‘Not so natural’ enemies ?

- Biocontrol agents on artificial diets

By

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(2018 – 11 – 120)

MSc. Agrl. Entomology

Seminar report

Submitted in partial fulfilment of requirement of the course

ENT. 591, Seminar (0+1)

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DECLARATION

I, G. Sravanthi (2018 – 11 – 120) hereby declare that the seminar report titled ‘Not so natural’ enemies ? - Biocontrol agents on artificial diets has been completed by me independently after going through the references cited here and I haven’t copied from any of the fellow students or previous seminar reports.

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CERTIFICATE

This is to certify that seminar report titled ‘Not so natural’ enemies ? - Biocontrol agents on artificial diets for the course ENT. 591 has been solely prepared by G. Sravanthi (2018 - 11 - 120) under my guidance, and she has not copied from seminar reports of seniors, juniors or fellow students.

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1. INTRODUCTION

In India, arthropod pests contribute to 30 % yield loss and an annual revenue loss of 36 billion dollars (ICAR, 2018). To regulate this, indiscriminate use of pesticides (54,000 tonnes/year) often leads to resistance, resurgence and residue problems (DPPQS, 2017). Biocontrol offers a viable solution which is relatively specific, eco-friendly, cost effective and permanent. Biocontrol of crop pests is fast evolving as the mainstay of pest management across the world. Annual growth rate of biocontrol market is 14.7% (IOBC, 2018). However, a serious challenge that biocontrol faces is mass production of arthropod natural enemies on live host. The emergence of artificial diets as alternatives to natural hosts could help biocontrol surmount this major obstacle and broad base it.

1.1 Artificial diet

Artificial diet for insects is defined as “a food which is not natural diet of the insect but has been synthesized or processed” (Ishii, 1974). Artificial diets are mainly composed of plant extracts, ground plant material, nutrient mixtures, synthetic additives. They are combined with nutrients and water or entirely of pure chemicals.

1.2 Significance of artificial diet

Artificial diets supports mass production of natural enemies round the year. They have prolonged shelf life of about 4 weeks as in case of *Podisus nigrispinus* (Fritsche, 2005). Frequent handling is not necessary, which results in reduction in mortality. Artificial diets provides cost effective mass production. Bortoli *et al.* (2011) concluded that cost per 250 eggs of *Podisus nigrispinus* on artificial diet was 60 dollars whereas, on natural host it was 133 dollars.

Cost of *Podisus nigrispinus* eggs

Type of diet	Cost per 250 eggs ($)
Artificial diet	60
Natural host	133

Plate 1. ***Podisus nigrispinus***

2. Artificial diets classification

Artificial diets are traditionally classified based on nature of components in to holidic, meridic and oligidic diets and based on nature of formulations into powders, liquid diets, semi-liquid diets and microencapsulations.

2.1 Chemical nature of components

2.1.1 Holdic diets

Holidic diets are chemically defined. They are composed entirely of known pure chemicals. These diets are most desirable for critical nutritional studies. Khanamani *et al.* (2017) studied that holidic diet composed of multivitamin enriched syrup as an alternative food source for rearing *Neoseiulus californicus.*

Plate 2. ***Neoseiulus californicus***

2.2.2 Meridic diets

Meridic diets are composed of a defined chemical and one or more poorly defined components *i.e.*, they are chemically not defined. Chen *et al.* (2011) developed meridic diet composed of tuna fish oil and vitamin complex for predatory pentatomid, *Arma chinensis.*

Most artificial diets used for insect rearing are of meridic type. Several entomophagus insects can now be conveniently reared on such artificial diets (Singh *et al*, 1982).

