SNS 2018 Draft Report

Team R14

Death Trap or Sweet Home

死亡陷阱或甜蜜家園

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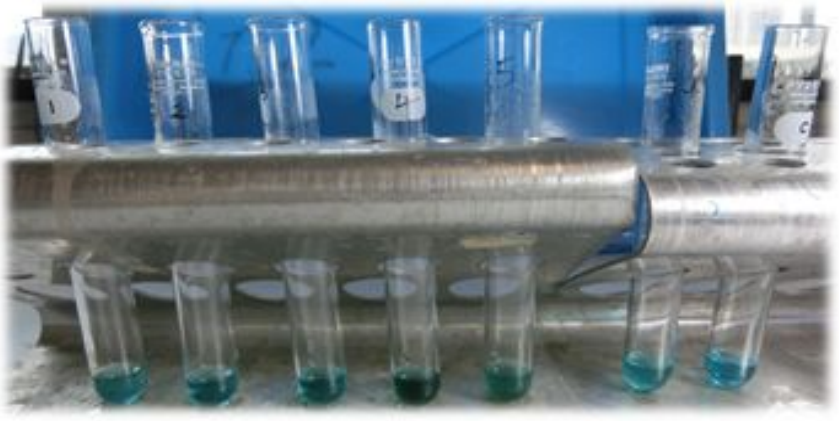

Content

1. Abstract

This project aimed at studying the physical environment of the microhabitat of the local species of pitcher plant, *Nepenthes mirabilis* (Lour.) Druce,奇異豬籠草, morphology of the pitchers of wild native *Nepenthes* species of Pitcher plant and commercial cultivated pot plant *Nepenthes* species and the the mechanism of trapping preys by pitchers. The types and abundance of insects inside the pitchers, both dead preys and living in-fauna was also studied. The ability to trap invertebrate preys by artificial pitfall traps on ground in the field were also investigated. Finally sugared and non-sugared pseudo-pitchers, half of them wrapped by red, green or both red and green colour cellophane were used to investigate whether sugar or colour may help to trap insects. From our observation the native species of pitcher plant were mainly growing along the bank of the freshwater reservoir. The lid was very efficient in preventing the entry of rain water into the pitcher avoiding the turning over of the pitcher due to over filling. The wax covered rim of the mouth of the pitcher, with a lot of nectaries was very slippery and ants were observed walking on it to reach the nectaries and slided into the digestive fluid of pitcher and drown. It was found out that the neutral to slightly acidic digestive fluid inside the pitcher carried very active protease for digesting the protein of the preys. There were two main types of insect preys trapped inside the pitchers, ants (Hymenoptera) and flies (Diptera), and two main types of living in-fauna, mosquito larvae (Diptera) and mites (Acarina) . It was found that sugared pseudo-pitchers trapped larger number of insect preys, whereas non-sugared pseudo-pitchers captured only few. Sugar also lured more invertebrates into the artificial pitfall traps on the ground in the field than non-sugared pitfall traps. The effect of red and green colour on luring insect preys was not able to be determined in our investigation.

我們的專題研究旨在探討本港豬籠草的唯一品種-奇異豬籠草所棲息的微生境的物理環境，野生豬籠草與人工培植豬籠草豬籠的形態及捕蟲的機制，與及豬籠內的昆蟲，包括蟲屍及存活的底內動物的數量與品種。同時，我們也研究了地面人工陷阱捕捉昆蟲的能力。最後，我們製作了兩種假豬籠，其中一種塗上糖水，而有一半假豬籠用紅色，綠色或紅及綠色的玻璃紙包裹起來，以研究糖分與顏色是否有助豬籠捕捉昆蟲。

實驗結果顯示，本地的野生豬籠草主要生長在淡水水塘的岸邊。人工培植豬籠草的籠蓋對於防止過量雨水流入，導致過重而翻側十分有效。其以蠟質覆蓋並含有大量蜜腺的外緣非常濕滑，當螞蟻受蜜腺吸引走在外緣上時會不慎滑落豬籠內的消化液並淹沒其中。這些帶微酸性的消化液內含活躍性極高的蛋白酶，用作分解獵物體內的蛋白質。此外，被困籠中的昆蟲主要有兩種，一為螞蟻（膜翅目），二為蠅（雙翅目）。底內生物亦有兩種，一為蚊的幼蟲（雙翅目），二為蟎（蜱蟎目）。添加了糖分的假豬籠比無添加糖分的假豬籠捕捉到更多數量的獵物。同時有糖水的比起沒有糖水的陷阱更能引誘獵物落入陷阱。顏色對於吸引昆蟲的效能在本次研究則無法測量。

2. Introduction

A few months ago, one of our members watched a short video about the insect trapping process of pitcher plants on Facebook and shared it to the rest of us. This has raised our curiosity on how the plants attract and capture its preys. Later, we wanted to find out more informations about the structure and behaviour of this plant, and therefore, carried out this project.

2.1 Brief description

Nepenthes mirabilis (or Common Swamp Pitcher Plant)(奇異豬籠草) is a carnivorous plant species which obtain minerals, especially fixed nitrogen, from insect prey for nutrient. It is the only species of *Nepenthes* inhabiting in Hong Kong[1]. Commercial cultivated potted plants of *Nepenthes sp.* are also available in local Flower shops. *Nepenthes* is the most diverse group of carnivorous plants and the regular species of this plant primarily gain nitrogen and phosphorus to supplement their nutrient requirements for growth, given these soil nutrients are typically lacking but moist is remained constantly.[2] It is a perennial herb, climbs by means of tendrils which are prolongations of the leaf midrib. The end of the tendril generally becomes swollen and hollowed out and develops into a pitcher with a lid on its mouth, which later opens as the pitcher matures. At the entrance of the pitcher, there are honey glands, below which the interior is extremely slippy. Insect preys are attracted by its honey. [3]Once they enter, they are unable to climb out due to the slippy surface and eventually drown in the enzymatic juice accumulated in the base of the pitcher. The plant absorbs the products of digestion. Living organisms such as mosquito larvae and mites inhabit the digestive juice inside the pitcher. They will not be killed by the enzymes inside the pitcher and are called infauna.[4] [5]

In this project we investigated the mechanism of trapping preys by pitchers, besides the common belief of pitchers acting as simple passive traps. This involves the lid, peristome teeth, waxy zone and digestive zone of the pitcher. We also investigated the trapping ability of artificial pitfall traps buried in ground and that of hanging pseudo-pitchers.

2.2 Structure of the pitcher of the pitcher plant

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[1] *Nepenthes* species recorded from China (including Hong Kong and Macau). :*N. mirabilis* * https://en.wikipedia.org/wiki/List_of_Nepenthes_species_by_distribution#China

[2] Barthlott, W., Porembski, S., Seine, R., and Theisen, I. 2007. *The Curious World of Carnivorous Plants.* Portland, Oregon: Timber Press.

[3] Moran, J.A. (2010). "The carnivorous syndrome in Nepenthes pitcher plants". *Plant signaling & behavior*. **5** (6): 644–648. doi:10.4161/psb.5.6.11238. PMC 3001552 . PMID 21135573

[4]Clarke, C.M. 1997. *Nepenthes of Borneo*. Natural History Publications (Borneo), Kota Kinabalu.

[5] Beaver, R.A. (1979). "Fauna and foodwebs of pitcher plants in west Malaysia". *Malayan Nature Journal*. **33**: 1–10.

