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EXPERIMENTAL EFFECT OF FEEDING ON *RICINUS COMMUNIS* AND *BOUGAINVILLEA GLABRA* ON THE DEVELOPMENT OF THE SAND FLY *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE) FROM EGYPT

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Abstract

Plants are promising sources of agents useful for the control of vectors of human diseases including leishmaniasis. The effect of *Ricinus communis* (Euphorbiaceae) and *Bougainvillea glabra* (Nyctaginaceae), on transmission of leishmaniasis was investigated using them as diets for *Phlebotomus papatasi* to monitor their effect on life-history traits. *P. papatasi* were allowed to feed separately on both plants then offered a blood-meal. Fed-females were observed daily for egg-laying and subsequent developmental stages. *P. papatasi* was able to feed on *B. glabra* (29.41% females and 46.30% males) and *R. communis* (5.80% females and 10.43% males). 34.28% of females died within 24-48 hours post-feeding on *R. communis*, whereas, it was 16.5% in females fed on *B. glabra*. Overall fecundity of surviving females was reduced compared to controls, reared on standard laboratory diet; however there was no effect on the sex ratio of progeny. Female *P. papatasi* in the control group had significantly longer life span compared to plant-fed group. Feeding on these plants not only decreased sand fly survival rates but incurred negative effects on fecundity. Findings indicate that planting high densities of *R. communis* and *B. glabra* in sand flies-endemic areas will reduce population sizes and reduce the risk of *Leishmania major* infections.

Keywords: *Phlebotomus papatasi* - *Ricinus communis* - *Bougainvillea glabra* - Egypt

Introduction

Phlebotomus papatasi (Scopoli) has a broad geographical distribution, and is the principal vector of *Leishmania major* throughout the Mediterranean basin, Middle East, Central Asia, and North Africa (Feliciangeli, 2004). Sand fly distribution within that range is determined by local environmental factors such as frequency of precipitation, land surface temperature, Normalized Difference Vegetation Index, physical barriers, habitat availability and the distribution and abundance of the sylvan reservoirs (Peterson and Shaw, 2003). *P. papatasi* was first reported in Egypt in 1917, and has been shown by several studies to be the predominant species in the country (Willcocks, 1917; Hoel *et al,* 2007). Cutaneous lesions resulting from infection by *L. major* typically self-heal within several months in immune-competent patients and injection with intra-lesional antimonals are often a prescribed treatment of the parasites (Porrozzi *et al,* 2004; Alborzi *et al,* 2006).

Plants have always been an important source of active compounds against insects and they have developed several compounds that are still used in modern day insecticides.

Insect evolution is characterized by rapid adaptation with selective pressures exerted by environment and is closely related to the evolution of flowering plants. The adaptations include feeding on plant sap (Moran *et al,* 2005; Janson *et al,* 2008). Studies have shown that some phytochemicals have a toxic effect on insect adults and larvae by interfering in their growth, development and/or reproduction, or by producing attractive or repellent scents (Amóra *et al,* 2009). The Neem *Azadirachta indica* oil had a repellency activity against *P. papatasi* when used for three days at concentrations of 1% and 2 % (Srinivasan and Kalyanasundaram, 2001) that was reported later to be due to the active ingredient azadirachtin, that was shown to kill sand fly larvae in laboratory experiments (Andrade-Coelho *et al,* 2009).