Plate 3. Tuna fish oil

Plate 4. *Arma chinensis*

2.2.3 Oligidic diets

These diets are composed of crude organic material. Meat based diet composed of ground beef, beef liver and chicken yolk is used for cost effective mass rearing of *Chilocorus nigritus. (*Hattingh and Samways, 2000)

Plate 5. *Chilocorus nigritus*

2.2 Nature of formualtions

2.2.1 Powders/fragments Eg : Wheat germ based diets for *Thanasimus dubius*

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Plate 8. *Orius thirboporus*

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Plate 9. *Propylea japonica*

3. Natural enemies on artificial diets - Examples

3.1 Predatory insects

Unlike parasitoiods, there is no special consideration for respiration to be met by artificial diets. Liquid diets that are fully or semi-defined are presented within wax capsules can be used for mass rearing of predators (Neuroptera and Hemiptera).

3.1.1 Artificial diet for *Mallada basalis* larva

Green lacewing, *Mallada basalis* has the potential to be a valuable biological control agent because of its predatory abilities, strong reproductive capacity, and broad prey range. Larva predatory on soft bodied arthropods while, adult feeds on pollen and nectar. Ye *et al.* (2017) analysed development, reproduction of *M. basalis* was on two different diets (AD1 and AD2) which are modified form of standard diet (AD0).

Table 1. Composition of *Mallada basalis* larval diet

Ingredients	Amount(g)		
	AD0	AD1	AD2
Chicken egg yolk	40	40	40
Beer yeast powder	30	30	30
Honey	20	20	20
Pottasium sorbate	0.1	0.1	0.1
Trehalose	1	1	1
Sucrose	9	9	0
Vitamin C	0.1	0.1	0
Sea water spirulina	0	1	1

Table 2. Development and reproductive parameters of *Mallada basalis* on different diets

Diet	Developmental time (days)	Preoviposition period (days)	Oviposition periods (days)	Fecundity (eggs/female)
AD1	22.02 ± 0.81	7.57 ± 1.25	38.86 ± 6.07	481.29 ± 54.40
AD2	26.62 ± 0.96	12.14 ± 1.87	31.57 ± 6.05	307.14 ± 60.77

The developmental time of *M. basalis* larvae reared on AD1 were significantly shorter than those reared on AD2. Oviposition period, fecundity were significantly higher on AD1 as sucrose enhanced fecundity of the predator.

Plate 10. Adult of *Mallada bas*alis

Plate 11. *Mallada basalis* larva

3.1.2 Artificial diet for adult green lacewing, *Chrysoperla carnea*

Green lacewing, *Chrysoperla carnea* is a voracious predator of insect eggs and various soft bodied insects. Experiments were conducted to find out a better and cheaper alternative for mass rearing of adult *C. carnea*. Ulhaq *et al.* (2008) tested three adult diets under laboratory conditions in comparison with standard diet; that was mixture of yeast extract, casein, honey, sugar and distilled water. Parameters were fecundity and adult longevity. The results revealed that the mixture of egg yolk, milk and honey was better than all other diets due to higher protein content in egg yolk.

Plate 12. Adult of *Chrysoperla carnea*

Plate 13. Stalked eggs of *Chrysoperla carnea*

Table 3. Composition of artificial diets for adult *Chrysoperla carnea*

Diet	Ingredients	Weight/ Volume

Standard diet	Sugar : yeast extract: honey: distilled water: casein	3.5g: 2.5g: 2.5g: 10 ml: 2.5g
A	Egg yolk: milk: honey	5ml: 10ml: 5ml
B	Egg white: milk: honey	5ml: 10ml: 5ml
C	Mixed egg: milk: honey	5ml: 10ml: 5ml