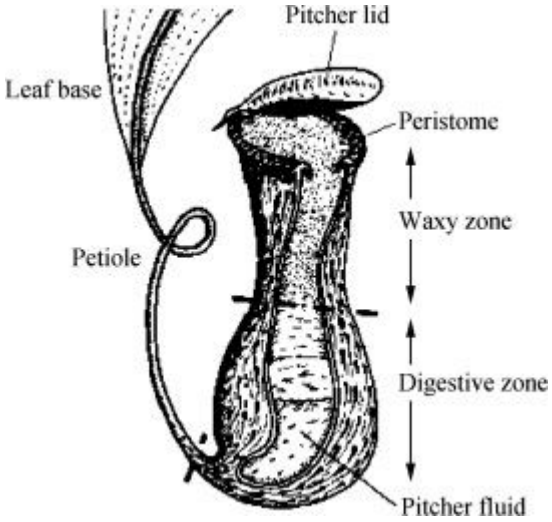


Figure 1

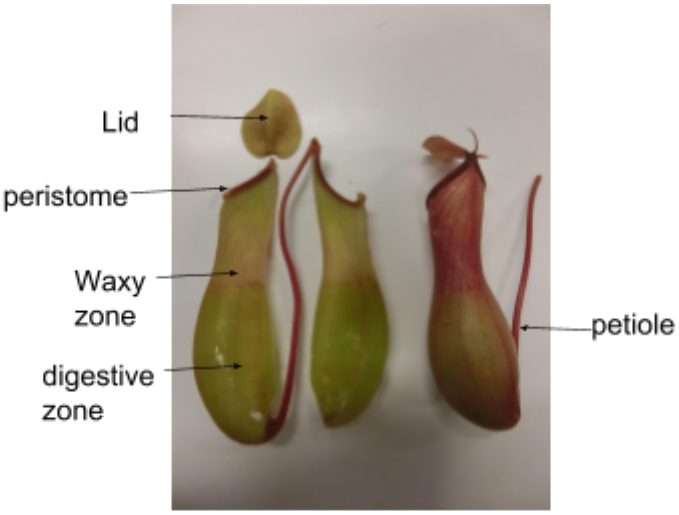
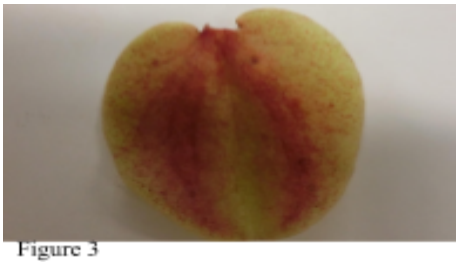
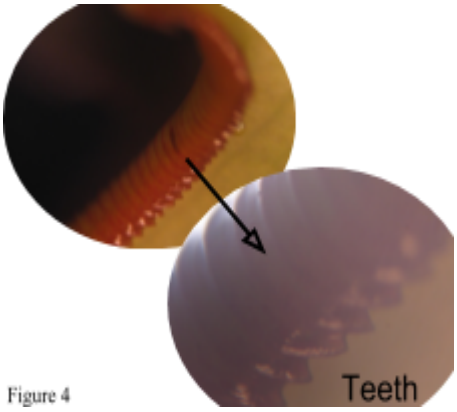


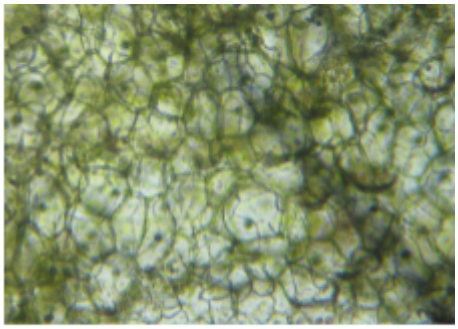
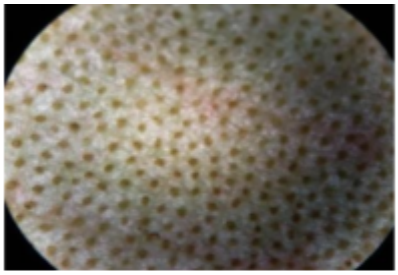
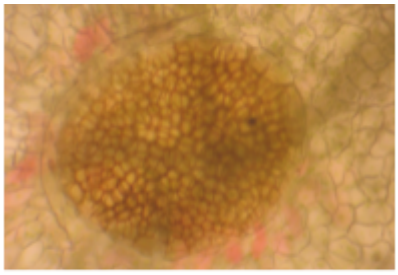
Figure 2

Most of the investigation was done using species of commercial cultivated pot plants as the local species is protected under the Protection of Endangered Species of Animals and Plants Ordinance.

2.3 Dissection of pitchers to investigate its internal structure[6]

Body Parts	Description
Pitcher lid :  Figure 3	❑ coloured (red, yellow) ❑ prevents entry of too much rainwater or other contaminants ❑ reduces evaporation of the enzymatic juice
Peristome :  Figure 4	❑ wax covered rim ❑ completely wetted by nectar secreted at its inner margin and by rain water, so homogenous liquid films cover the surface under humid weather conditions ❑ extremely slippery for insect We had performed further experiment on how it can help capture insects. More details are shown in *Experiment 4*.

[6] https://www.sciencedirect.com/science/article/pii/S1002007109002998 Retrieved on 3/7/2018

Waxy zone(with smooth wall) :  Figure 5 H.P. of waxy zone peel surface	Waxy zone ❏ located below the peristome ❏ slippery to prevent the escape of preys Procedures in dissection: 1. The pitcher cup was cut into 2 halves longitudinally by a pair of scissors. 2. At the waxy zone and digestive zone of the cup, a small part of the epidermis was peeled out by a pair of fine forceps. 3. The peel was observed under the microscope
Digestive zone(with glandular wall) :  Figure 6 L.P. of Digestive zone peel surface  Figure 7 H.P. of Digestive zone peel surface	Observation: ❏ Under lower power (x40). , many dots were seen on the surface, which were the digestive glands ❏ Under high power(x400). a digestive gland was mganified ❏ These glands secretes enzymes to digest their prey ❏ These enzymes don't harm the leaves, but break down the protein in the insect's body until it can be absorbed by the plant

3. Field Trips - Study of Pitcher Plant Habitat

Table 1

1st field trip	2nd field trip	3rd field trip
Hung Shui Hang Irrigation Reservoir	Hung Shui Hang Irrigation Reservoir	Hung Shui Hang Irrigation Reservoir
21/12/17	28/1/18	20/2/18
9:00 am to 2:00 pm	10:30 am to 1:30 pm	3:30 pm to 5:30 pm
Searching for local Pitcher plants, *Nepenthes mirabilis* (Lour.) Druce 奇異豬籠草	Measuring the physical environment of the microhabitat of *Nepenthes mirabilis* (Lour.) Druce, collection of fluid in sample pitchers, setting pitfall traps near sample pitcher plants	Collection of pitfall traps, collection of digestive fluid in more pitchers

3.1 Procedure of field work

1. An area with pitcher plants was found.
2. The light intensity, temperature, relative humidity and wind speed were measured by light meter, digital thermo hygrometer, anemometer and compass.
3. (6 - 8) representative pitchers were chosen and their length, diameter were measured.
4. The digestive fluid (including the prey captured inside) from each pitcher was collected in vials for later investigation.
5. After taking out all the fluid inside, some distilled water was poured back into the pitcher.
6. Step 1 to 5 were repeated in a new area.

3.2 Data and Analysis

3.2.1 **Physical environment of the microhabitat of the Pitcher plants (on 28-1-2018)** **Table 2**

Pitcher plant (No.)	Height (cm)	No. of pitchers on plant	Light intensity (Lux)	Temperature (˚ C)	Relative humidity(%)	Wind Speed (ms^{-1})	Soil Colour	Soil pH
1	125	6	76100	20	74	0	yellow brown	8
2	100	5	91500	21	73	0	yellow brown	8
3	47	4	49100	20	70	0	dark brown	7
4	200	2	70000	20	74	0	brown	7
5	75	5	77400	20	69	0.1	brown	7
6	28	4	5500	20	71	0	light brown	7
Average	**95.8**	**4-5**	**6993**	**20.2**	**71.8**	**0**	/	**7.3**

From the result above, it was found that pitcher plants lived in a relatively high humidity region (R.H. of 72%) and warm temperature. We also tested the soil pH. The soil for growing pitcher plants was generally neutral.

