UGC MAJOR RESEARCH PROJECT
ON

DEVELOPMENT OF CHEAPER AND ECO-FRIENDLY MYCO-INSECTICIDE FOR EFFECTIVE MANAGEMENT OF MAJOR PESTS IN RICE

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Submitted by

Dr. Y.HARIPRASAD

Professor in Entomology and

Principal Investigator


WITH COURAGE AND FAITH

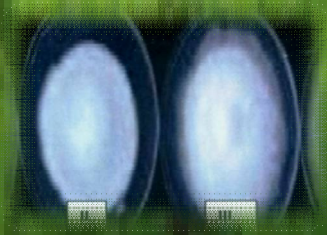
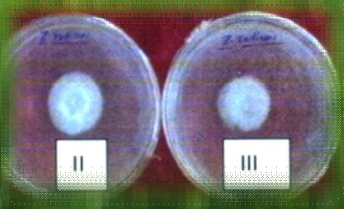
DEPARTMENT OF ENTOMOLOGY

FACULTY OF AGRICULTURE

ANNAMALAI UNIVERSITY

ANNAMALAINAGAR – 608 002

TAMILNADU

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Development of cheaper and eco-friendly myco-insecticide for effective management of major pests in rice

FINAL REPORT

Submitted By

Dr. Y. HARIPRASAD

Professor & Head, Principal Investigator

DEPARTMENT OF ENTOMOLOGY

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TAMIL NADU – INDIA

DEVELOPMENT OF CHEAPER AND ECO-FRIENDLY MYCO-INSECTICIDE FOR EFFECTIVE MANAGEMENT OF MAJOR PESTS IN RICE

Introduction

Rice is the most important crop of India, which occupied 39.16 million hectares area with a production of 85.59 million tonnes and average yield of 2.2 t ha^{-1} (Anonymous, 2013). Productivity of rice in Punjab is 3.8 t ha^{-1} with a production of 10.5 million tonnes from an area of 2.8 million hectares (Anonymous, 2013). Rice is a stable food for more than 65 per cent of the people in India and cultivated under diverse agro-climatic conduction. Based on the present rate of population growth of 1.5 per cent, the demand for rice is expected to be about 110 million tonnes (mt) by 2020

which involves an annual increase of 2.0 – 2.5 mt in milled rice production (Dass, 2006). To achieve this target it requires increasing the productivity per unit area by the adoption of tested methods, cultural and management practices. Insect-pests and diseases pose a very serious challenge in improving the productivity and achieving sustainability.

The chemical pesticides have so far used to contain this menace. To avert the heavy input of pesticides and their effect on non target organisms, use of biological substances sought for. Among the biological substances, microbials play major role in suppressing pest populations.

Of the several microbial pathogens *viz*., bacteria, fungi, viruses, protozoans and entomopathogenic nematodes reported, only a few have been studied systematically for their usefulness. A careful evaluation of this beneficial pathogen can lead to gainful exploitation in microbial control programmes (Burges, 1998). India is bestowed with a rice biodiversity of entomopathogens and exploitation of this natural and renewable resource is essential in a successful bio control strategy.

Not long after the discovery of fungi as the cause of some insect diseases, it was proposed to use fungi as bio control agents. Hence, major motivations still exist for the exploitation of entomopathogenic fungi in the management of insect pests. Entomopathogenic fungi are specific natural enemies of insects pests and infect their hosts by penetration through the cuticle and growth of hyphae. The entomopathogenic Zygomycete *Zoophthora radicans* (Brefeld) Batko was encountered worldwide infecting diverse hosts of economic importance such as rice leaffolder, *Cnaphalocrocis medinalis* Guenee, spruce budworm, *Choristoneura fumiferana* (Clem.) and the brown planthopper *Nilaparvata lugens*

Entomopathogens was mass produced using the diphasic liquid-solid fermentation technique developed for the LUBILOSA (Lutte Biologique contre les Locustes et Sauteriaux, www.lubilosa.org) project to produce *Metarhizium flavoviride*. The liquid phase provides active growing mycelia and

blastospores, while the solid phase provides support for development of the dry aerial conidia. The conidia produced by these fungi was used directly as natural granules or extracted through sieving and formulated.