Fig 1. Effect of artificial diets on the fecundity of *Chrysoperla carnea*

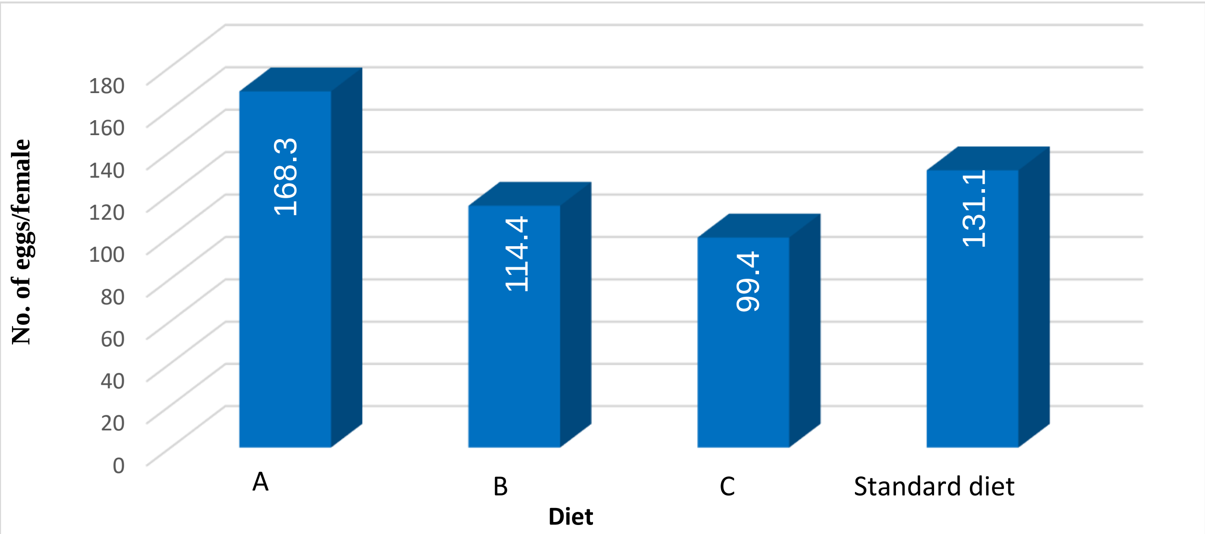
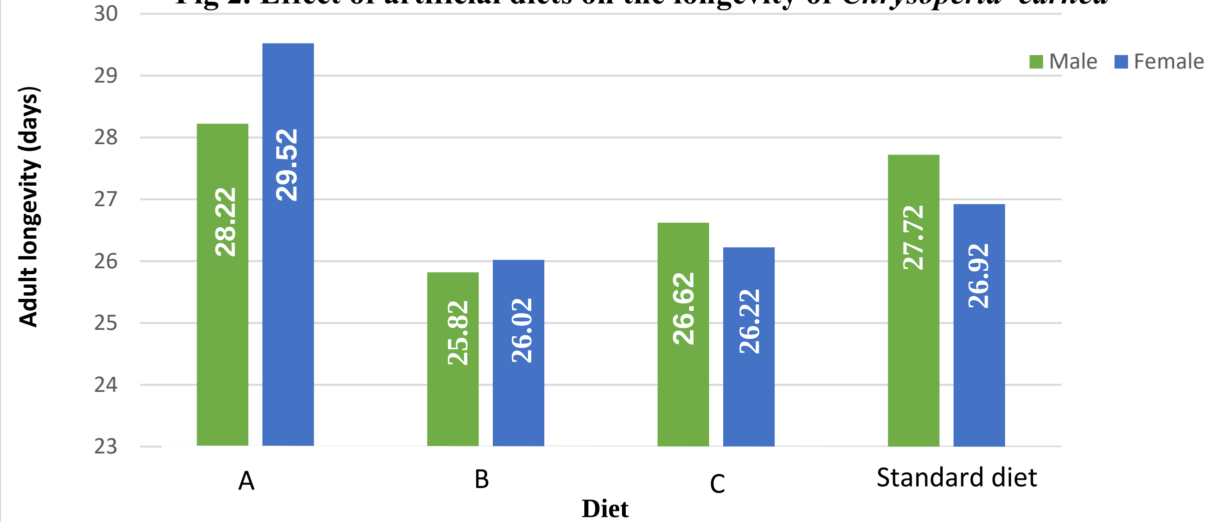


Fig 2. Effect of artificial diets on the longevity of *Chrysoperla carnea*



3.1.3 Artificial diet for Australian ladybird beetle, *Cryptolaemus montrouzieri*

The specialist predatory ladybird *Cryptolaemus montrouzieri* is an effective natural enemy of mealybugs.. However, its mass production is complicated by the dependence on parallel cultures of mealybugs. Xie *et al*. (2017) developed a pollen based artificial diet as an alternative food for the predator. The artificial food supported the development and reproduction of *C. montrouzieri*. Although the developmental time and preoviposition period of the predator on the artificial food were longer than on the natural prey.