3.2.2 **Pitchers in the field (on 28/1/18)** **Table 3**

Pitcher No.	**pH of fluid**	**Colour of fluid**	**Length of pitcher (cm)***	**Width of pitcher (cm)***	**Volume of pitcher (cm^3)***	**Volume of fluid in pitcher (cm^3)***	**% volume of pitcher filled up with fluid**
1	7	Pale brown	15	3	105.9	11	10.4
2	7	Light yellow	12	3	84.8	10.7	12.6
3	7	Pale brown	13	3.5	125.0	16.6	13.3
4	6	Pale brown	15	4	188.4	6.5	3.5
4	6	Brown	16	5.5	379.9	13.8	3.6
6	6	Pale yellow	15.5	4	194.7	1.1	0.6

3.2.2 **Pitchers in the field (on 20/2/18)**

Pitcher No.	pH of fluid	Colour of fluid	Length of pitcher (cm)*	Width of pitcher (cm)*	Volume of pitcher (cm^3)*	Volume of fluid in pitcher (cm^3)*	% volume of pitcher filled up with fluid
7	not recorded	brown	13	3	91.8	9	9.8
8	not recorded	colourless	12.5	3	88.3	1.9	2.15
9	not recorded	Yellow	11.5	2.5	56.4	2.8	5
10	not recorded	Light brown	13	3	91.8	9.8	10.7
11	not recorded	Light yellow	13	4	163.3	12.3	7.5
12	not recorded	Light yellow	13	3.5	125.0	11	8.8
13	not recorded	colourless	4	1	3.14	1	31.8
14	not recorded	Light yellow	13.5	2.1	46.7	10	21.4
Average	/	/	**4 - 16**	/	/	**8.39**	**10.08**

***Length and width are mesasured by measuring tape. Volume is measured by measuring cylinder, the sample fluid was sucked out by a syringes**

Findings:

1. Colur of fluid in the pitchers was colouless to light yellow to light brown.
2. pH of the fluid in pitcher was slightly acidic to neutral .
3. Length of pitcher ranged from 4 - 16 cm.
4. Average volume of digesive fluid inside the pitcher = 8.39 cm^3
5. Average % volume of pitcher filled up with digestive fluid = 10.08 %

3.2.3 **Study of pitchers of cultivated pitcher plants**

I. General information of the day of study

Place of investigation: Biology Lab. & School Garden

Date of investigation: 25-6-2018

Time of investigation: 9:30 am - 12:30 pm

Recent weather conditions: cloudy / shower

II. Digestive fluid and reducing sugar analysis

Pitcher no.	1	2	3	4	5	6
Volume of digestive fluid (cm^3)	22.5	18.5	39.5	21	13	18.5
Colour of digestive fluid	colourless	colourless	colourless	colourless	colourless	colourless
pH of digestive fluid	7	7	7	7	7	7
Presence of Protease	-	-	-	+	-	-
Presence of reducing sugar in peristome	+	-	-	-	-	-

Presence of Reducing sugar in lid	++	-	-	-	-	-
Presence of Reducing sugar in leaf	-	-	-	-	-	-
Time required to drown an ant* (min s)	19 s	2 min.50 s	2 min.13 s	26 s	10 min.	5 min

Table 4 Key: ++ (present in small amount), + (present in minute amount) , - (absent)

*Observation: the ants sunk to the bottom and lost their mobility

Time required to drown an ant in distilled water (1st test) = 52 min 43 s
(2nd test) = escaped
(3rd test) = escaped
(4th test) = escaped

Time required to drown an ant in distilled water (Average) = usually escaped

Time required to drown an ant in detergent solution (1st test) = 5 s
(2nd test) = 4 s
(3rd test) = 13 s
(4th test) = 14 s

Time required to drown an ant in detergent solution (Average) = 9 s

Findings:

From the experiment, we found out that the digestive fluid in the pitchers of cultivated plants was colourless and neutral. Protease was present in one of the six samples. The samples could drown a ant within 10 minutes while most of the ants dropped on distilled water could escape. The digestive fluid had lower surface tension than water and could not support the weight of the preys. As a result, the prey is less likely to escape once it fall onto the digestive fluid.

3.2.4 Invertebrates found inside the pitchers in the field (on 21-1-18 & 20-2-18)

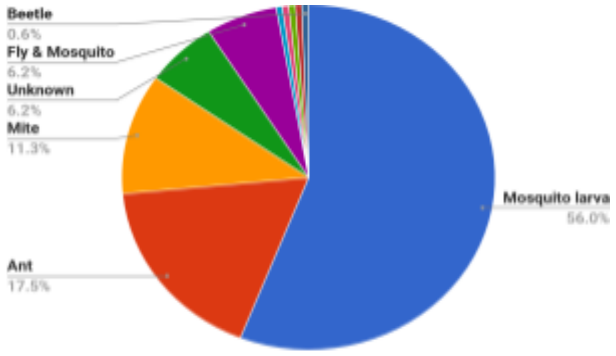


Figure 8 Invertebrates(%) found inside the Pitchers in the field (on 28-1-18 & 20-2-18)

Total number of invertabrates : 177
Ant: 31
Fly & Mosquito : 11
Mosquito larva: 99
Mite: 20
Springtail : 1
Spider:1
others----grasshopper: 1
wasp: 1
beetle: 1
unknown: 11
black - dead (prey)
red - living (infauna)

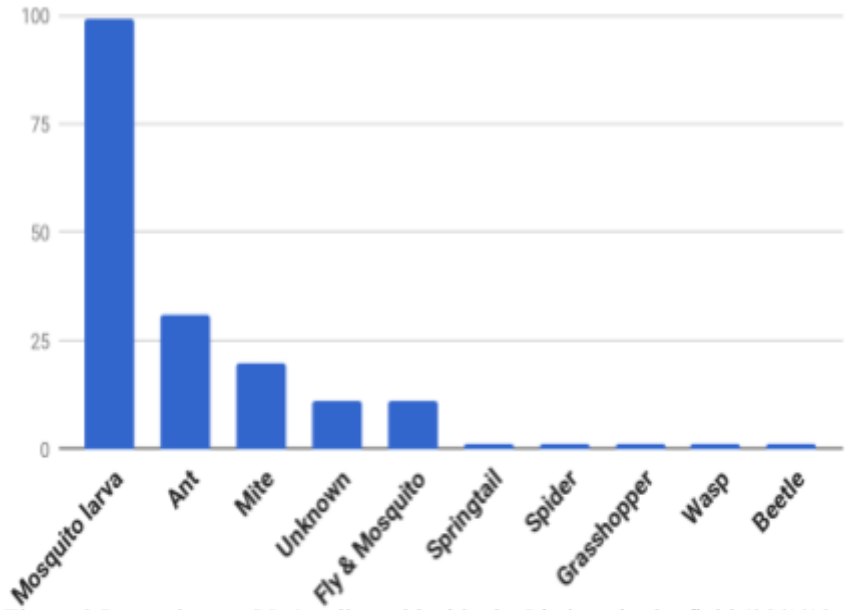
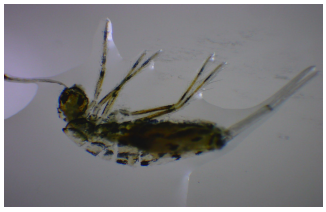
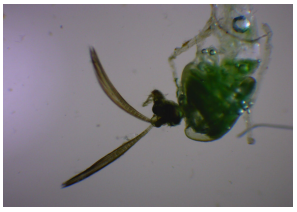


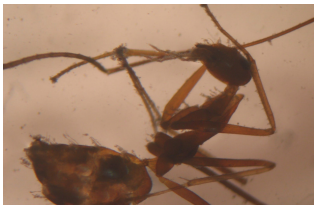
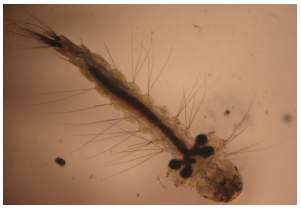
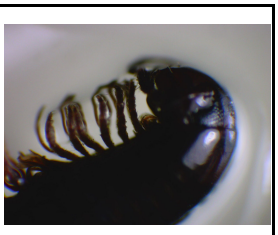
Figure 9 Invertebrates (No.) collected inside the Pitchers in the field (28/1/18 & 20/2/18)