Other plant extracts are highly toxic to the sand fly *Lutzomyia longipalpis*, such as dried leaf extracts of *Antonia ovata* (Loganiaceae) and *Derris amazonica* (Papillionaceae) killing 80% and 100% of females, respectively, 72 h after exposure (Luitgards-Moura *et al,*2002). The repellency effect of the garlic (*Allium sativum*) oil was also evaluated on *P. papatasi* females, at 1%, the oil had a repellency effect of 97% (Valerio and Maroli, 2005). Some plants, such as *Solanum jasminoides*, *Ricinus communis*, *Bougainvillea glabra*, are toxic to adult sand flies and could therefore represent a readily available alternative to the commercial insecticides for sand fly control (Schlein *et al,* 2001). Certain plants (e.g.; *Capparis spinosa*, *Ricinus communis*, *Bougainvillea glabra* and *Solanum luteum*) used as sources of sugar by sand flies are toxic to *L. major*, and these are also able to kill sand flies **(**Schlein and Jacobson, 1994; Jacobson and Schlein, 1999; Schlein and Jacobson, 1999). Host plant is a key determinant of the sand fly life cycle (Schlein *et al,* 2001) and plants diet can also affect insect reproductive strategies, and the life history traits. Male sand flies feed mainly on plant sap as their major source of nutrients, however, females require a blood meal to support the egg development, and use the sugar meal as a source of the energy (Killick-Kendrick, 1999; Benkova and Volf, 2007). *R. communis* is a natural vegetation of the Egyptian fauna, while *B. glabra* is cultivated.

The present study investigates the effect, if any, of 1) feeding on two different plant species, *R. communis* and *B. glabra* and accessing resulting mortality, 2) Interference of feeding on the two plants with *P. papatasi*'s survival and fecundity, and 3) Monitoring subsequent changes in the sand fly's life cycle. This study was conducted as an initial step to highlight the use of natural sources as plants to control diseases-causing vectors in endemic populations.

Materials and Methods

The laboratory colony was initiated in 1989 using adult wild caught sand flies from El-Arish in the North Sinai Governorate in Egypt by the U.S. Naval Medical Research Unit No. 3 (NAMRU-3); sand flies used in the current experiments were the 106th generation from the initial colony. Rearing was done in NAMRU-3 according to standard methods of Modi and Tesh (1983) with minor changes, at a stable monitored temperature of 27 ± 1°C, 70-80% relative humidity (RH), and a 12:12 (Light: Dark) photoperiod. Syrian golden hamsters (*Mesocricetus auratus)**, with an average weight of 160±40 g, were used as the blood-meal source for colony rearing. Prior to sand flies feeding process, hamsters were previously anaesthetized by the intra-peritoneal (IP) injection of Ketaset® (0.1 ml/100 g Body Weight, Fort Dodge®, USA). After the blood meal, fed females were separated to a new cage, given a 24 h recovery period and then aspirated individually into a 50 ml polymethyl-pentene rearing vials at the controlled (70-80%) relative humidity to oviposit.

Plant seeds originating from South of Egypt were brought to the Botanical Garden of Ain Shams University, where they had been grown without fertilizers, pesticides or other chemical treatments. Branches of *R. communis* and *B. glabra* were readily available for feeding processes. Freshly-harvest branches were cut under water and transferred to the laboratory within 1 h. To track feeding by sand flies, branches were first suffused with blue food dye (Kamina, Egypt) by placing their stems in 200 ml Erlenmeyer flasks filled with 1% dye and 0.127 M sucrose solution, then left for 24-48 h as described by Schlein and Jacobson (2008).

For each sand fly feeding trial of the seven trials done for this study, 100-150 newly emerged *P. papatasi* (1-3 days old) adults were placed in cages containing blue dyed-branches of either *R. communis* or *B. glabra* for 24-48 h. Sand flies used in the feeding trials, were a total of 2130 flies, of which, 996 flies were allowed to feed on *R. communis*, 594 flies were allowed to feed on *B. glabra* and 540 flies were used as controls and were fed 30% sucrose solution. Flies were monitored daily to determine if feeding had occurred; feeding was detected by the presence of blue food-dye in the guts of fed *P. papatasi* (Fig. 1). For control experiment, female and male adults of the same age were fed on 30% sucrose solution and received a blood meal at the same time with the experimental groups. Plant-feeding trials covered a period of 13 months of the study, during which a feeding trial was repeated every 1.5 month, which resemble a sand fly's complete life cycle.