Table 4. Composition of artificial diet for *Cryptolaemus montrouzieri*

Ingredients	Weight (g)
Bee pollen	6
Mediterranean flour moth eggs	2
Sucrose	1.5
Yeast extract	1

Table 5. Effect of different diet on reproductive biology of *Cryptolaemus montrouzieri*

Diet (larval + adult)	Pre-oviposition period (days)	Egg production (mean no.)	Egg hatchability (%)
Planococcus citri + *P. citri*	**9.47 ± 0.35**	225.60 ± 38.26	83.20 ± 1.17
P. citri + Artificial diet	12.72 ± 1.04	230.55 ± 31.38	81.19 ± 0.93
Artificial diet + *P. citri*	**9.40 ± 0.81**	221.50 ± 21.03	83.50 ± 1.51
Artificial diet +Artificial diet (F0)	15.10 ± 1.59	188.20 ± 23.08	81.60 ± 1.46
Artificial diet + Artificial diet (F1)	13.20 ± 1.59	195.50 ± 14.81	83.25 ± 3.35

Table 6. Artificial diets for predators

Predator	Type of diet	Reference
Orius laevigatus (Hemiptera: Anthocoridae)	Oligidic	(Arijs and Clercq, 2004)

Rhynocoris marginatus (Hemiptera: Reduviidae)	Oligidic	(Sahayaraj, 2006)
Amblyseius swirskiii *(*Mesostigmata: Phytoseiidae)	Oligidic	(Nguyen *et al.*, 2014)
Propylea japonica (Coleoptera: Coccinellidae)	Meridic	(Tan *et al.*, 2014)
Chilocorus nigritus (Coleoptera: Coccinellidae)	Meridic	(Cheng *et al.*, 2011)

3.2 Parasitoids

Unlike predators, rearing of parasitoids on artificial diets is complex. Besides nutritional requirements, medium must provide for respiration and excretion (Grenier, 1994). *Itoplectis conquisitor*, an endoparasitoid was the first natural enemy to be reared on artificial diet (Yazgan, 1972).

Plate 16. Artificial diet medium

Plate 17. *Itoplectis conquisitor*

3.2.1 Artificial diet for *Exorista larvarum*

Plate 18. Larva of *Exorista larvarum*

Plate 19. *Hermetia illucens*

Table 7. Effect of artificial diets on development of *Exorista larvarum*

Sl. No	Medium	Egg hatching (%)	Puparium weight (mg)	Egg to pupa (days)	Pupa to adult (days)
1	BEM	61.30	45.65	13.00	9.20
2	BEM+ haemolymph of *H. illucens*	67.20	**50.80**	10.00	9.00
3	BEM+ haemolymph of *A. pernyi*	**70.20**	38.96	**9.57**	**9.00**

Table 8. Effect of artificial diets on reproductive parameters of *Exorista larvarum*

Sl. No	Medium	Ovipositing female (%)	Pre-ovipostion (days)	Oviposition in 10 days (eggs/ female)	Adult emergence (%)
1	BEM	60.00	4.67	18.00	72.2
2	BEM+ haemolymph of *H.illucens*	**100**	3.56	54.85	88.2
3	BEM+ hamemolymph of *A. pernyi*	**100**	**3.00**	**58.00**	**90.2**

Table 9. Artificial diet for parasitoids

Natural enemy	Type of artificial diet	Reference
Trichogramma brassicae (Hymenoptera: Trichogrammatidae)	Meridic	(Grenier, 1994)
Campoletis sonorensis (Hymenoptera: Ichneumonidae)	Meridic	(Vinson and Hu, 1998)
Bracon hebator (Hymenoptera: Braconidae)	Oligidic	(Magro and Parra, 2004)