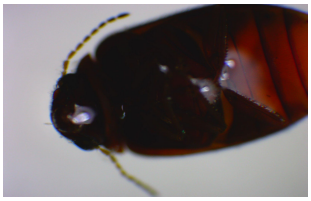
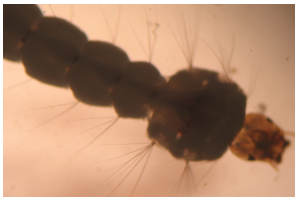
Finding:

Ants were the primary prey trapped inside the pitchers while fly and adult mosquito were the second abundant prey trapped inside the pitcher; mosquito larvae were the most abundant **living infauna** inside the pitchers. The mosquito larvae may belong to the Genus *Culex* and *Armigeres* (Barbara, 1984)(further discuss on ***Further Studies and Questions***)

Photomicrographs of some invertebrates of different Taxa collected in the Pitchers / Pitfall traps

				
Taxa	Collembola (Springtail) 1. 彈尾目 (跳蟲)	Diptera (mosquito) 2. 雙翅目 (蚊)	Diptera (fly) 3. 雙翅目 (蠅)	Araneae (Spider) 4. 蜘蛛

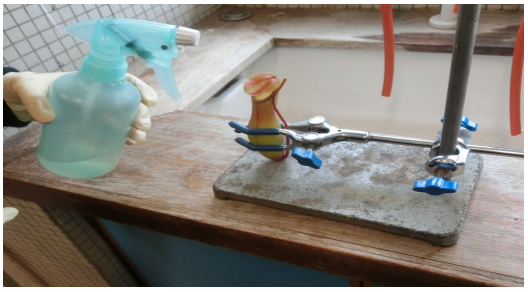
				
Taxa	Hymenoptera (Ant) 5. 膜翅目(蟻)	*Diptera* (small mosquito larva) 6. 雙翅目 (小蚊幼蟲)	*Acarina* (mites) 7. 蜱蟎 (蟎)	Diplopoda (millipedes) 8. 多足類 (馬陸)

				
Taxa	Coleoptera (Beetle) 9. 鞘翅目 (甲蟲)	*Diptera* (large mosquito larva) 10. 雙翅目(大蚊幼蟲)	Orthoptera (grasshoppers) 11. 直翅目 (蚱蜢)	Hymenoptera (wasp) 12. 膜翅目 (黃蜂)

Black title (dead) Prey獵物 (死)
Red title (living) Infauna 底內動物 (活)

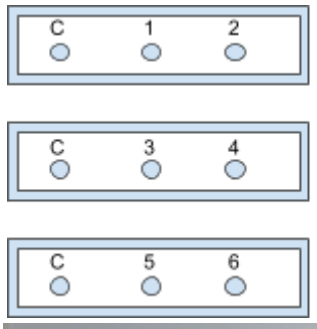
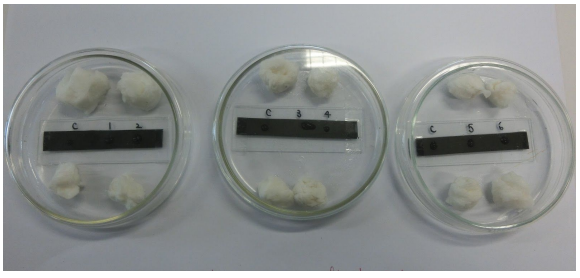
4. Investigation by experiment

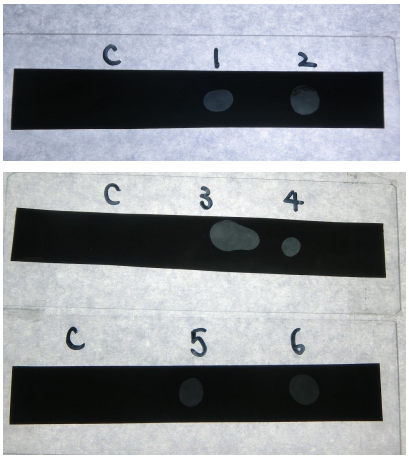
Experiment 1 : Prevention of entry of rain water into the pitcher by the Lid

Aim of the experiment	To find out how efficient is the lid of pitcher plant in preventing the entry of rain water into the pitcher
Experimental design	We want to find out how pitcher plants prevent the entry of the rainwater by their lids. Therefore we splashed a measured volume of water above the lid to imitate raining and see how much water could enter the pitcher.
Date	2/2/2018 (FRI)
Venue	School laboratory
Procedure  Figure10	1) A pitcher with a lid from cultivated plant was fixed vertically in a stand with clamp.
 Figure 11	2) 100 cm^3 of water was all splashed at different angles above the lid. (we try to simulate a rainy environment by splashing water from all directions) The volume of water collected inside the pitcher was measured.

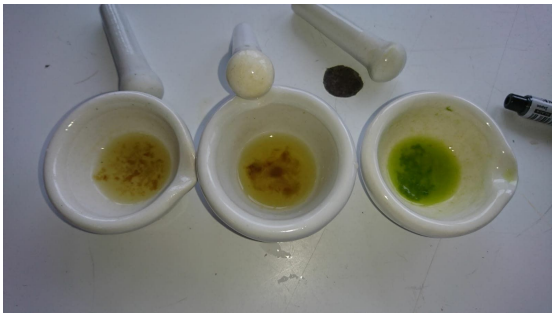
	3) The experiment was repeated using different volume of water (200 cm^3, 300 cm^3 and 500 cm^3)
Result (i)	We could see the lid plays an essential role in preventing too much rainwater to enter the pitcher cup

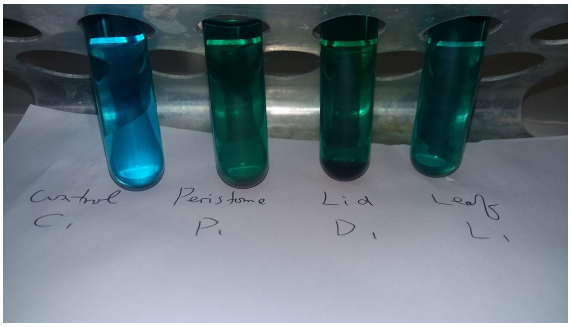
Experiment 2 : Digestive fluid analysis (presence of protease)

Aim of the experiment	To find out whether protease is present in the digestive fluid of pitcher
Hypothesis	We assumed the digestive fluid contain protease as they need to digest the proteins of insects.
Experimental design	We place a drop of digestive juice on some developed black X-ray film strips for testing the presence of protease since they have protein gelatin coating the black silver salt inside. If the juice contain protease enzyme, the gelatin would be digested so the black pigment of the film could be washed away and become transparent.
Date	5/2(MON)
Venue	school laboratory
Procedure Photo/ diagram	1) 3 strips of photographic negatives were secured onto 3 microscopic slide respectively with cellotape.
 Figure12  Figure 13	2) 1 drop of digestive juice from pitcher No. 1 was dropped onto the surface of the negative. 1 drop of digestive juice from Pitcher no. 2 to 6 was put onto the photographic negatives respectively. Their relative position was labelled as 1, 2, 3, 4, 5 and 6 respectively. Distilled water was used as control (labelled as C) 3) The photographic strips were put in a moist chamber (in a petri dish containing a piece of moist tissue paper) and incubated at 37°C.

 Figure 14	4) After 4 hours, the strips were washed with water. The size of clear spots was noted and the enzyme activity were compared.
Result (ii)	All 6 juice drops gave a clear spot except the distilled water (C), which indicates all digestive juice from pitcher no. 1-6 contained protease.

