"A NOVEL APPROACH OF FUNGUS DELAYED PREPARATION OF GONGYLIDIA DETERMINED THE LCAs FORAGING DECISION AND THE INVOLVEMENT OF BETA CAROTENE AS A HYPOTHETICAL ASSUMPTION ABOUT COMMUNICATION BETWEEN LCAs AND FUNGUS".

Paul Inbaraj
MSW(NET).,M.Sc (Zoo).,B.Ed.,MBA.,MA(Psy)
Administrator, Academic Head and Counselor
Address: St.John's Mission School
Channi Morh, Udhampur, Jammu & Kashmir-182102.

Abstract: This hypothetical study had been formulated for Jena Microbial School for communication, Jena, Germany on 2009. Leaf Cutter Ants are major pest in the South and Central America causes damage in various fruit plants, weeds and grasses, ornamental plants and cereal crops. Introduced Beta carotene as a nutrient to establish the fungal colony within two days using simple diffusion method by adding mixture in the normal leaves in the form of cyclohexaamide a fungicide, chloroform, antimony tri-chloride to check the LCAs foraging decision and mulch preparation, selection of Beta carotene by the fungus over various mixture and it response to quickened and delayed gongylidial preparation, colony establishment and refused activities eventually to get hypothetical assumption to control the LCAs but allow the fungus to grow and make gongylidia not suitable for LCAs pupa.
Key Words:- Leaf Cutter Ants (LCAs), Fungus, Beta Carotene, Cyclo hexaamide, Chloroform, Antimony tri chloride, Mulch, Gongylidia, Forage.

INTRODUCTION

The leaf cutter ants are of immense importance in tropical and sub tropical ecosystem and are also major pests in cultivated fields of central and south America and harvest about 85-470 Kg (Dry weight) total plant biomass per colony per year, not feasible even for human species to harvest and process such enormous amount of plant material as carried leaf- cutter ants.

A cook (Fungus) is responsible for preparing good meals but the nutritive material (Beta Carotene) and its related goods are being bought by house owner (because he or she has an energy in the form of currency), if the house owner (LCAs) does not chosen a good nutritive material how can cook prepare a good meals even if the food prepared by a cook how can the children grown with hale.

By the way -of, the nutritious edible materials external structure may shows a good appearance but the house owner does not knows the internal one sometimes. At the time of preparation a cook knows the materials with contamination then he does not prepare the food and also he avoids it.

Now the house owner wake up, here after he chosen the good nutritive materials rather than the bleak one finally the fore most activity is relinquished.

MATERIALS AND METHODS

1.Study the Animal:

Leaf cutter Ants found in warmer regions of India ,it comprise two Genera Atta and Acromyrmex ,the width of the head may vary from 1mm to 7mm. Animals are fed with leaves and acclimatized to the laboratory conditions

Division of labor:

1. Sections of leaves are cut from vegetation around the nest by specialized workers and carried away by trails.
2. Another group of specialized workers that process the leaves.
3. The processing of labor reduce the leaf fragments to a mulch which is used to feed the colony's fungus.
4. The queen sits among the fungus garden laying her eggs, when the eggs hatch, the larvae that emerge will eat gongylidia while they are being cared for by specialized nurse workers.
5. The ants provide the fungus with a suitable environment, in that is cultured in nearly axenic or pure gardens (Craven et al., 1974)

2.Study the Fungus:

The fungi cultivated by Leaf Cuttig Ants can be pylogenetically divided into three groups called G1,G2 and G3 by chapela et al (1994).The groups G1 and G3 belong to the Leucocoprineae (Lepiotaceae, Agaricales) symbionts associated with the leaf-

Cutting Ants are basidiomycetous fungi (Leucocoprineae : Basidiomycotina) of the G1 group (Chapela et al., 1994). G1 having evolved gongylidia . The fungus feeds on the mulch and uses the nutrients to grow.

3.Study of Beta carotene:

Beta carotene is a fat soluble compound and also precursor of vitamin A, it has high Bio accessibility and digestive stability (Mark L. Failla, Department of human nutrition , The ohio state university, Columbus, ohio) Beta carotene riches in leaves like Moringa leaves, Rosa leaves, Portulaca leaves and oleaceae leaves (in oleaceae leaves lacking the beta carotene during dry season and it recovered by rainfall) and also present in various leaves with minimum and maximum amount. Chemical formula of beta carotene C40H56 and its molecular weight 536.89. Beta carotene occurs as red purple color to dark red crystals or crystalline powder, having a slight, characteristic odor and taste.