Based on the above facts, present study is planned to develop cheaper and ecofriendly myco insecticide for the effective management of major pests of rice like leaffolder, stem borer and brown plant hopper.

OBJECTIVES

1. To map and identify the potential fungal pathogens available in coastal rice ecosystem of Tamilnadu and its correlationship with weather parameters
2. To enhance the efficacy of promising fungal pathogens through mutagenesis
3. To evaluate various natural substrates *i.e.* Prawn & fish scales and other agro wastes for mass production of major potential fungal pathogens
4. To formulate and study the virulence and efficacy of selected entomopathogenic fungi under lab and field conditions
5. To test the safety levels of fungal formulations on non target organisms
6. To develop cheaper and eco friendly myco insecticide for management of major rice pests to minimize the losses.

Experiments for first year

Experiment. I - Survey and collection of Rice pests to identify potential major rice pests

Experiment. II - Survey and collection of cadavers to identify promising Entomofungal pathogen

Experiment. III - Correlation studies of potential cadaver incidence with weather parameters

Experiment. IV - Pathogenicity test (Lab studies)

Experiment. V- Evaluation of cheaper and coarse food grains, fish and prawn scales waste for mass production and growth characteristics of Different fungal pathogen

Experiment. V - Genetic improvement of strains to produce virulent and resistant spores

Experiment. V- Evaluation of cheaper and coarse food grains and Agrowastes for mass production of different fungal pathogens

Objectives for Second and third year

1. To evaluate various natural substrates *i.e.* Prawn & fish scales and other agro wastes for mass production of major potential fungal pathogens
2. To formulate and study the virulence and efficacy of selected entomopathogenic fungi under lab and field conditions
3. To test the safety levels of fungal formulations on non target organisms
4. To develop cheaper and eco friendly myco insecticide for management of major rice pests to minimize the losses.

METHODOLOGY

1.Survey to identify and document the major rice pests and fungal pathogens available in coastal rice ecosystem of Tamilnadu

Preliminary surveys were conducted to identify the major rice pests in three districts of Tamilnadu. Based on physical observation, damaged symptom, sweep net sampling and collection in the random survey revealed presence of Rice leaffolder, Rice Stem borer and Brown plant hopper in the fields. Based on overall samples counting Rice leaffolder was found to be the higher incidence in the samba season. As the occurrence of the entomopathogenic fungi is promoted by the humid climate in the cold season of the year, field surveys were taken up during Rabi (Samba) season during October 2013 – February 2014 from different rice fields in coastal areas of Tamilnadu. Collected cadavers were stored in sterile petriplates and glass vials with proper labeling.

Isolation

The infected insect pests were collected from the rice fields and surface sterilized in 70% ethanol for 10 seconds followed by 2% sodium-hypochlorite solution for two minutes and washed with sterile distilled water for two minutes. The cadavers are then transferred to synthetic medium, Sabouraud Dextrose Agar with yeast extract (SDAY) (Keller, 1994).

Identification

The isolated fungi were ascertained through reliable sources and standard procedures. Fresh cadavers of different pathogenic forms with characteristics of the original specimen were gathered both from the rice fields and glasshouse and each specimen was arranged in a clear, sterile plastic vial and tightly packed following usual precaution to avoid contamination. Besides, fungal cultures in slides, plastic petridishes and flask with broth were also sent for identification.

Storage

To assure viability, isolation of the pathogen was made from the diseased cadavers as soon as collected from the field and the fungal cultures were protected under refrigerated conditions to avoid

saprophytic fungal and bacterial contaminants occurring over entomophathoralean cadavers. Freshly inoculated media were kept at 20-25^{o}C for two weeks in order to secure consistent growth of the initial fungal inoculums. The cultures were transferred to fresh media at fortnight interval. Further the virulence of the culture also maintained by passing it through the live host after every fourth successful sub-culturing to obtain a fresh culture as per standardized procedures (Udayaprabhakar, 1995).