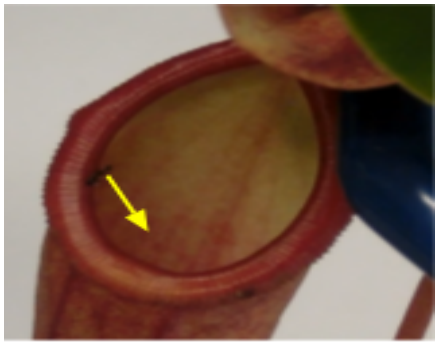
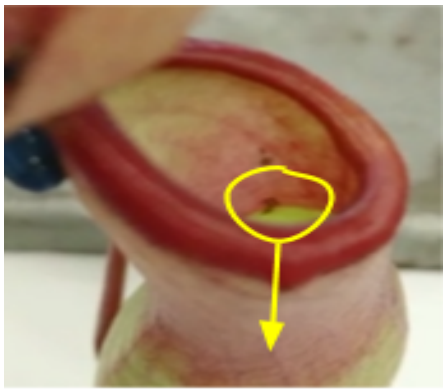
Experiment 3: Presence of reducing sugar in different regions of the pitcher plant

Aim of the experiment	To find out whether reducing sugar is present in different regions (lid, peristome and leaf) of the pitcher plant
Hypothesis	We assumed only the lid and peristome contain sugar to attract insects
Experimental design	
Date	28/6/2018
Venue	school laboratory
Procedure Photo/ diagram	1) Exactly 1.5 g of the tissue of the lid, peristome and the leaf were weighed respectively by an electronic balance.
	2) Each tissue was grinded in a mortar and pestle by adding 5 cm^3 of distilled water. 3) The mixture was filtered to collect the filtrate.

	4) 3 cm^3 of the filtrate of each tissue was transferred to a test tube. 3 cm^3 of distilled water was added to the fourth tube as a control. 3 cm^3 of Benedict's solution was added to each test tube. All test tubes were boiled in a beaker of boiling water for 5 minutes. They were then left overnight to wait for precipitation.
Result (iii)	Very minute amount of red ppt. was formed in the tube of the Peristome, small amount of red ppt.was formed in the tube of the Lid. The results was negative in both the distilled water (control) and the leaf.

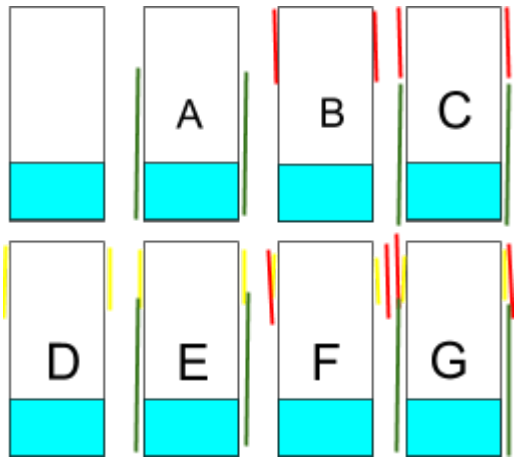
Experiment 4 : Pitcher Plant's "eating" process

Aim of the experiment	To study how the pitcher plant capture insects
Experimental design	We want to find out how the pitcher plant capture insects by the slippery peristome and also the waxy inner wall.
Date	5/2(MON)
Venue	school laboratory
Procedure Photo/ diagram	1) Some ants were collected from the school garden
 Figure15	2) An ant was transferred to the surface of the peristome by tissue.

 Figure 16	3) After a few seconds, the ant fell down into the pitcher cup
 Figure 17	4) The ant couldn't escape and was drown by the digestive juice. After a moment of struggling, it finally stop moving. The experiment was repeated with another ant.
Result (iv)	The ant fell down in the pitcher cup easily due to the slippery peristome.

Experiment 5 : Pseudo-pitchers in trapping preys (in the school garden)

Aim of the experiment	To investigate whether colour or nectar may lure invertebrates to death in the pitchers (compare the amount and variety of insects captured)
Experimental design	Sugared and non-sugared pseudo-pitchers, half of them wrapped by red, green or half red and half green colour cellophane. These were used to investigate whether sugar or colour help to trap insects.
Date	20/2
Venue	school laboratory
Procedure	1) 8 plastic sample bottes were prapared.

	*Control : No colour* *A: wrapped with green paper* *B: wrapped with red paper* *C: wrapped with half green and half red paper* *D: some honey was painted on the bottle surface, with no colour* *E: some honey was painted on the bottle surface,(green)* *F: some honey was painted on the bottle surface, (red)* *G: some honey was painted on the bottle surface,(½ red ,½ green)* 15 cm^3 of alcohol was put into each bottes.
 Figure 19	2) The pseudo-pitchers were hung next to the real pitchers of a cultivated potted pitcher plant and the whole plant was hung back to the school garden.
Result (v)	After 2 weeks it was found that real pitchers trapped more insects than pseudo-pitchers. Sugared pseudo-pitchers trapped more insects than non-sugared pseudo-pitchers.

Experiment 6 : Pitfall traps in trapping preys (in the field trip site:Hung Shui Hang Irrigation Reservoir)

Aim of the experiment	To compare the ability of sugared and non sugared artificial pitfall traps on ground in trapping invertebrates
Experimental design	Artificial pitfall traps (sugared & non-sugared) were buried in the soil adjacent to the pitcher plants to act as model pitchers.
Date	28/1
Procedure Photo/ diagram	1) 20 cm^3 of alcohol was poured into each of the six plastic vials.

 Figure 20	2) Sugar solution was painted on the mouth of 3 vials(sugared pitfalls). No sugar was painted on the mouth of the remaining 3 vials. (non-sugared pitfalls)
 Figure 21	3) Near the site where pitcher plants were found, 6 holes were dug in the soil by using a shovel. The 6 vials were buried into the holes respectively.
 Figure 22	4) A tree bark or a flat stone was used to cover the mouth of the pitfall loosely.
 Figure 23	5) The whole pitfall trap was covered by withered leaves. The whole process was repeated by using the sugared and non-sugared pitfall traps in another area.
Result (vi)	The total number of invertebrates trapped in non-sugar pitfall traps was smaller than that in sugar pitfall traps. Hence sugar lured the invertebrates into the pitfall traps.

5. Results and discussion

(i) Prevention of the entry of rain water by the lid (Experiment 1)

Pitcher no.	1	2	3	4
Volume of water sprayed (cm^3)	100	200	300	500
Volume of water collected in the pitcher (cm^3)	0.2	0.64	1.54	1.25
% of water collected in pitcher	0.2	0.32	0.51	0.25

Table 6

From the result in experiment 1, we concluded that the lid of the pitcher plant are useful for preventing the entry of rain water. Physically, the surface wall of the lid is slippery as the rain drop is being flicked off which prevent the pitcher from receiving too much rainwater.

(ii) Study of the presence of protease in digestive fluid of pitcher (Expeiment 2)

From the result in experiment 2, we confirmed the presence of protease collected in the digestive juice from the pitchers in field. The pH values of the digestive juice were slightly acidic (pH 6) to neutral (pH 7) which is probably the optimum pH value of the protease After capturing the prey and soaking them in digestive juice, the protease inside would help to digest the proteins in the prey into amino acids which will be absorbed as a source of Nitrogen.

(iii) Study of the presence of reducing sugar in different parts of the pitcher plant (Experiment 3)

From the result in experiment 3, reducing sugar was present in both the lid and the peristome to attract insects.

(iv) Study of the special features of peristome of the pitcher in capturing insects (Experiment 4)

From the result in experiment 4, we confirmed that the slippery peristome is useful for capturing insects. Physically, waxes significantly reduces the attachment force of insects compared with reference surfaces, such as glass and the wax-free surface.When the peristome is wet, it will cause the ants to fall into the pitcher. Also the humidity of the surface area of peristome is the most influential part which affects how the pitcher plant capture the insects. (Lixi Wang, 2009)[7]

[7] https://www.sciencedirect.com/science/article/pii/S1002007109002998 Retrieved on 3/7/2018

(v) The effect of the sugar and /or colour in capturing preyss in pseudo pitchers and artificial pitfall traps (Experiment 5) & (Experiment 6)

From the beginning of the experiment, we assumed that bright colour, usually red, of pitcher plant and the presence of nectar can attract more preys. However, from our results, in the pseudo-pitchers the number of prey captured with red or without red colour didn't have a big difference. From literature search, we find out that actually red coloration didn't affect the number of ants – the pitchers' primary prey – that were captured.(Aaron Ellison of Harvard University and his coauthor Katherine Bennett)[8]. Since ant see the colour red as grey which may only weakly contrasts with the green base color of the pitcher. Therefore, the bright red colour won't work on them. So, our assumption is incorrect.