Identification:

1.A solution of beta carotene in cyclohexaamide solution (1-400) does not have an optical rotation.

2.To 0.5 ml of a solution of beta carotene in chloroform (1- 250),. The solution exhibits absorbance maxima at wavelengths of 455-457 nm and 482-484 nm.

3.Dissolve 10 mg of beta carotene in 10 ml of chloroform, which is range in color, add 1 ml of antimony trichloride solution. A green – blue color develops

LCAs and fungus mutualism depend on trophical oriented paraphernalia, the vital and noticeable action is energy transfer from locomotory organism to non locomotory organism vice versa. How the communication is maintained by both of them this is what we have to discuss.

Methodological experiences and experimental methods

a) Ant Sub colonies :

All sub colonies (Acromyrmex) were set up at least one week prior experiments to allow the garden reestablishment. Experiments were performed with sub colonies, establishing approximately 100 cm 3 of fungus garden and approximately 500 workers from large laboratory colony.

In experimental wise the LCAs laboratory and its colony must be separated into 3.The main aspects of laboratory established by 3 plastic boxes and it connected (400 cm 3 each) with by plastic tubes (10 mm diameter 6cm length). The first box served as the feeding areana, the second central box contained the fungus garden and the third box was used by the ants as refuse chamber or dump chambers where dead ants and dead fungus are also placed.

b) Select the leaves for the experimental purpose

(Moringa leaves, Rosa leaves ,Portulaaca leaves, oleaceae leaves)

Chosen Moringa leaves for our experimental purpose; we could separate the leaves three different level.

1.Normal leaves of Moringa oelifera for control.

2.Treated leaves and normal leaves mixed with each other.

3. Only treated leaves.

We offered leaves as disks (6mm in diameter) punched with a paper clipper without any contamination.

c) simple diffusion method – plant physiological studies : Here after we must treat the leaves with fungicidal property cycloheximide solution (Sigma- Aldrich, Disenhofen, Germany) Taken a beaker without any contamination and add 100 gm of 0.02 % cyclo heximide solution in this beaker (high dose of fungicide is expected to lethal for fungus).Then overwhelmed the leaves in cycloheximide solution for half an hour, this time diffusion occurred between leaves and solution. Hence, the cycloheximide successfully invade the leaves. Here after inject the solution with syringe and dry the leaves with paper tissue**.**

d) Presented the leaves at the foraging arena:

There were 3 sets of sub colonies maintained in different laboratory. Normal leaves were introduced and kept in the foraging arena of 1st laboratory. Mixed leaves were introduced into foraging arena of 2nd laboratory. Treated leaves were introduced into foraging arena of 3rd laboratory.

The collected leaf disks were readily processed and incorporated into the fungus garden. During this process gardening and shredded the disks into minute fragments of 1-2 mm 2 in sizes.

e) My experimental methods in columba livia (pigeon) and lactobacillus, experiments can give the idea and natural understanding of beta-carotene.

(i)Experiment with pigeon: The extraction of Moringa oelifera pods and leaves containing beta carotene it has lot of bio accessibility and bio stability. I introduced this extraction intra muscularly (0.5 ml) to pigeon (Columba livia) with susceptible to disease and abnormal (with disease) , the abnormal one died within 24 hours and first one escape from this disease and has been survived**.**

The disease having pigeon has had large yellow color warts (like erysipelas) on throughout its body. I concluded that the carotene was helping the susceptible pigeon from the disease.

Because the extraction contains beta carotene (selected pigeons were same family members and did this experiment with other two pigeons the results were the same)

(ii) Experiment with Lactobacillus : Lactobacillus is a probiotic and it is more helpful in cheese industry and for experimental purpose. 25 ml of Moringa leaves extraction poured to Petri disc without any contamination with suitable environment and introduced the Lactobacillus in the extraction containing Petri plate (using microbiological method). Within 18 to 24 hours the carbon source of beta carotene utilized by the Lactobacillus and established its own colonies**.**

By the way of, I had some questions beyond this experiment.