Dinocampus coccinellae (Hymenoptera: Braconidae)	Meridic	(Silva *et al.*, 2009)
Cotesia flavipes (Hymenoptera: Braconidae)	Holidic	(Bortoli *et al.*, 2012)

3.3 Predatory mite

3.3.1 Artificial diet for predatory mite, *Amblyseius swirskii*

Amblyseius swirskii (Acari: Phytoseiidae) is a generalist predatory mite against thrips and whiteflies in a range of greenhouse crops. However, the rearing procedures of phytoseiid mites based on these factitious foods are often time-consuming and allergy problems can be generated using storage mites. The survival, development and reproduction of the predatory mite were assessed when fed on artificial diets composed of honey, sucrose, tryptone, yeast extract and egg yolk, supplemented with haemolymph of dried fruit mite. The females that were fed with the enriched diets from the adult stage on had higher oviposition rates and fecundities than those maintained on the basic diet (Nguyen *et al.*, 2012).

Table 10. Composition of *Amblyseius swirskii* artificial diet

Ingredients	AD1 (%)	AD2 (%)
Honey	5	5
Sucrose	5	5
Tryptone	5	5
Yeast extract	10	10
Egg yolk	10	10
Distilled water	70	70
Haemolymph of dried fruit mite	-	20

Table 11. Effect of different diets on developmental and reproductive parameters of *Amblyseius swirskii*

Diet	Developmental time (days)	Oviposition period (days)	Fecundity (eggs/female)	Female longevity(days)

Dried fruit mite	**7.00 ± 0.07**	24.68 ± 1.65	29.03 ± 1.72	35.90 ± 2.05
AD1	7.57 ± 0.11	22.81 ± 3.02	25.94 ± 2.26	29.56 ± 3.52
AD2	7.26 ± 0.11	**25.67 ± 2.05**	**38.26 ± 2.89**	**42.07 ± 2.63**

4. Advances in artificial diets for natural enemies

Advances in artificial diets for natural enemies include wax artificial eggs and microencapsulations.

4.1 Wax artificial eggs (WAEs)

The Chinese first developed *in vitro* rearing of Trichogramma in commercial production by using wax artificial eggs. An artificial host egg was developed as a first attempt to improve the techniques for *in vitro* rearing of *Trichogramma galloi* Zucchi and *T. pretiosum* Riley (Hymenoptera: Trichogrammatidae). The eggs are composed of oligidic diet containing 25-70% *Manduca sexta* egg liquid. Plastic membranes (polyethylene, polypropylene and polystyrene) of different thickness are used in manufacturing artificial eggs, using a thermal system to produce semi-spherical capsules on the plastic surface. High-density polyethylene 7–8 and 9–10 µm thick resulted in the best acceptance The size, shape and surface texture of the artificial eggs were found to provide enough cues to elicit parasitization behavior in Trichogramma females, since no chemical stimulus was provided.

Table 12. Composition of wax artificial egg (WAE) for *Trichogramma minutum*

Component	Proportion (%)
Yeast Extract solution (7 percent)	10
Free amines	5
Suspension of non fat dry milk (10 percent)	15
Chicken egg yolk	25
Chicken embryo extract	15
Manduca sexta egg liquid	30

4.2 Production of Wax artificial eggs (WAEs)

The paraffin and Vaseline mixture in the ratio of 3:1 along with liquid artificial diet is taken in a glass tube. It was subjected to thermal treatment of approximately 65°c. Later, the mixture was pippeted on to a glass slide to form wax artificial eggs of 2-3 mm in diameter. These eggs are exposed to trichogrammatids for oviposition. The parasitized eggs along with some diet were transferred on to a culture plate and kept in the dessicator for the excess moisture to evaporate. After 10-12 days, pupae of trichogrammatids will be formed on wax artificial eggs.

