcane+pollen, Sugarcane + pollen + Chitosan, Sugarcane + Chitosan and Chitosan) effect on *Proctolaelaps gizaensis sp. nov.* a) female life cycle, b) female longevity, c) female life span, d) female fecundity, e) male life cycle, f) male longevity and g) male life span, at significant level = 95%.

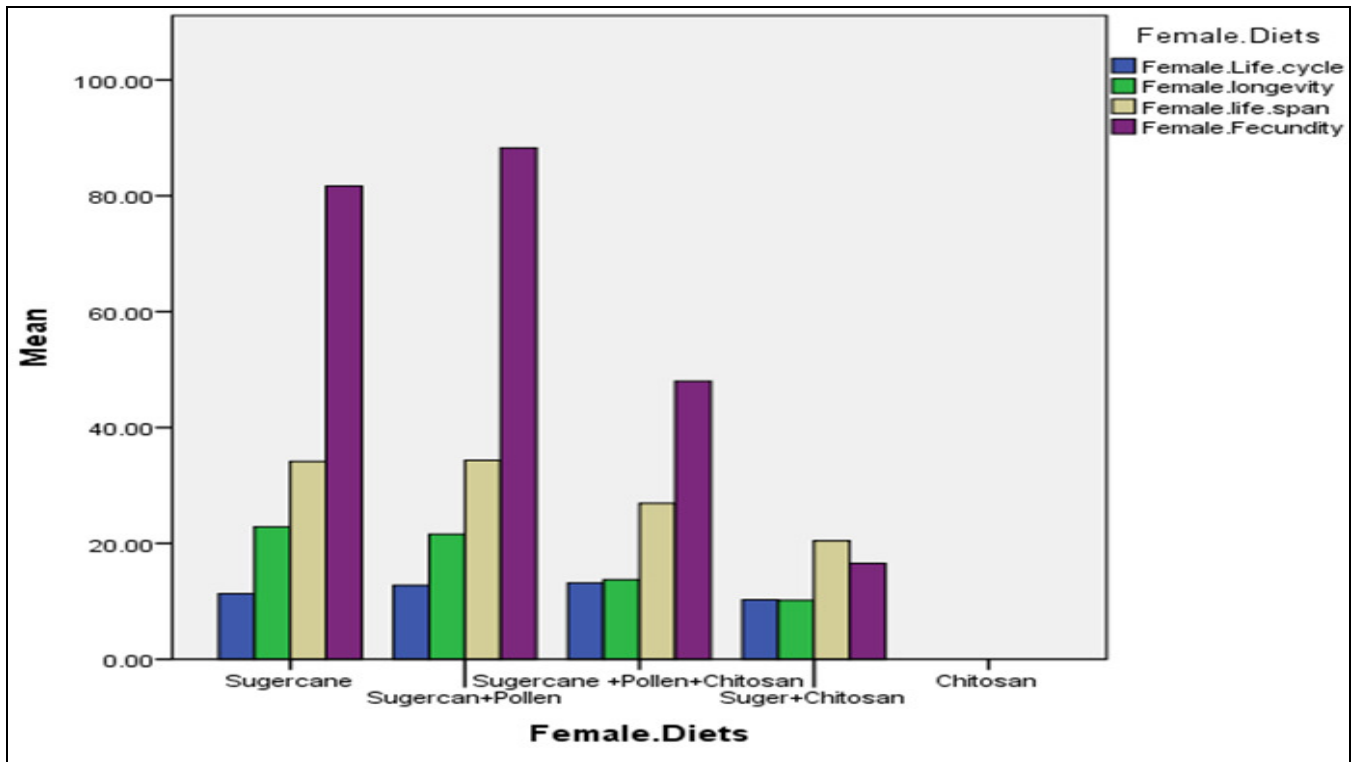


Fig. 2 : Effect of alternative diets; Sugercane, Sugercane + pollen, Sugercane + pollen + Chitosan, Sugercane + Chitosan and Chitosan on *Proctolaelaps gizaensis* post-embryonic developmental duration (days), adult female longevity (days), female life span (days) and female total fecundity (eggs/♀), under laboratory conditions at $27\pm 2^{\circ}\text{C}$. and 65-70% R.H..