1. **If the extraction (Beta carotene) merely act as a medium in a susceptible pigeon how can it survive:** The bio accessibility of this carotene may suppress the disease (it meant low quantity of pathogens) and immunologically dissociation constant (Kd) less as well as the antibody affinity is high. Dynamic equilibrium is constant.

2. **If the leaves extraction may toxic (assuming) how can it would be act as a nutritive medium for lactobacillus:** Simultaneously apply this question with treated leaves with low quantity or meager amount of fungicide. We must know low the treated leaves accepted only by the LCAs (Ant can tolerate over the fungicide) not by the fungus, hence foraging decision choice maintained by fungus. Beta carotene acts as a nutritive medium so that Lactobacillus survives and also spread its own colonies.

   Basically the leaves containing carotene, so that foraging workers first communication is tied with the leaves whether treated or not. Two types of carotene transfer.1)Through the excretory droplets of workers (it spreader over by LCAs on the fungal).2)Through fresh leaves shredded by LCAs on the fungal colonies, after that ant prepare the mulch through fresh leaves for the growth of fungus and the fungus directly obtained the carotene through excretory droplets of the ants.

   Thus, two ways predominantly transfer the carotene in abundant level .If the fungus consuming the beta carotene per square centimeter can calculate. Preferably the LCAs select the carotene containing leaves, this action might be an evolutionary related aspects.

Gongylidial preparation with contradiction:

Gonglydial preparation and its starting time were depending on the leaves chosen by the ants, so that fungus determines the suitable leaves to prepare the gonglydia and for their food. So we here clarify one doubt that beta carotene presented in the leaves may after abate one ,so we apply this clarification with my pigeon experiments , the attacked (profuse number of pathogens) pigeon was died due to the abate of beta carotene. Beta carotene act as a nutrient for the growth of fungus and gonglydial preparation at the same time leaves with fungicide does not attacked the ants, so now the beta carotene consumed by the ants without any selection because it may eaten both treated and normal but the selection an intriguing one .,why?
The gonglydial preparation is procrastinated by fungus due to it could not derive the nutrients from normal leaves.
Obviously fungus are highly sensitive to fungicide but the LCAs are tolerate over the fungicide at the same time they could accept treated and normal leaves but fungus avoid the mulch prepared by ants through mixed leaves and treated leaves.

LCAs learning:
Foraging decision also a sensitive one because the LCAs next generation only depend on gongylidia for food or the gongylidia feed the young one of LCAs.

LCAs learning based on the below question:

1. Why the gongylidial preparation is delayed?
2. Why were the mixed leaves or treated leaves (nutrients) not accepted by fungus?
3. Which leaves would be the best one for fungus?

So LCAs learn by hook or crook and it to make good to the fungus needs.

RESULTS:

Box No 1 **Feeding arena**	Normal Leaves With Beta Carotene (1A)	Beta carotene+ Cyclohexamide (1B)	Beta carotene+ Chloroform (1C)	Beta Carotene+ Antimony tri-chloride (1D)
	LCAs Pick up time quickened 5 seconds	Delayed 30 seconds	Moderate 10 seconds	Moderate 20 seconds
Box No 2 **Fungus Garden** **Fungal Colony Established time** **LCAs and Fungus growth**	Accepted (gongylidial formation) (2A) 2 days Normal growth	Hypersensitive/ Delayed (Fungal growth stopped, higher Mortality rate) Selection of beta carotene over Cyclohexamide (2B) Not established LCAs pupa and fungal colony died.	Sensitive, (Used chloroform as a carbon source) (2C) 5 days Both are benefitted	Sensitive (synthetic organic transformation when combined with Vitamin A) test indicates a green blue color. (2D) 6-7 days LCAs pupa growth stopped but fungal colony established
Box 3 **Refused chamber used by LCAs**	Less number of debris.	More number of debris (dead ants and dead fungus)	Moderate	Moderate

	(3A)	(3B)	(3C)	(3D)

Experimental series 1: Pick up time procrastinated due to the first time selection from sub colonies 2 and 3.
LCAs did not learn to segregate the leaves pattern so that pick up time for normal leaves were procrastinated because the fungus still not started the gongylidial preparation**.**

Experimental series 2: Immediate rejection of mulch (from mixed or treated leaves) by fungus.
Hyper sensitivity of fungus to fungicide, so it avoided the mulch because it contains both normal and treated leaves and it might be raising a problem so that fungus protect its own colony from the fungicide property through rejection

Experimental series 3:Fungal gongylidial preparation delayed.
Ant does not learn the necessacity of normal leaves for fungus, so that the gongylidial preparation could not ameliorate by fungus**.**

Experimental series 4: LCAs learning.
LCAs learning was based on, 1) mulch rejection by fungus 2) delayed gonglydial preparation, this reason had to be obliged the LCAs to select normal one.