Observations recorded

Date, Place of collection, Cadaver name, Number of cadavers collected from each location, Variety, etc

Practical utility

It provides the information on natural incidence of major pests and different fungal pathogens on rice pests in coastal ecosystem of Tamilnadu.

2. Correlation studies on influence of weather parameters on the incidence of Entomopathogenic fungus

Based on information collected, Annamalainagar was found to be ideal for further studies. Initial studies on weather parameters of five selected locations of the hot spots were collected from Cuddalore district to correlate the natural incidence of cadavers (Fungal infected dead insect pests). Correlation and regression studies were carried out to study the relationship between natural occurrences of leaffolder and BPH and stem borer cadavers with climatic factors like maximum and minimum temperature, relative humidity, rainfall, wind speed and sunlight hours.

Observations recorded

Regular weather data related to temperature, relative humidity, rainfall, wind speed and sunlight hours were collected along with number of cadavers available at regular intervals

Practical utility

This experiment was facilitated to learn the influence of weather factors including various levels of temperatures and relative humidity on incidence, multiplication of fungal pathogens in particular location. The results from the study were led to the development of the corrective measures and strategies to identify the hot spots of maximum incidence of cadavers.

It was found from the three districts surveyed, maximum incidence of fungal cadavers was noticed in Cuddalore district especially leaffolder. It may be due to higher relative humidity and low temperature in this coastal district which were ideal climatic factors for incidence and multiplication of leaffolder. Leaffolder infection was very high in Cuddalore district (Coastal area) when compared to Thanjavure district (80 km) and Ariayalure (120 km) which were far away from the Cuddalore.

3. Pathogenicity test to evolve promising entomopathogens

Different isolates of the fungus isolated from different places were grown on the Sabouraud Dextrose Agar Yeast extract (SDAY) medium separately and incubated at 25^{o}C for 7 days to attain complete sporulation. Spore suspension of each isolate was obtained by washing the culture plates with 75-100 ml of sterile distilled water containing 0.02% Tween 80, a spreading agent (Rombach *et al*., 1986) and filtered through double layered muslin cloth and centrifuged 3000 rpm for 20 minutes and then resuspended in 0.02% Tween 80 solution. The appropriate spore suspension was ascertained with an improved Neubaer haemocytometer and arrived at as 107 spore's ml-1 (Narayanasamy, 1994). The per cent mycosis of the test insect over the control was computed. The isolate showing highest percentage

mycosis were re isolated in pure culture from the diseased cadavers showing typical mycosis while confirming the pathogenicity of the fungus.

Observations recorded

Number of infected cadavers from each isolate of each fungus and data recorded at regular intervals, Spore count of various fungal pathogens at different concentrations.

Practical utility

The potential fungal pathogen spores were identified. This experiment was enable to identify ideal and potential fungal pathogen to suppress the rice pests and thereby preventing damage loss and increase the yield and above all minimizes the use of chemical pesticide.

4. Genetic improvement of strains to produce virulent and resistant spores

Rearing insects:

The rice leaffolder, *C. medinalis* (L.) larvae cadavers were collected from rice fields of coastal districts of Cuddalore district and *Zoophthora radicans* was isolated from rice leaf folder cadaver and cultures were maintained at Department of Entomology, Annamalai University for further studies.

Irradiation technique:

Gamma cell irradiated unit located at Centre for Application of Radioisotopes and Radiation Technology in Mangalore University. Cobalt was the irradiation source of gamma rays used in the present study with a dose rate of 50, 100 Gy were applied to the *Z. radicans* culture to induce mutation and also to improve its virulence.