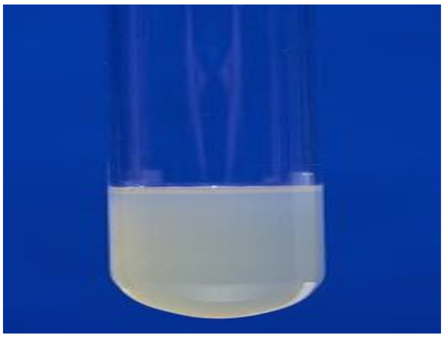


Plate 22. Paraffin - Vaseline mixture and diet

Pippeting

Plate 23. WAEs

Plate 24. *T. minutum* laying eggs on WAE

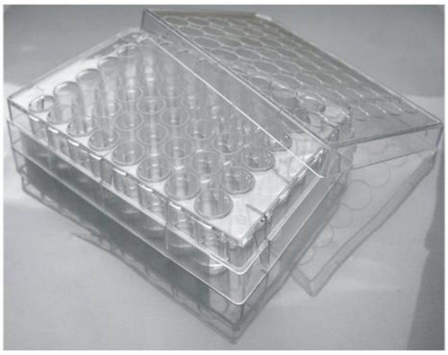


Plate 25. Culture plate

Plate 27. Dessicator

Plate 26. Pupae in WAE s

4.1.3 Automated production WAEs

A computer controlled machine automatically completes all five egg production processes: 1. Setting up the synthetic membrane, 2. Forming the egg shells, 3. Injecting the artificial medium in to the shells, 4. Sealing the double layered membrane, 5. Separating the egg cards. The production capacity of the machine is 1,2000 egg cards/hour, which can be used to produce 6-7×10² Trichogramma. About 5× 10² Trichogramma can be reared on one litre of the oligidic diet. The existing form fill seal machine operating for six hours per day and seven days per week, the weekly production would be 4.2 billion. A more modern machine with five times that capacity would increase production to 21 billion per week.

Plate 28. Conventional mass rearing unit Plate 29. Automated production unit of WAEs

4.2 Microencapsulation

Artificial diet optimization is a key aspect in mass rearing of natural enemies since it influences the quality and feeding effectiveness, and thus the success of the biological control program. Although most artificial diets have been made in liquid form due to ease of mixture and insect ingestion, the adhesive nature of the material also increases mortality (Zhang et al., 2008). Furthermore, moisture loss in liquid and solid artificial diets is a problem when exposed during storage and the course of feeding. Without any protective packaging both liquid and solid artificial diets can be easily contaminated and lose nutritional value during storage. These shortcomings have hindered development of artificial diets in scalable biological control. To address some of these negative features of liquid artificial diets, the microencapsulation technique was introduced for improving physical properties (Thompson, 1999).

4.2.1 Microencapsulated diet for *Orius sauteri*

The predacious flower bug, *Orius sauteri* (Heteroptera: Anthocoridae) can be efficiently used to control hemipteran, thysanopteran and lepidopteran pests. However, a key limitation to the extensive application of *O. sauteri* as a means of biological control, is the lack of understanding of the requirements for rearing stable laboratory populations of *O. sauteri*, which is required for the mass production of these insects. However, a natural prey diet was unable to support the *O. sauteri* population throughout the year because of environmental condition limitations and the need to rear prey. Tan *et al.* (2013) developed an integrated artificial diet recipe using microencapsulation for the mass rearing of *O. sauteri* for commercial biological control application.