To identify which sand flies had fed on the plants; individuals were aspirated from the feeding cages, anesthetized using CO_2 and observed on a cold plate to detect the presence of the blue marker dye (Fig. 1). Successful feeders were released into new cages, starved for 2-4 h then allowed to feed on Syrian golden hamster as the blood meal source using the same blood feeding procedure and precautions as mentioned above. Each blood-fed female sand fly was separated in rearing vial, where it was observed daily for oviposition. Each rearing vial was observed daily to measure life-history parameters for each stage of the life cycle, starting with counting the numbers of oviposited eggs, hatched larvae, developed pupae and finally adults. The timing of developmental stages was also recorded. Flies were kept under controlled insectary conditions throughout the whole study. Standard batches of larval food, prepared as described by Modi and Tesh (1983) were used to maintain the survival of hatched eggs.

Statistical analysis: The statistical analysis of the data was performed using the one-way ANOVA, by GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). Normal (Z-test) for proportions was applied to access the influence of feeding on *R. communis* or *B. glabra* and the rate of mortality in the flies. Results were expressed as mean ± SE and considering 95% as significance level between averages. "*Post Hoc*" Tukey's HSD test for the multiple comparisons was utilized to ascertain the extent of the difference made by different plant meal sources on each variable of the life-history parameters of the sand flies. The control group was compared against both treatments of *R. communis* and *B. glabra*.

Results

A total of 2130 flies (1279 females and 851 males) were allowed to feed on *R. communis*, *B. glabra* or 30% sucrose solution. A total of 996 flies (603 females and 393 males) were allowed to feed on *R. communis*, of these, 35 females (5.80%) and 41 (10.43%) males were able to feed. whereas, a total of 594 flies (391 females and 203 males) were allowed to feed on *B. glabra*, of these, 115 females (29.41%), and 94 males (46.30%) were able to feed. For the 540 flies (285 females and 255 males) that were allowed to feed on 30% sucrose solution, 265 females (93%) and 228 males

(89.41%) were able to feed (Table 1). Sand fly feeding rates varied significantly among the three sources (*R. communis*, *B. glabra* and control; F= 5.26, df= 2, P<0.05). The present findings showed that there was a noticeable difference in the feeding of *P. papatasi* sand flies on *B. glabra* and *R. communis*, with *B. glabra* being the highest, either in female or male sand flies. Some sand flies died 24-48 hours post-feeding on the plants, notably after feeding on *R. communis* where the mortality rate was 34.28% (12 out of 35) for females and 14.63% (6 out of 41) for males (Fig. 2). Mortality rates were 16.52% (19 out of 115), and 24.46% (23 out of 94) for females and males, respectively after feeding on *B. glabra*. Mortality rates did not exceed 1 % for males (1/228, 0.43%) or females (1/265, 0.37%) fed on the control solution. The mortality rates in either female or male *P. papatasi* were highly significant (p<0.005) after feeding on *R. communis* and *B. glabra* when compared to morality rates in sand flies due to feeding on the 30% sucrose solution, by the normal test (Z-test) for proportions.

Phlebotomus papatasi females fed on sugar solution and blood laid significantly more eggs (43.49±3.704) than those fed on *R. communis* or *B. glabra* (Table 2) which retained fewer eggs after oviposition. Few females fed on *R. communis* survived till the oviposition (11 out of 35). These females laid a mean ±SE of (9.34±3.027) eggs. A similar decrease in fecundity occurred in the sand fly *P. papatasi* fed on *B. glabra*. The females fed on *Bougainvillea* laid an average number±SE of eggs of (29.80±2.903) which is more than the eggs laid by the female fed on *Ricinus,* however, eggs laid in both cases were fewer than those laid by females which received a normal diet regimen (Sugar 30% and blood). *Post hoc* (Tukey HSD) analysis showed complete significant difference (P<0.05) in the mean number of eggs laid in all plant treatment groups and also in the control.