[9]

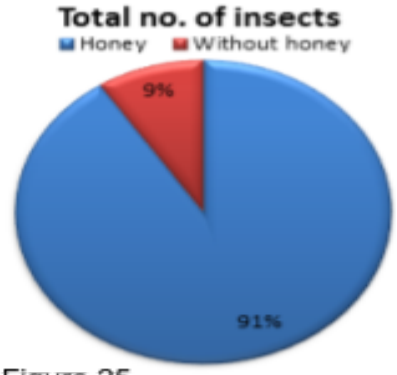


Figure 25

No. of insects captured in Pseudo- pitcher
(with honey): 59
(without honey):6
Hence sugar should be the primary means for the plant to attract preys.

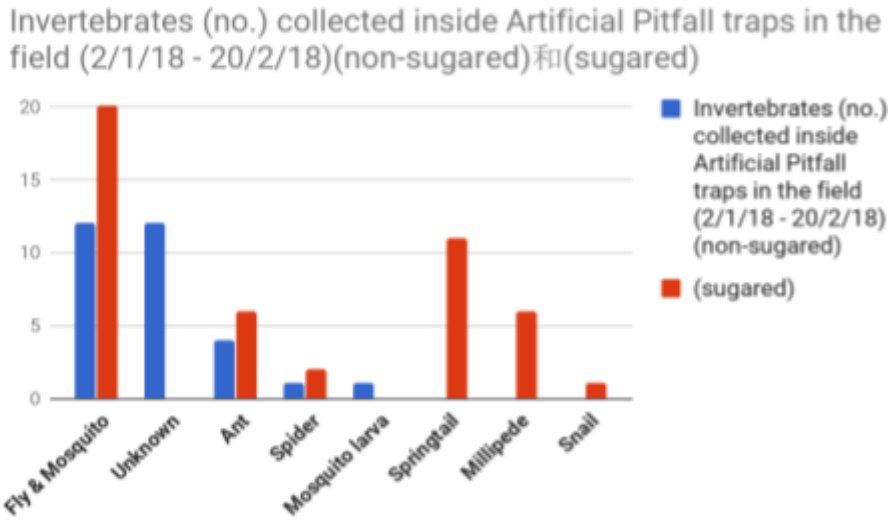


Figure 26

From the results in the artificial pitfall traps in the field

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[8] https://www.sciencedaily.com/releases/2009/08/090804081545.htm Retrieved on 3/7/2018

[9] ant see the colour red as grey which may only weakly contrasts with the green base color of the pitcher Pitcher Plants' Red Colors Don't Attract Prey

1. Total number of invertebrates trapped in non-sugared Pitfall traps = 30 was smaller than the total number of invertebrates trapped in sugared Pitfall traps = 46
2. Sugar lured the invertebrates into the pitfall trap.

We found that number of invertebrates collected in sugared pitfall traps were more than that of non-sugared. That means, sugar is necessary in attracting prey. In real pitcher plants, they will produce sugary nectars secreted by the nectar-producing glands in order to attract more prey.

6. Conclusion

From our observation, the native species of pitcher plant, *Nepenthes mirabilis* (Lour.) Druce were mainly growing along the bank of the freshwater reservoir. The lid of commercial cultivated potted *Nepenthes sp.* was efficient in preventing the entry of rain water into the pitcher, avoiding the turning over of the pitcher due to over filling. The wax covered rim of the peristome with a lot of nectaries was very slippery and ants were observed walking on it to reach the nectaries and slided into the digestive fluid of pitcher and drown. It was found out that the neutral to slightly acidic digestive fluid inside the pitcher carried active protease for digesting the protein of the preys. The surface tension of the digestive fluid in the pitchers was much lower than that of water and preys dropped onto the surface of the fluid could not be supported and was drown quickly. There were two main types of dead insect preys trapped inside the pitchers of *Nepenthes mirabilis*, ants (Hymenoptera) and flies/mosquito (Diptera), and two main types of living in-fauna, mosquito larvae (Diptera) and mites (Acarina). It was found that sugared pseudo-pitchers could trap larger amount of insect preys, whereas non-sugared pseudo-pitchers captured only few. Sugar also lured more invertebrates into the artificial pitfall traps than non-sugared pitfall traps on the ground. The effect of red and green colour on luring insect preys wasn't able to be determined by pseudo-pitchers in our investigation.

7. Limitations

1. Only a limited number of samples of pitchers can be taken for investigation as we do not want to disrupt the habitat by obtaining a large number of sample at one time. The finding obtained may not be totally statistically reliable.

8. Questions and Further Studies

From literature search the following areas are interesting topics worth further studies.

a) Why can mosquito larvae and mites survive inside the pitcher fluid (Sweet home of mosquito larvae)?[10]

Actually, there are lots of species of insect larva lived inside the pitcher plant while mosquito larvae are the most common species. These are called the infauna of the pitchers. The presence of these larvae is essential to the plant, because while the pitchers produce digestive enzymes, they don't produce that much, especially with the level going down as the individual pitcher ages. Also, it is difficult to break down a whole insect into the essential nutrients that the plant needs to sustain itself. Therefore, this is what these larvae do----to tear the prey into smaller pieces. As the prey's surface area increases, the rate of enzyme-substrate reaction can be fasten. While these larvae hang out in the pitcher in order to eat the prey, it seems like they are stealing the plant's meal, yet they are doing their favours. Moreover, the prey eaten will ultimately pass out as faeces, which the plant can also use. This greatly speeds up the digestion process of the plant, and at the same time, provides food and shelter for these larvae. This is called **mutualism**.

b) Why are ants the primary prey of pitcher plants?(Death trap of ants)[11]

Ants are a kind of 'scout' animal. They explore new food sources and subsequently recruit nest-mates in a scouting habit. This habit causes them to find food (that is the nectar) in the pitcher of a pitcher plant and leading most of them to fall into the trap of the pitcher[12].That is why ants are massively found dead in pitcher plants, as one ant fall into the trap at instance, the others follow the former's route. [Dr.Ulrike Bauer,14 January 2015]

c) How do pitcher plants enable more prey (ants) to visit the pitchers ?

What we know is that, pitcher plants' key trapping surface, the pitcher rim (peristome), is highly slippery when wetted by rain, nectar or condensation, but not when dry. When the inner wall of pitcher plant is wet, there is no room for the prey to escape by climbing the peristome and the prey is drowned to death. However, this situation doesn't work when the inner wall is dry. The peristome of a pitcher can be dry and safe to walk on for up to 8 hours during the day. Therefore, insect-eating pitcher plants temporarily 'switch off' their traps in order to trap their prey from escaping[13]. Besides, ants are the predominant prey as mentioned above. From obtaining all the 'Scout' ants at one time by covering their lids at all the dry period, the pitcher plant cleverly left a gap of dry period, so that this allows safe access for 'scout' ants under dry conditions, thereby promoting

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[10] https://oleaeuropea.wordpress.com/2011/06/02/the-fascinating-microcosm-of-the-pitcher-plant/ Retrieved on 3/7/2018

[11] http://www.bris.ac.uk/news/2015/january/ants-and-pitcher-plants.html Retrieved on 3/7/2018

[12] Nectar, not colour, may lure insects to their death, Katherine F. Bennett1,2 and Aaron M. Ellison2,*