Plate 30. *Orius sauteri*

Table 13. Artificial diet for *Orius sauteri*

Diet ingredients	Material for microencapsulation
Egg yolk (40 g)	Sodium alginate 2%
Tussah pupa (50g)	Chitosan 0.6%
Sucrose (5g)	Calcium chloride 1%
Honey (5g)	
Rapeseed pollen (10g)	

4.2.2 Microencapsulation process

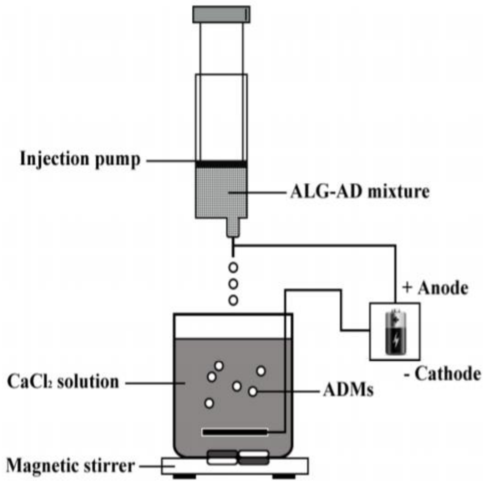


Plate 31. Microencapsulation

A general complex coacervation method was used to make the artificial diet microcapsules (ADMs). The optimal basic chemical materials for artificial diet microencapsulation for feeding *O. sauteri* to be as follows: sodium alginate 2%, chitosan 0.6% and a 13:1 ratio of core material to wall-forming material. The ADMs were made as follows: a 2% mass concentration calcium chloride solution was prepared using distilled water. A prepared artificial diet solution and quantificational sodium alginate were mixed using a magnetic stirrer for 5 min at 3000 rpm to ensure uniform mixing. The mixture was then added to a Top-5300 model medical micro-injection pump. After turning on the power, the injection pump dropped the mixture into the calcium chloride solution under 5 bars. The dropping speed could be controlled by adjusting the pressure of the compressed air. The ADMs prepared were maintained in calcium chloride solution for at least 5 min for stability, and were then moved to chitosan solution and shocked regularly for 13 min. This resulted in calcium alginate-chitosan-sodium alginate colloid particles that were kept in 0.15% sodium alginate solution for 30 min and then put into a 0.055 mol/L sodium citrate solution for 10 min. Finally, the ADMs were conserved in airproof plastic bags and stored at 561uC until required.

5. Challenges in the use of artificial diet

Artificial diets for entomophagous arthropods contain a balanced set of nutrients suitable for the growth of contaminant microbial agents such as bacteria and fungi.. In addition, metabolic by products produced by the microbial contaminants are often toxic for the arthropods. Cohen (2004) listed 17 species of bacteria and nine species of fungi commonly found in insect artificial diets. The same publication, presents an excellent list of chemicals that have been utilized to control microbial_growth in insect artificial diets. Artificial diets likewise should not be used during host-specificity testing of entomophagous natural enemies because behavioral characteristics, triggered in response to kairomones or other semiochemicals produced by the host insect or host plant, may be altered. Chelonus *sp.* parasitoids of pink bollworm exhibited diapause when mass reared on artificial diet (Biobest, 2005). The loss of genotypes in natural enemies reared on artificial diet is considered especially dependent on the founder effect, a random event where there is initially a very restricted gene pool resulting from the selection of few founder individuals. The initial variation in the new laboratory culture will depend on the size of the sample "the larger the original sample, the smaller the deviations of the sample from the original gene frequencies; the smaller the sample, the greater the observed deviation"

6. Conclusion

Mass rearing of arthropod natural enemies using artificial diets is a nascent science. However, with the advances in insect genetics, nutrition and ethology, the day is not far off when mass production of arthropod natural enemies on artificial diets will be the norm rather than exception….

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8. Discussion - [Questions & Answers]

8.1 How many species of arthropod natural enemies can be mass reared on artificial diets?

Approximately 130 entomophagous insects can be mass produced on artificial diets, out of which, more than 20 species are trichogrammatids. Nearly, 15% of artificial diets are concentrated on natural enemies of order Diptera and Hymenoptera.

8.2 Expalain the difference between artificial diet requirements for predators and parasitoids?

Artificial medium for parasitoids must provide for respiration and excretion besides satisfying the nutritional requirements. In case of egg parasitoids, artificial egg shell has to be permeable to oxygen and carbon dioxide, but not to water vapour, in order to avoid desiccation. For predators, there is no special requirement for respiration to be met by artificial diets.