Fungal isolation and preparation:

Spores of *Z.radicans* were harvested by rising with sterilized distilled water, collected spores were filtered through muslin cloth to reduce clumping spores suspended in sterilized water were counted using a haemocytometer. Two different concentrations were prepared; 10^4 and 10^8 spores per ml. Each concentration was used for treatment for the larvae of rice leaffolder, *C. medinalis*

Larval mortality determination:

The larvae of rice leaf folder which were fed on potted rice plants treated with both fungal concentrations and gamma irradiation different doses were daily observed to calculate the surviving larvae. Larval mortality percentage was recorded daily after treatment for four periods; 24, 48, 72 and 96 h and each treatment were triplicated. Mortality percentages were corrected with Abbott's formula.

Biological studies:

After larval mortality calculation, the survived ones were transferred into small cages (25×25×15 cm) and kept under laboratory conditions to determine survived larvae, pupation, emergence and sex ratio percentages.

Statistical analysis:

The data obtained from the present study were statistically analyzed, whenever the calculated "F" values were significant at 5% level.

Observations to be recorded

Variations in the performance of different strains under different levels of Gamma rays, To record formation of extra spores due to mutants, comparison of active period, pathogencity with non treated strains. The best strain identified from above tests will be re tested for its enhanced performance.

Practical utility

These studies will provide stronger, resistant strains of fungal pathogens to produce more number of spores viable for longer duration.

5. Evaluation of cheaper and coarse food grains and Agrowastes for mass production of different fungal pathogens

The crushed grains of sorghum**,** Bajra, Maize, Ragi, Broken rice and Wheat with 1 per cent yeast extract were assessed for their suitability as substrates for mass production of individual fungus. In addition to grains, agro waste *viz.*, Crushed maize cobs, Wheat bran, Rice bran, Baggase and Press mud with and without molasses were also tested. To each of these substrates, sterile distilled water was added in order to bring the moisture content to 50 per cent. After thorough mixing, the bottles were plugged with cotton and autoclaved at 15 psi and 121°C for 30 minutes. Circular agar discs of 5 mm diameter were taken from the eight day old fungal culture grown on SMAY plates. One disc was inoculated to each bottle and mixed with it to disperse the inoculum. The bottles were incubated in BOD incubator at 25±1°C. Four replications were maintained for each treatment. The spores were harvested from sixth day onwards at definite intervals of up to 25 days by sampling 1 g of the digested material. The spore suspension of each sample was made by dispersing the inoculum in 10 ml sterile water blank with one drop of 0.02 per cent Tween-80, serially diluted and the spore count estimated using a haemo cytometer.

Observations to be recorded

1. Growth of fungus on each substrate
2. Spore counting on each substrate
3. Best substrate for each fungus will be identified based on spore load and cost

Practical utility

1. Great scope for the farmers to get the myco insecticide at cheapest cost

2. Training to the farmers about the advantages of mycoinsecticde when compared to

3. Farmers can handle the mycoinsecticides safely.

RESULTS (Part 1)

1. Survey for identifying major rice pests and hot spot for conducting further research studies

Preliminary surveys were undertaken to collect rice pests from rice fields. To evaluate the incidence of major rice pests in twelve selected spots falling under three districts *viz.,* Cuddalore, Thanjavur and Ariyalur were surveyed at fortnight intervals during samba season of 2013- 2014(Among three major pests of rice, rice stem borer, rice leaffolder and brown plant hopper, incidence percentage of rice leaffolders were maximum in Annamalainagar due to consumption of minimum pesticides in the rice fields compared to other places).

1.1. Survey of cadavers of Entomofungal pathogens in rice ecosystem of Cuddalore, Thanjavur and Ariyalur districts.

Preliminary surveys were undertaken to collect cadavers of naturally occurring entomopathogenic fungi on rice pests from rice fields. To evaluate the incidence of cadavers major rice pests in twelve selected spots in three districts *viz.,* Cuddalore, Thanjavur and Ariyalur were surveyed at fortnight intervals during samba season of 2013-2014. Diseased cadavers were gathered during early morning hours between 6.00 and 8.00 am (Table 1&2).