Experimental series 5: Fungal expectation.
High content of carotene in normal leaves. Fungus expecting the nutritious mulch with carotene for their growth and establishing the colony and it avoids the nutritious mulch with fungicidal property. Synthetic organic transformation using antimony trichloride combined with beta carotene.

Experimental series 6: Ant's excretory droplets utilized by fungus. (Flavonoid metabolism)
LCAs excretory droplets used by fungus because it contains high carbon sources and also little amount of beta carotene, hence fungus establishing the its own colony apart from fresh leaves**.**

Experimental series 7: Bio accessibility of beta carotene.
Enzymes of fungus obviously mingled with beta carotene through the bio accessibility of beta carotene and then they prepared gongylidia as a humus and nutritive material.

REFERENCES:

Aylward FO, Burnum KE, Scott JJ, Suen G, Tringe SG, Adams SM, et al. Metagenomic and metaproteomic insights into bacterial communities in leaf-cutter ant fungus gardens. ISME J. 2012;6 1688–701. pmid:22378535.

Fiona M. Soper,Benjamin W. Sullivan, Brooke B. Osborne,Alanna N. Shaw,Laurent Philippot and Cory C. Cleveland (2019), Leaf-cutter ants engineer large nitrous oxide hot spots in tropical forests, https://doi.org/10.1098/rspb.2018.2504.

Gina R Lewin et al.,(2016). Cellulose-Enriched Microbial Communities from Leaf-Cutter Ant (*Atta colombica*) Refuse Dumps Vary in Taxonomic Composition and Degradation Ability, https://doi.org/10.1371/journal.pone.0151840.

Haines BL. Element and Energy Flows through Colonies of the Leaf-Cutting Ant, *Atta colombica*, in Panama. Biotropica. 1978;10: 270–277.

Herz H, Beyschlag W, Hölldobler B. Assessing herbivory rates of leaf-cutting ant (*Atta colombica*) colonies through short-term refuse deposition counts. Biotropica. 2007;39: 476–481.

Hudson TM, Turner BL, Herz H, Robinson JS. Temporal patterns of nutrient availability around nests of leaf-cutting ants (*Atta colombica*) in secondary moist tropical forest. Soil Biol Biochem. 2009;41: 1088–1093.

Isabelle Boulogne,et al.,(2014). Leaf cutting Ants, Biology and control; Springer International Publishing, DOI: 10.1007/978-3-319-00915-5_1.

Poulsen M, Hu H, Li C, Chen Z, Xu L, Otani S, et al. Complementary symbiont contributions to plant decomposition in a fungus-farming termite. Proc Natl Acad Sci. 2014;111: 14500–5. pmid:25246537.

Rainer Wirth and Wolfram Beyschlag et al.,(2003)Herbivory of Leaf-Cutting Ants: A Case Study on Atta Colombica in the Tropical Rainforest of Panama, DOI: 10.1007/978-3-662-05259-4.

Raphel Vacchi Travaglini, et al.,(2018). Leaf cutter Ants and microbial control DOI:10.5772/intechopen.75134.

Scott JJ, Budsberg KJ, Suen G, Wixon DL, Balser TC, Currie CR. Microbial community structure of leaf-cutter ant fungus gardens and refuse dumps. PLoS One. 2010;5: e9922. pmid:20360970.

Weber NA. Fungus-Growing Ants. Science. 1966;153: 587–604. pmid:17757227.

Wirth, R., **Herz**, H., **Ryel,** R.J., **Beyschlag**, W., **Hölldobler**, B. (2003), Herbivory of Leaf-Cutting Ants A Case Study on Atta colombica in the Tropical Rainforest of Panama, Springer Publishers.