Sand flies feeding on *R. communis* or *B. glabra* produced fewer larvae compared to control groups (P< 0.005, Table 2). The number of the larvae was reduced to a mean of (6.34±2.225) and (20.71±2.606) larvae for *R. communis* and *B. glabra*, respectively, whereas, the mean number of larvae in control females was (29.43±3.649) (Table 2). *Post hoc* analysis showed significant difference (P<0.05) in mean number of hatched eggs in *R. communis* against *B. glabra* and control groups, thus producing fewer fragment numbers of larvae. Similar observations were reported for the larvae pupated into the next pupal stage; Fewer numbers of pupae were developed for the females fed on both exotic plants, with mean number of pupae of (5.57±2.000) and (16.43±2.118) for the *R. communis* and *B. glabra*, respectively, whereas, a higher number of pupae populations were developed in the control group with a mean of (25.23±3.471) (P<0.005). *Post hoc* analysis showed a significant reduction in the number of larvae, and pupae that revealed the effect of exotic plant feeding by the mother on the progeny's development to larval and pupal stages. The significant difference (P<0.05) in mean number of pupae was detected in *R. communis* against *B. glabra* and control group.

There was a difference in the number of males, and females emerging from pupae developed from females that received the *R. communis* (4.91±1.762) or *B. glabra* (14.29±1.828) treatments compared with the control (23.11±3.229). The number of males and females was highly reduced particularly upon feeding on *R. communis* treatment. *Post hoc* analysis showed significant difference (P<0.05) in adult emergence in *R. communis* against *B. glabra* and control groups. There was no difference in the emerging adult sex ratio (Female: Male) in each treatment and was found close to 1: 1 ratio in all treatments.

The duration of the life cycle in *P. papatasi* females fed on sugar solution and

blood was significantly longer than the life cycles of those fed on the plants; *R. communis* or *B. glabra* (Table 3). In females

Table 1: Total number and percentage of feeding of *P. papatasi* sand flies on *Ricinus communis, Bougainvillea glabra,* and 30% sucrose solution (control)

Type of meal	Total number of flies per Experiment			Fed females (%)	Fed males (%)
	Female	Male	Total		
R. communis	603	393	996	35 (5.8)	41(10.43)
B. glabra	391	203	594	115 (29.41)	94 (46.3)
Control (30% sucrose solution)	285	255	540	265 (93)	228 (89.41)

Table 2: Life-history consequences of female *P. papatasi* sand fly after feeding on *R. communis*, *B. glabra* or 30% sucrose solution

Life-history stage/ Type of meal	*Ricinus communis* Mean number ± SE	*Bougainvillea glabra* Mean number ± SE	30% sucrose soln. Mean number ± SE	F-value	P-Value
No. of laid eggs	9.34 ± 3.027	29.80 ± 2.903	43.49 ± 3.704	28.291	<0.005
No. of larvae	6.34 ± 2.225	20.71 ± 2.606	29.43 ± 3.649	16.186	< 0.005
No. of pupae	5.57 ± 2.000	16.43 ± 2.118	25.23 ± 3.471	14.164	< 0.005
No. of adults	4.91 ± 1.762	14.29 ± 1.828	23.11 ± 3.229	14.735	< 0.005
No. of females	2.91 ± 1.083	6.91 ± 0.945	11.83 ± 1.741	11.740	< 0.005
No. of males	2.00 ± 0.691	7.37 ± 1.019	11.29 ± 1.557	16.553	< 0.005

F: ratios

Table 3: Effect of feeding on *R. communis*, *B. glabra* or 30% sucrose solution on pre-oviposition, oviposition, larval, pupal, life cycle, and egg incubation periods

Duration/Type of meal	*Ricinus communis* Mean duration ± SE	*Bougainvillea glabra* Mean duration ± SE	30% sucrose soln. Mean duration ± SE	F-Value	P-Value
Pre-oviposition duration	2.89 ± 0.784	8.97 ± 0.637	10.20 ± 0.122	44.449	< 0.005
Oviposition duration	0.43 ± 0.131	1.11 ± 0.114	1.51 ± 0.103	22.03	< 0.005
Larval duration	4.83 ± 1.412	15.09 ± 1.086	17.46 ± 0.898	33.958	< 0.005
Pupal duration	2.74 ± 0.804	6.89 ± 0.500	8.86 ± 0.496	25.593	< 0.005
Life Cycle	7.57 ± 2.209	22.03 ± 1.561	26.40 ± 1.300	32.345	< 0.005
Egg incubation period	1.77 ± 0.522	4.97 ± 0.359	5.74 ± 0.305	26.932	< 0.005