8.3 You have mentioned about artificial eggs. Is there any resaerch on artificial larva?

Artificial larvae are used for rearing of the predatory stinkbugs Podisus *spp.*, cylindrically shaped “artificial larvae”, were produced by bringing thawed or fresh diet onto a stretched Parafilm M sheet and wrapping a single layer of the Parafilm around the meat-based diet paste. For *Orius laevigatus*, the diets were encapsulated in Parafilm M to form small hemispherical domes sealed with transparent tape. The artificial larva increased the acceptance and reproductive parameters of the predator.

8.4 Does the classification of artificial diets similar to that of insecticides?

Artificial diets are classified based on nature of formulations in to solid diets, liquid diets and microencapsulations in the same way insecticides are classified in to granules, dusts, wettable powder formulations.

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ENT 591: Masters Seminar

Name	:	G. Sravanthi	Venue	:	Seminar Hall
Admission No	:	2018 - 11- 120	Date	:	31- 10- 2019
Major Advisor	:	Dr. Madhu Subramanian	Time	:	11.30 am

'Not so natural' enemies ? - Biocontrol agents on artificial diets

Abstract

Biocontrol of crop pests is fast evolving as the mainstay of pest management across the world. However, ensuring the availability of bioagents, particularly arthropod natural enemies at a reasonable cost remains a serious challenge. Arthropod bioagents are predominantly multiplied on live hosts which limits the possibilities of scaling up the production and also leads to increased costs. The emergence of artificial diets as alternatives to natural hosts could help biocontrol surmount this major obstacle and broad base it.

Artificial diet for insects is defined as "a food which is not natural diet of the insect but has been synthesized or processed" (Ishii, 1974). They are classified based on chemical nature of components in to holidic, meridic and oligidic diets and based on nature of formulations into powders, liquid diets, semi-liquid diets and microencapsulations.

Several predators and parasitoids have been successfully reared on artificial diets. *Itoplectis conquisitor*, a hymenopteran parasitoid was the first arthropod natural enemy to be multiplied on artificial diets (Yazgan, 1972). Several studies have underscored the significance of composition of artificial diets on the performance of the natural enemies reared on them. Ye *et al.* (2017) evaluated artificial diets for the larvae of *Mallada basalis* and observed that sucrose enhanced fecundity of the predator. The fecundity and adult longevity of *Chrysoperla carn*ea likewise were significantly higher on diet with egg yolk (Ulhaq *et al.*, 2006).

Rearing of parasitoids is more complex compared to that of predators. The immature stages of many parasitoids practically live in their diet and the diet, therefore, has to meet not only the nutritional need, but also respiratory and excretory needs of the parasitoid. A number of reports suggest that compounds in the host haemolymph are of significance to the quality

attributes of the parasitoid. The tachinid parasitoid *Exorista larvarum*, for instance, showed greater fecundity when mass multiplied on basic diet supplemented with haemolymph of its host, *Antheraea pernyi* (Dindo *et al.,* 2016). Similarly, Nguyen *et al.* (2012) concluded that the predatory mite, *Amblyseius swirskii* when fed on artificial diet containing haemolymph of dried fruit mite *Carpoglyphus lactis*, recorded higher fecundity and female longevity.

Advances in formulation of artificial diets, such as the wax artificial eggs (WAEs) for trichogrammatids (Greenberg *et al.,* 2000) and microencapsulated diet for the predatory *Orius sauteri* (Tan *et al.,* 2013) opens up exciting possibilities in mass multiplication of arthropod natural enemies.

In spite of their obvious advantages, use of artificial diets for mass production of biocontrol agents does involve challenges like microbial contamination, cannibalism, diminishing biological attributes *etc.,* Mass rearing of biocontrol agents on artificial diets is a nascent science. Yet, with advances in insect genetics, nutrition and ethology, the day is not far off when mass production of arthropod natural enemies on artificial diets will be the norm rather than the exception.

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