Surveys showed that highest infection percentage was noticed during second fortnight of December (92.00%) 2013 (2013-2014) in Annamalainagar (Table-1). In 2013-2014, among the five places surveyed in the Cuddalore district, overall infection percentage of leaffolder was maximum at Annamalainagar (65.25%) followed by B. Mutlur (59.75%), Periyapattu (54.00%), Bhuvanagiri (52.87%)

and Sethiathoppu (48.00%)(Table-1). Among the three places surveyed in Thanjavur district, infection percentage of rice leaffolder in Aduthurai was high during February 2014 (57.00%) when compared to Papanasam and Kuttalam. In 2013-2014, among the three places surveyed in the Thanjavur district, overall infection percentage of leaffolder was maximum at Aduthurai (39.00 %) followed by Kuttalam (30.50%) and

Table:-1.Survey of Entomofungal pathogens of rice leaffolder larvae in Cuddalore District during Samba season of 2013-2014.

S.No.	Month @ fort night intervals	Fungal Infection Percentage				
		Cuddalore District				
		Annamalainagar	Bhuvanagiri	B.Mutlur	Periyapattu	Sethiathoppu
1	Nov I	31.00	23.00	26.00	25.00	27.00
2	Nov II	35.00	31.00	35.00	29.00	41.00
3	Dec I	62.00	55.00	55.00	44.00	67.00
4	Dec II	92.00	61.00	74.00	64.00	69.00
5	Jan 1	91.00	72.00	86.00	68.00	64.00
6	Jan II	92.00	78.00	79.00	79.00	45.00
7	Feb I	71.00	64.00	69.00	74.00	38.00
8	Feb II	54.00	39.00	54.00	49.00	33.00

	Average	**65.25**	**52.87**	**59.75**	**54.00**	**48.00**

I - Fortnight

II - Fortnight

Table:-2.Survey of Entomofungal pathogens of rice leaffolder larvae in Thanjavur and Ariyalur districts during Samba season of 2013-2014.

S.No.	Month @fort night intervals	Fungal Infection Percentage						
		Thanjavur District			Ariyalur District			
		Aduthurai	Papanasam	Kuttalam	Jayamkondam	Ariyalur	Meensuruti	Anaikarai
1	Nov I	26.00	10.00	17.00	6.00	-	-	9.00
2	Nov II	32.00	16.00	21.00	8.00	-	-	17.00
3	Dec I	29.00	22.00	24.00	11.00	5.00	7.00	19.00
4	Dec II	36.00	37.00	28.00	18.00	11.00	21.00	26.00
5	Jan 1	42.00	28.00	32.00	24.00	12.00	27.00	29.00
6	Jan II	49.00	44.00	37.00	26.00	24.00	19.00	35.00
7	Feb I	57 .00	49.00	46.00	12.00	18.00	13.00	29.00
8	Feb II	41.00	33.00	39.00	9.00	10.00	-	25.00
	Average	**39.00**	**29.87**	**30.50**	**14.25**	**10.00**	**10.87**	**23.62**

I - Fortnight

II - Fortnight

(-) - Mycosed insect not found

Table:-3.Incidence percentage of Entomopathogenic fungi on the rice leaffolder larvae in the hot spots of Cuddalore, Thanjavur and Ariyalur Districts during 2013-2014

S.No.	Month @fort night intervals	Cuddalore District	Thanjavur District	Ariyalur District
		Annamalainagar	Aduthurai	Anaikarai
		2013-2014 Preliminary survey	2013-2014 Preliminary survey	2013-2014 Preliminary survey
1	Nov I	31.00	26.00	9.00
2	Nov II	35.00	32.00	17.00
3	Dec I	62.00	29.00	19.00
4	Dec II	92.00	36.00	26.00
5	Jan 1	87.00	42.00	29.00
6	Jan II	91.00	49.00	35.00
7	Feb I	71.00	57 .00	29.00