[13] Pitcher Plant Captures Prey in Batcheshttps://www.scientificamerican.com/article/pitcher-plant-captures-prey-in-batches/

recruitment and ultimately higher prey numbers.This mechanism is an adaptation for ants capturing.[Pitcher plants 'switch off' traps to capture more ants,14 January 2015]

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7.Ecological Society of America , pitcher plant's red colour don't attract preys https://www.sciencedaily.com/releases/2009/08/090804081545.htm Retrieved on 3/7/2018
8.The Fascinating Microcosm of the Pitcher Plant https://oleaeuropea.wordpress.com/2011/06/02/the-fascinating-microcosm-of-the-pitcher-plant/ Retrieved on 3/7/2018
9.Pitcher plants 'switch off' traps to capture more ants http://www.bris.ac.uk/news/2015/january/ants-and-pitcher-plants.html Retrieved on 3/7/2018
10.Pitcher plants 'switch off' traps to capture more ants,14 January 2015
11.Dr.Ulrike Bauer,14 January 2015
12.Nectar, not colour, may lure insects to their death, Katherine F. Bennett1,2 and Aaron M. Ellison2,* http://rsbl.royalsocietypublishing.org/content/roybiolett/5/4/469.full.pdf
13.Pitcher Plant Captures Prey in Batches.https://www.scientificamerican.com/article/pitcher-plant-captures-prey-in-batches/

12.Appendix

12.1 a) Invertebrates found inside the pitcher in the field (on 28//1/18)

Pitcher No.	Ants	Fly & Mosquito	*Mosquito larva*	*Mite*	Springtail	Spider	Others
1	1		12	2			Enormous amount of ant fragments

2			2				
3	13		26			1	Enormous amount of ant fragments
4	1		3				With ant fragments
5			33				1 (wasp) Enormous amount of ant fragments
6	2						

b) Invertebrates found inside the pitcher in the field (on 20//2/18)

Pitcher No.	**Ant**	**Fly & Mosquito**	***Mosquito larva***	***Mite***	**Spring tail**	**Spider**	**Others**
7	1		3				
8		1	2	3			
9		1			1		
10			1				
11			1				1 (beetle)
12		1					
13							
14	7	6	1	2			
15		2					
16							3 (unknown)
17			15				
18	6			13			1 (grasshopper) 8 (unknown)
Total	31	11	99	20	1	1	1 (grasshopper) 1 (wasp) 1 (beetle) 11 (unknown)
%	17.5	6.2	55.9	11.3	0.56		0,56 (grasshopper) 0.56 (wasp) 0,56 (beetle) 6.21 (unknown)

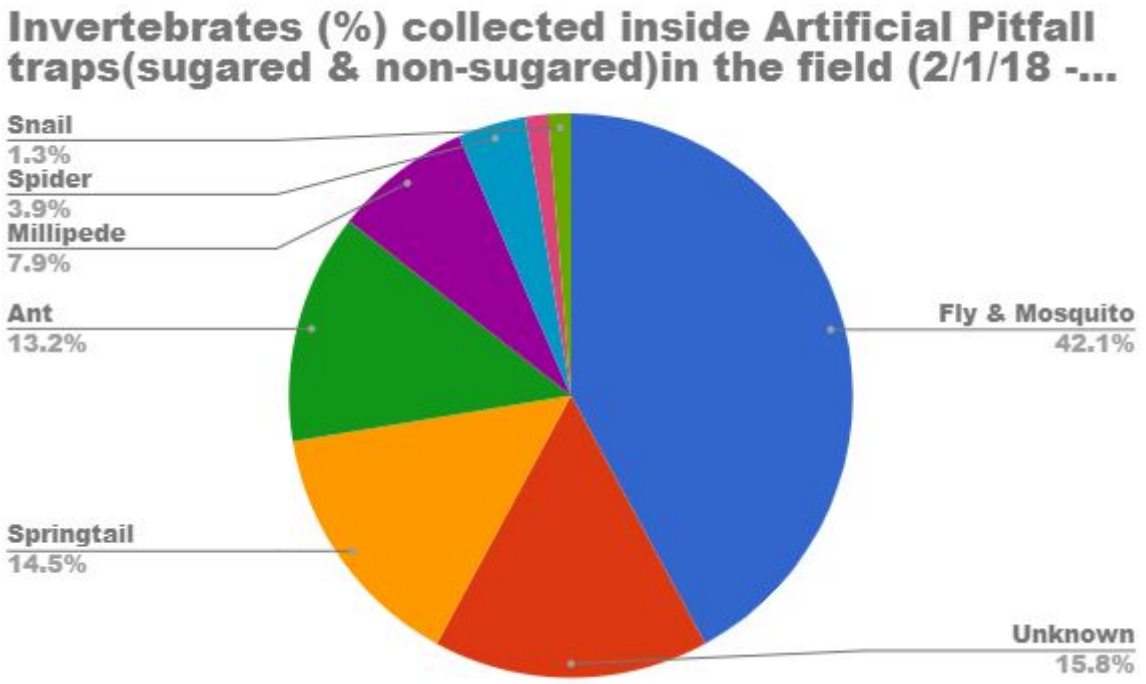
black - dead (prey) *red* - living (infauna)

c)Invertebrates collected inside the pitfall traps on the ground in the field from 28//1/18 - 20/2/18 (23 days)

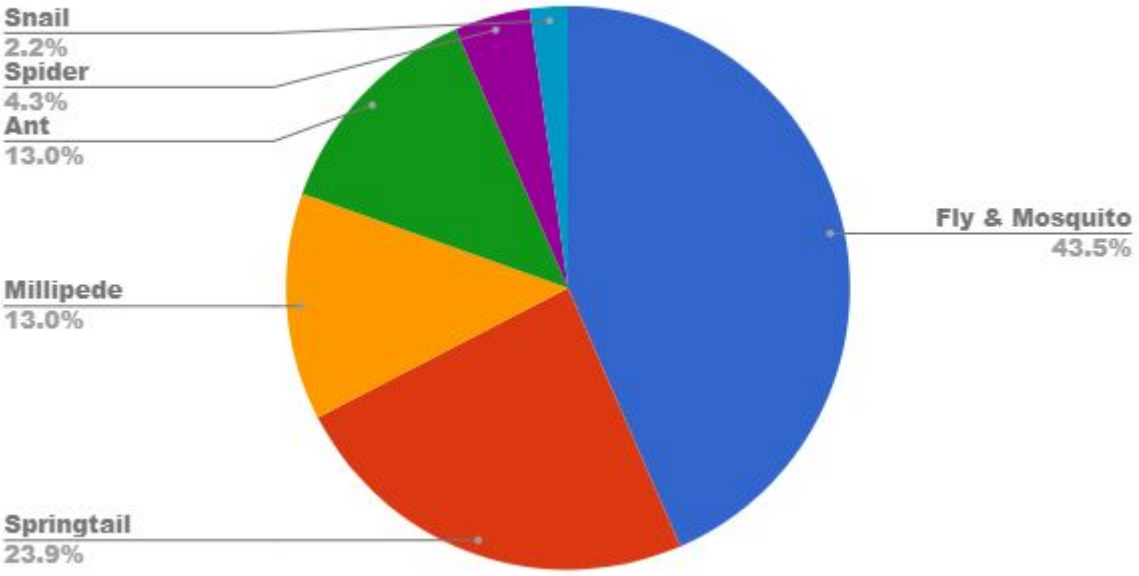
Pitfall No.	**Ants**	**Fly & Mosquito**	**Mosquito larva**	**Mite**	**Springtail**	**Spider**	**Others**
1a	**2**	**7**					

1b	2	5				2	1 (snail)
2a	1	2					12 (unknown)
2b		8					
3a	1	3	1			1	
3b	4	7			11		6 (millipede)
Total	10	32	1	0	11	3	1 (snail) 6 (millipede) 12 (unknown)
%	13.2	42.1	1.3	0	14.5	3.9	1.3 (snail) 7.9 (millipede) 15.8 (unknown)