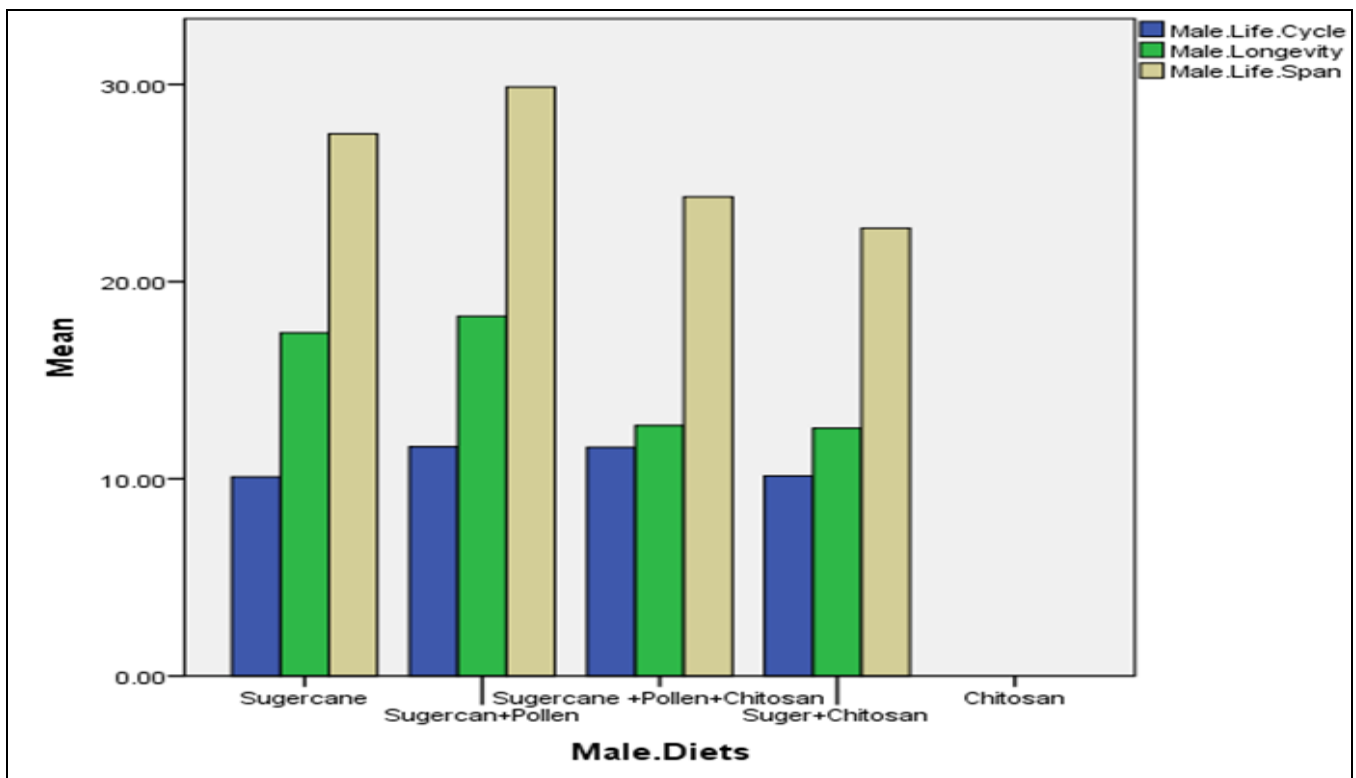


Fig. 3 : Effect of alternative diets; Sugercane, Sugercane + pollen, Sugercane + pollen + Chitosan, Sugercane + Chitosan and Chitosan on *Proctolaelaps gizaensis* post-embryonic developmental duration (days), adult male longevity (days) and male life span (days), under laboratory conditions at $27\pm 2^{\circ}\text{C}$. and 65-70% R.H..