8	Feb II	54.00	41.00	25.00
	Average	**65.25**	**39.00**	**23.62**

I - Fortnight

II - Fortnight

Papanasam (29.87%). Among the four places surveyed in Ariyalur district, infection percentage of rice leaffolder in Anaikarai was high (35.00%) during January second fortnight of 2013-2014 when compared to Jayamkondam, Meensuruti and Ariyalur. In 2013-2014, among the four places surveyed in the Ariyalur district, over all infection percentage of leaffolder was maximum at Anaikarai (23.62%) followed by Jayamkondam (14.25%), Ariyalur (10.00 %) and Meensuruti (10.87%). Finally, hot spot in each district was selected to know the effect of weather parameters on the incidence of entomopathogenic fungi on the rice leaffolder larvae. Correlation studies were conducted.

3. Multiple correlations between incidence of entomopathogenic fungi on rice leaffolder larvae and weather parameters in Annamalainagar during 2013-2014. (Cuddalore District)

Multiple correlations between minimum temperature and infection percentage in Annamalainagar during 2013-2014 were highly significant (- 0.724) followed by RH (0.849) indicating that these factors

exhibited definite and appreciable influence on the infection percentage. However correlation with rainfall (- 0.597) and maximum temperature (-0.623) was not significant (Table 5&6).

Among three major pests of rice, Rice stem borer, Rice leaffolder and Brown plant hopper, incidence maximum number of leaffolders were needed in the form larvae, cadavers and adults in the Annamalainagar because of pesticides were sprayed in the rice field compared to other places.

With a view to bring out relationship among incidence of fungi on rice leaffolder and abiotic factors, multiple regressions were worked out. The fitted equation was for the year 2013-2014 was

$Y= 65.25 + 0.681\ NS\ (x_1) - 21.42^*\ (x_2) + 12.642^*\ (x_3) - 0.462\ NS\ (x_4)$

x_1 = Maximum Temperature x_2 = Minimum Temperature

x_3 = Relative Humidity x_4 = Rainfall

Table 4.Percentage of rice leaffolder larvae infection in relation to weather parameters during 2013-2014

S. No.	Period	Annamalainagar					Aduthurai					Anaikarai				
		Max. T (°C)	Min. T (°C)	R. H (%)	R.F (mm)	P.I (%)	Max. T (°C)	Min. T (°C)	R.H (%)	R.F (mm	P.I (%)	Max. T (°C)	Min. T (°C)	R.H (%)	R.F (mm)	P.I (%)
1.	November I	30.4	21.0	79	302	31.0	30.8	24.2	74	170.5	26.0	31.5	25.3	70	75.0	9.0
2	November II	28.1	23.0	86	400	35.0	28.4	23.6	76	340.7	32.0	29.4	24.9	73	350	17.0
3	December I	27.9	22.5	88	406	62.0	28.3	23.9	75	150.2	29.0	29.2	24.0	74	80	19.0
4	December II	28.0	20.5	96	205	87.0	28.0	22.4	78	50.0	36.0	28.9	24.1	76	50	26.0
5	January I	27.3	20.0	94	36.6	90.0	27.9	22.4	81	60.6	42.0	28.5	23.4	78	16.6	29.0
6	January II	27.1	19.9	95	5.4	92.0	28.1	22.1	80	5.4	49.0	29.0	22.5	83	0.4	35.0
7	February I	29.8	20.0	86	-	71.0	29.9	22.0	81	-	57.0	29.8	23.1	76	-	29.0
8	February II	30.4	22.4	79	-	54.0	30.1	23.4	76	-	41.0	31.1	24.3	74	-	25.0

I – Fortnight Max. T-Maximum temperature, Min. T-Minimum temperature, R.H-Relative humidity, R.F-Rainfall

II - Fortnight P.I-Percentage of infection

Table:-5. Over all correlation and regression between percentage of leaffolder infection and weather parameters in Annamalainagar during 2013-2014

Particulars	Correlation coefficient (r)	Regression coefficient (b)	Constant (a)
Maximum temperature Vs P.I	-0.623 NS	-4.572 NS	21
Minimum temperature Vs P.I	-0.724*	-10.542*	17
Relative humidity Vs P.I	0.849*	3.874 NS	26
Rainfall Vs P.I	-0.597 NS	2.412 NS	12