F: ratios

fed on *R. communis*, the pre-oviposition time was (2.89±0.784) days, and the oviposition duration was (0.43±0.131) days, whereas, the larval duration, and pupal duration were (4.83±1.412) and (2.74±0.804) days, respectively. On the other hand, females fed on *B. glabra* had longer pre-oviposition and oviposition time (8.97±0.637) and (1.11±0.114), respectively if compared with *R. communis*, also the same applies for larval and pupal durations (15.09±1.086) and (6.89±0.500) days, respectively. Egg incubation period was shorter in the treatment with *R. communis* (1.77±0.522) than *B. glabra* (4.97±0.359) days. Overall life cycle duration was significantly different in *R. communis* than the control treatment (7.57±2.209) and (26.40±1.300) days, respectively. Also, life cycle was significantly different in *B. glabra* treatment (22.03±1.561) days. *Post hoc* analysis showed significant differences ($P<0.05$) in oviposition duration between both plant meals and the control, while it showed significant difference ($P<0.05$) in pre-oviposition duration, egg incubation period, larval duration, pupal duration and life cycle for *R. communis* against *B. glabra* and control group.

Discussion

There is not a lot of attention directed at research to assess influence of plant meals on *P. papatasi* egg production and developpment of the sand fly throughout the life cycle led to the current study which aims to understand the post-feeding effect of two control-candidate plants *R. communis* and *B. glabra* on the survival and development of *P. papatasi*, the vector for zoonotic cutaneous leishmaniasis in the Mediterranean region. To our knowledge and according to the literature reviews, no studies had examined such effect and the present results support the idea that strategically chosen plant species can be used as means of local sand fly control. *R. communis* and *B. glabra* have been proven to be attractive and of preference when they were available in the vicinity of *P. papatasi* sand flies in natural settings (Schlein and Yuval, 1987; Schlein and Jacobson, 1994), they also previously reported feeding on branches of *R. communis* by 71% of *P. papatasi* in the laboratory. In the same context, feeding on branches of *R. communis* was reported in 62.1% of *P. papatasi* and 54.2% on *B. glabra* in the field. Schlein *et al,* (2001) reported feeding for both *P. papatasi* sexes. The latter concluded that there was no significant difference in the overnight feeding rates between male and female flies. Our findings agreed with the previously mentioned research in the proven feeding of *P. papatasi* on both plants, however indicated that 5.80% and 29.41% of female flies of *P. papatasi* were able to feed on the *R. communis* and *B. glabra*, respectively. Feeding rates of males were higher than that of females (10.43% and 46.30% for *R. communis* and *B. glabra*, respectively), and also showing higher feeding rates for *B. glabra* meal against *R. communis* in both sexes. Sand flies fed on either plants suffered mortality within 1-2 nights post-feeding. Mortality rates were 34.28% for females and 14.63% for males after feeding on *R. communis*, and 16.52% for females and 24.46% for males after feeding on *B. glabra*. These results agree with Schlein *et al,* (2001), who observed that one night feeding on branches of *R. communis* or *B. glabra* drastically shortened the life span of *P. papatasi*.

Depending on the availability of host and the type of the blood meal, there is great influence on biological parameters of the sand fly especially on the fecundity (Noguera *et al,* 2006). The present data demonstrated that there was an effect of feeding of flies on the two plants on such biological parameters including fecundity, number of larvae, and number of pupae produced by each female in the study. This pattern has not been previously described to monitor the influence of the plant meal on

the sand fly, according to literature. Few females were able to survive for the oviposition post-feeding on *R. communis* or *B. glabra*, and these females laid significantly fewer eggs in comparison to those laid by the females fed on the 30% sucrose solution.