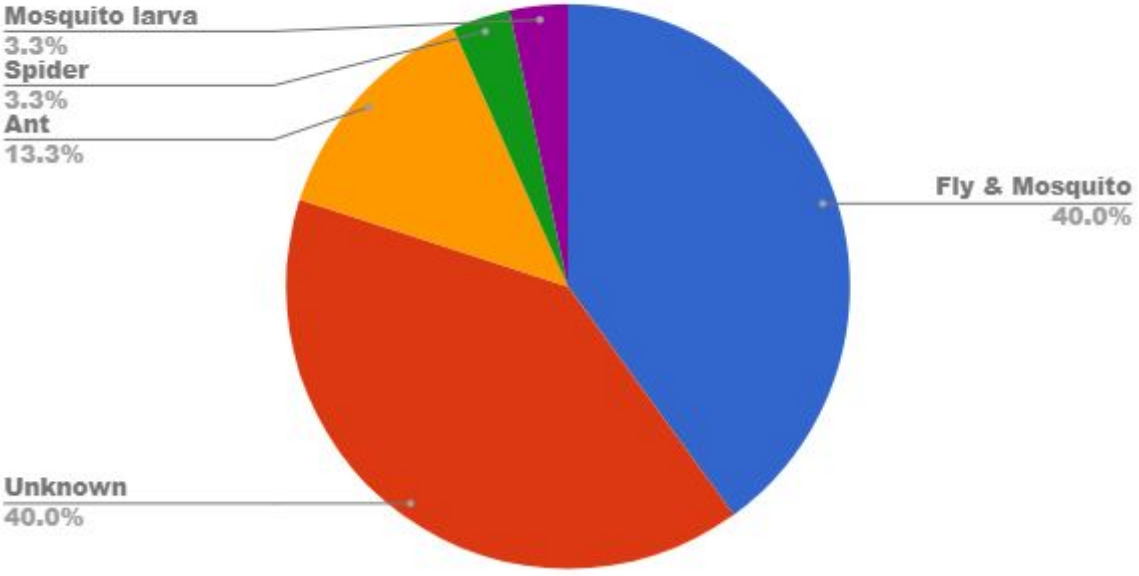
a - not sugared,
b - sugared

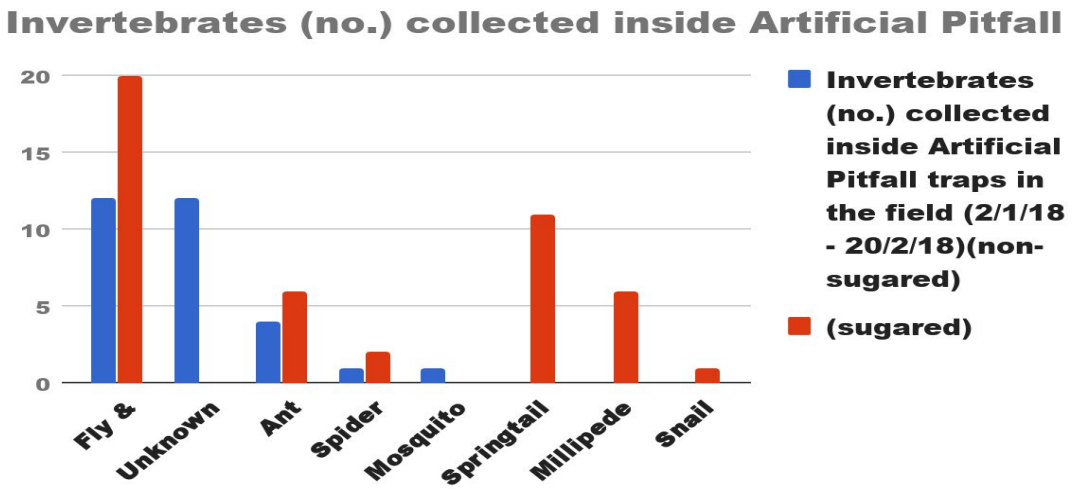


Invertebrates (%) collected inside Artificial Pitfall traps(sugared)in the field (2/1/18 - 20/2/18)



Invertebrates (%) collected inside Artificial Pitfall traps(non-sugared)in the field (2/1/18 - 20/2/18)





12.2) Number of insects in the pseudo-pitcher (28/1/2017)

Pseudo-pitcher	Treatment	Ants	Wasp	Fly	Mosquito larva	Total no. of insects	Total no. of insects
Control (H)	No colour	0				0	6
A	green	1				1	
B	red	4				4	
C	Green + red	1				1	
D	Honey + no colour	3				3	59
E	Honey + green	13				13	
F	Honey + red	34				34	
G	Honey + red + green	9		2		9	

12.3)Number of insects in the pseudo-pitcher(25/6/2018)

Pseudo- pitcher	Treatment	Ants	Wasp	Fly	Mosquito larva	Others	Total no. of insects
Control H1	No colour						0
Control H2	No colour					Spider (1)	1
A1	green						
A2	green						
B1	red						
B2	red						

C1	**Green + red**						
C2	**Green + red**						
D1	**Honey + no colour**						
D2	**Honey + no colour**						
E1	**Honey + green**				1		1
E2	**Honey + green**						
F1	**Honey + red**						
F2	**Honey + red**						
G1	**Honey + red + green**	3					3
G2	**Honey + red + green**						
pitcher 1		2				Cockroach (1) Springtail (1)	4
pitcher 2						Spider (1) Mosquito (1)	2
pitcher 3		2				Mosquito(1) Bee (1)	2
pitcher 4		2				Unknown (1)	3
pitcher 5		3					3
Real pitcher 6		5				Spider (1) Moth ()	7

12.4)Pitcher in the field (on 28/1/18)

Pitcher No.	**pH of fluid**	**Colour of fluid**	**Length of pitcher (cm)**	**Width of pitcher (cm)**	**Volume of pitcher (cm^3)**	**Volume of fluid in pitcher (cm^3)**	**% volume of pitcher filled up with fluid**
1	7	Pale brown	15	3	105.9	11	10.4
2	7	Light yellow	12	3	84.8	10.7	12.6
3	7	Pale brown	13	3.5	125.0	16.6	13.3
4	6	Pale brown	15	4	188.4	6.5	3.5
5	6	brown	16	5.5	379.9	13.8	3.6

6	6	Pale yellow	15.5	4	194.7	1.1	0.6

13. Equipments used

a) in field works

Item	Name	Quantity
1	Light Meter	1
2	Digital thermohygrometer	1
3	Anemometer (wind meter)	1
4	Compass	1
5	Trowel(spade)	2
6	Plastic bag (transparent)	6
7	Vial, large	8
8	Vial, medium	8
9	Gloves, medical	several pairs
10	Ruler, 1/2 m	1
11	Polythene rubbish bag, black	2
12	Cutter, plant	2
13	Sign pen / marker small for lablling	2
14	Labels, small	1 sheet
15	Camel hair brush(large)	1
16	Camel hair brush(small)	2
17	Ethyl alcohol, 50% (250 ml)	2
18	Syringe (50 ml) + rubber tubing	1
19	Syringe (10 ml) + rubber tubing	2
20	Measuring cylinder, plastic (10 ml)	2
21	Ethyl alcohol, 75% (250 ml)	1
22	Washing bottle (50 ml)	1
23	Torch (with batteries)	1

24	Droppers (plastic)	4
25	Plastic ruler (30 cm)	2
26	Forceps, blunt	2 pairs
27	Sign pen, large (bold)	2

b) in experiments

Item	Name	Quantity	Experiment
1	Stand and clamp	1 pair	1
2	plastic plate	1	1
3	sprinklierx	1	1
4	pitcher plant	3 plants	1, 3
5	photographic negatives	3 pieces	2a
6	microscopic slide	3	2a
7	glass dics	3	2a
8	distill water	/	2a
9	incubator	1	2a
10	digestive fluid	/	2a, 2b
11	dropper	1 6	2a 2b
12	pH paper	2 pieces	2b
13	plastic sampling bottler	6	2b
14	paintbrush	1	3
15	petri dish	3	3
16	ants	/	3
17	tissue paper	/	3