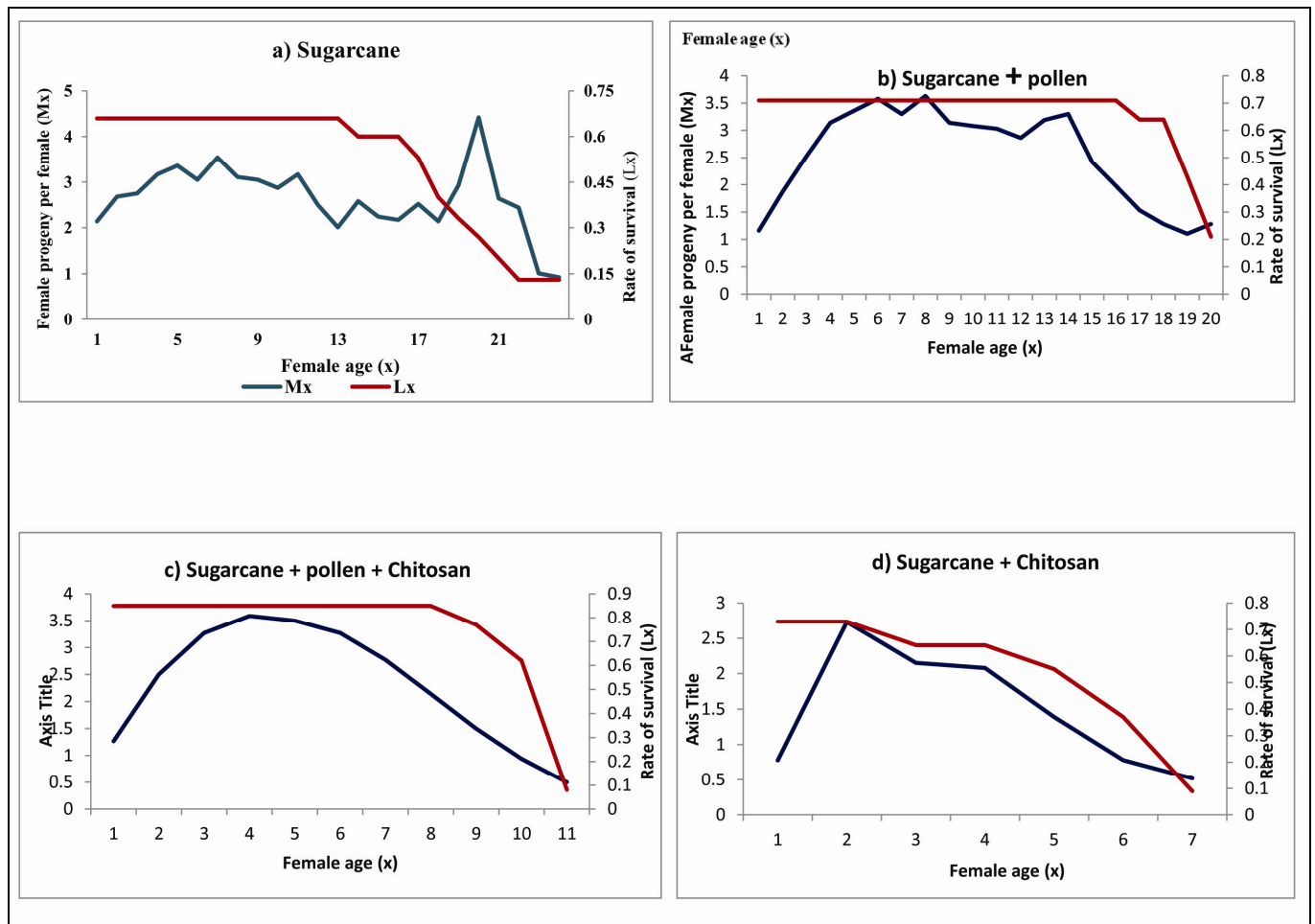


Fig. 4 : Female progeny per female (Mx) and Rate of survival (Lx) of *Proctolaelaps gizaensis* on four alternative diets; Sugarcane, Sugarcane + pollen, Sugarcane + pollen + Chitosan, Sugarcane + Chitosan and Chitosan at 27±2°C, 65-70% RH

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

SFA designed the study plan and revised manuscript, RAM conducted the methodology, obtain data and write the draft of manuscript, MFH and HAT were the head supervisors who arrange and revise the whole plan of work,

Drs MFH and HAT were also read and confirm the final draft of research.

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