Hatchability, larval and pupal development of the progeny were also drastically influenced post-feeding on either plant meals, reduced number of larvae and pupae were observed following feeding on *R. communis* or *B. glabra* than on sucrose solution. Previous reports had communicated the effectiveness of aqueous extracts from leaves of *R. communis* as larvicides against 2nd and 4th instar larvae of four mosquito species, *Culex pipiens*. *Aedes caspius*, *Culiseta longiareolata* and *Anopheles maculipennis* (Aouinty *et al,* 2006). A similar situation was reported by Kumar *et al,* (2012), where crude extracts of *R. communis* possessed significant larvicidal potential against *Aedes aegypti* larvae. Leaf extracts of *R. communis* were tested against *Rhipicephalus microplus* ticks. The extract significantly affected the mortality rate of ticks in a dose-dependent manner and the survivors laid fewer eggs. When they tested the efficacy of solvent guided fractions of the extract, among the four fractions, mortality was only recorded in ticks treated with n-butanol and hexane guided fractions but not with the chloroform and water soluble fractions. The highest oviposition inhibition was observed in the chloroform guided fraction. In addition, the extract showed significant efficacy against diazinon, deltamethrin and multi-acaricide resistant ticks (Ghosh *et al,* 2013).

Adult emergence of males and females was highly reduced, particularly of *P. papatasi* fed on *R. communis*. Females fed on *B. glabra* had longer pre-oviposition, larval and pupal durations if compared with *R. communis*. Similarly, the eggs incubation period was longer after feeding on *B. glabra* compared to *R. communis*. The detailed recording of each developmental stage of *P. papatasi* from the Sinai region of Egypt based on plant intake has not been previously well described. The prior observational studies of adult sand flies from the vicinity of plants had only suggested a possible control relationship between sand flies and plants. The effectiveness of the two tested plants in reducing mean numbers of immature stages and progeny's adult emergence of *P. papatasi* is likely due to the presence of secondary metabolites.

Phytochemical analysis of methanolic extractions of *R. communis* leaves has reported a variety of tannins, saponins, flavonoids, alkaloids and terpenoids (Rondon *et al,* 2011). Finger printing profile of *R. communis* leaf extract showed the presence of quercetin, gallic acid, flavone and kaempferol (Ghosh *et al,* 2013). Quercetin, a flavonoid isolated from leaves of *R. communis* was reported to interfere with the iron metabolism in *L. donovani*, and was found to reduce splenic burden in infected hamsters by 75-95% (Sen *et al,* 2008). Fractions of *R. communis* leaf extracts confirmed the presence of saponins, which are steroid substances with various biological activities, saponins increase membrane permeability of the parasite leading to their lysis (Rondon *et al,* 2011).

Ethanolic extractions of *B. glabra* leaves have been reported to contain a variety of tannins, saponins, flavonoids, steroids, alkaloids, anthraquinones, glycosides, proteins, reducing sugar and starch (Joshny *et al,* 2012). Also, quercetin, kaempferol and isohamnetin have been detected in *B. glabra* extracts (Su-Xia *et al,* 2010). Anthraquinones, are secondary metabolites belonging to the group of quinones and are found in fractions of *B. glabra* leaves, were reported to inhibit the synthesis of nucleic acids and therefore inhibit the formation of proteins in bacteria (Levin *et al,* 1988), which was believed to be the cause of the effect on *L. infantum* promastigotes (Rondon *et al,* 2011).

Controlling sand fly populations is challenging. The main reason is that the immature stages are dispersed and inaccessible as in rodent burrows. Barrier treatments with insecticides to control adult populations are the most common method used. The latter should be repeated at frequent intervals during the high seasons to manage the long life span of the sand flies; however, this is neither economically nor environmentally sound. In addition to complications resulted from the development of insecticide resistance. The present study investigated the first steps of an alternative approach, one whereby the plant communities in areas with high populations of sand flies could be cultivated to favor species whose presence would naturally decrease the sand fly population. Plants are rich sources of complex, yet unexplored mixtures of bioactive phytochemicals that can be further exploited to act as vector control measure tools. We and others, Schlein and Jacobson (1999) have shown that although *R. communis* and *B. glabra* are suitable sugar sources for *P. papatasi*, this feeding incurs a strong, deleterious effect on sand fly survival. The present experiments have further shown a significant effect on egg production, larval and pupal development and adult emergence. Although further studies are required to determine the precise impact of creating barrier zones with such plants (*R. communis* and *B. glabra*), our results to date present a plausible, cost effective and sustainable alternative to insecticides. Consequently, it can be considered an important asset in all the Integrated Vector Control Management Programs aimed at reducing *Leishmania* infections by decreasing their vectors population.

Continued studies investigating the sand fly-plant relationship with special emphasis on the development of *Leishmania* spp. is highly needed for better understanding of effective natural tools that could be widely used in sand fly control measures and to decrease the risk of leishmaniasis in the endemic areas.

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Legends for figures

Fig. 1: A blood-fed female *P. papatasi* showing blue-dyed gut indicating plant meal uptake

Fig. 2: Number of fed and fed-dead *Phlebotomus papatasi* after feeding on *Ricinus communis*

Fig. 3: Number of fed and fed-dead *Phlebotomus papatasi* after feeding on *Bougainvillea glabra*

Fig. 4: Number of fed and fed-dead *Phlebotomus papatasi* after feeding on 30 % sucrose solution

Fig. 1: A blood-fed female *Phlebotomus papatasi* showing blue-dyed gut indicating plant meal uptake

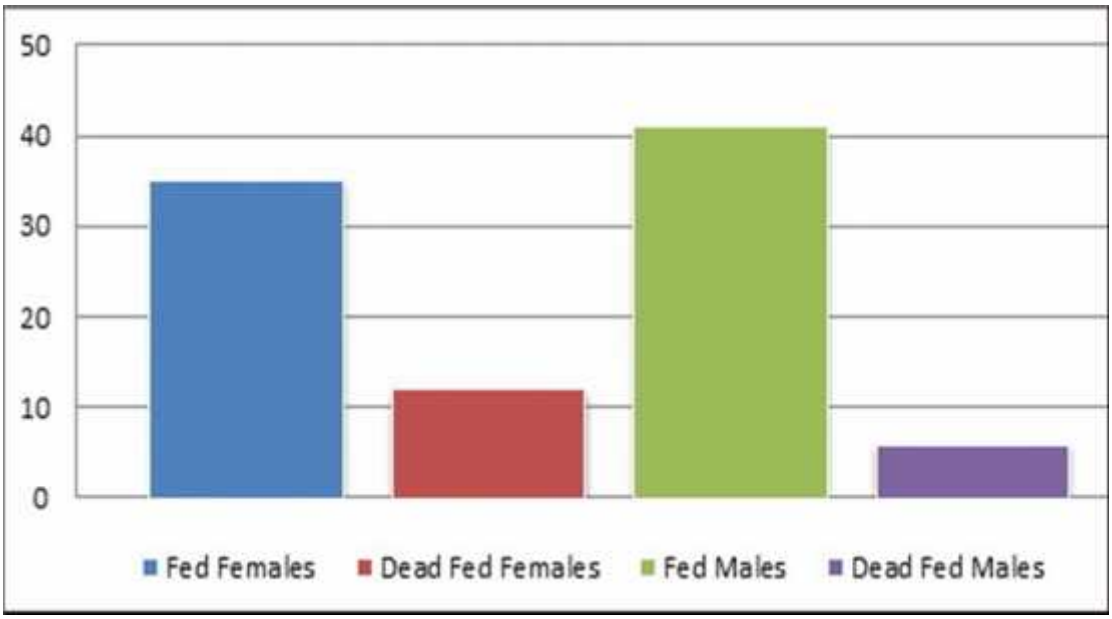


Fig. 2: Number of fed and fed-dead *Phlebotomus papatasi* after feeding on *Ricinus communis*

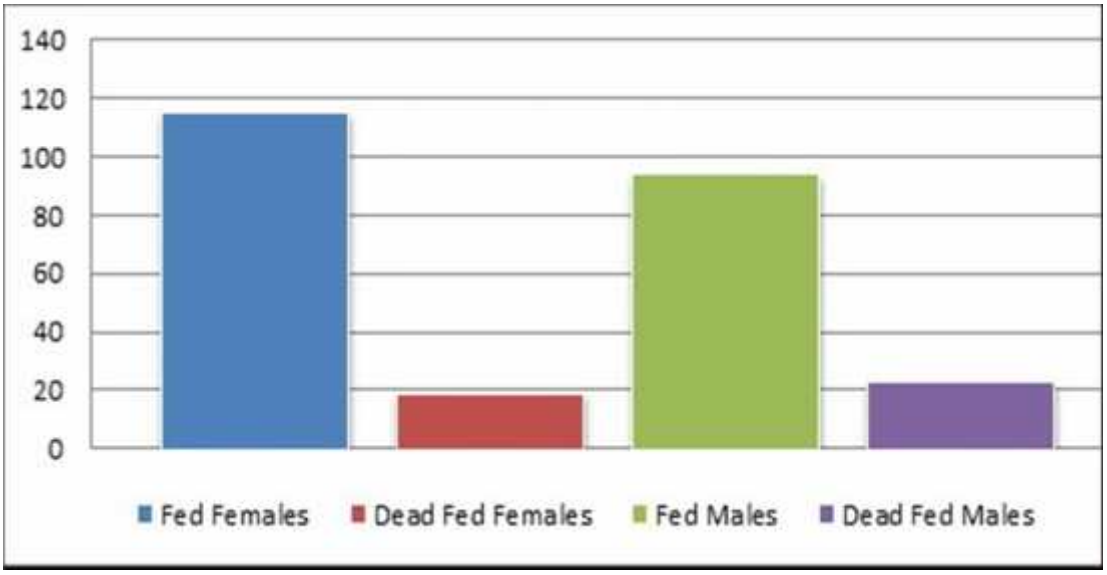
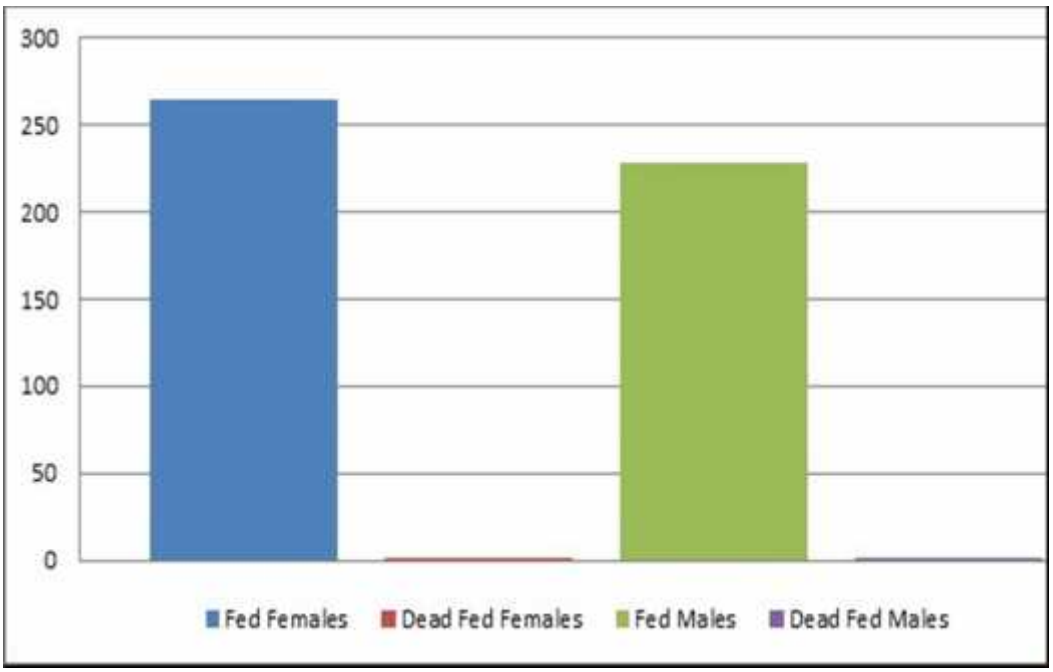


Fig. 3: Number of fed and fed-dead *Phlebotomus papatasi* after feeding on *Bougainvillea glabra*



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