*Significant at P=5%
NS=Not significant
P.I. =Percentage of infection

Table:-6.Multiple linear regression - interaction of leaffolder infection percentage with weather parameters in Annamalainagar during 2013-2014.

	x_1 = Max. temperature	x_2=Min. temperature	x_3=Relative humidity	x_4=Rainfall
Y= 65.25	0.681 NS (x_1)	-21.42* (x_2)	12.642* (x_3)	- 0.462 NS (x_4)
Tb	-0.972	-1.493	2.972	1.424

$R^{2=}$ 0.963

Multiple correlations between incidence of entomopathogenic fungi on rice leaffolder larvae and weather parameters in Aduthurai (Thanjavur District) during 2013-2014.

In Aduthurai, minimum temperature was significant (-0.943) during 2013-2014. Other parameters like rainfall and maximum temperature were not significant. Frequent heavy rainfall in the early November was decreased the fungal infection (Tabale 7&8).

With a view to bring out relationship among incidence of fungi on rice leaffolder and abiotic factors, multiple regressions were worked out. The fitted equation was for the year 2013-2014 was

$Y = 39 - 0.0490\ NS\ (x_1) - 0.693\ NS\ (x_2) + 8.643^*\ (x_3) - 27.931^*\ (x_4)$

x_1 = Maximum Temperature x_2 = Minimum Temperature

x_3 = Relative Humidity x_4 = Rainfall

Among the different weather parameters during 2013-2014, maximum temperature, minimum temperature and rainfall were negatively correlated whereas, relative humidity showed positive correlation with percentage of infection.

Multiple correlations between incidence of entomopathogenic fungi on rice leaffolder larvae and weather parameters in Anaikarai (Ariyalur District) during 2013-2014.

In Anaikarai minimum temperature (-0.814) was significant during 2013-2014 helps in the spread of fungal pathogen and the rainfall also was significant, less and drizzling rainfall during the season may favour the growth of the fungus to certain extent. However relative humidity and maximum temperature were not significant(Table 9&10).

With a view to bring out relationship among incidence of fungi on rice leaffolder and abiotic factors, multiple regressions were worked out. The fitted equation for the year 2013-2014 was

$Y = 23.62 - 0.343\ NS\ (x1) + 34.726\ NS\ (x2) - 17.631^*\ (x3) + 0.648^*\ (x4)$

x_1 = Maximum Temperature x_2 = Minimum Temperature

x_3 = Relative Humidity x_4 = Rainfall

Table 7. Over all correlation and regression between percentage of leaffolder infection and weather parameters in Aduthurai during 2013-2014

Particulars	Correlation coefficient (r)	Regression coefficient (b)	Constant (a)
Maximum temperature Vs P.I	-0.37 NS	-3.026*	27
Minimum temperature Vs P.I	-0.943*	5.432 NS	79
Relative humidity Vs P.I	0.154 NS	-1.479*	32
Rainfall Vs P.I	-0.694 NS	0.055 NS	88

*Significant at P=5%
NS=Not significant
P.I.=Percentage of infection

Table:-8.Multiple linear regressions -interaction of leaffolder infection percentage with weather parameters in Aduthurai during 2013-2014.

	x_1=Max. temperature	x_2=Min. temperature	x_3=Relative humidity	x_4=Rainfall
Y=39	-0.049 NS(x_1)	- 0.693 NS (x_2)	8.643 * (x_3)	-27.931*(x_4)
Tb	-0.346	-6.643	-2.463	2.874

$R^{2=}$ 0.972

Table 9. Over all correlation and regression between percentage of leaffolder infection and weather parameters in Anaikarai during 2013-2014.

Particulars	Correlation coefficient (r)	Regression coefficient (b)	Constant (a)
Maximum temperature Vs P.I	-0.579 NS	-2.474 NS	27
Minimum temperature Vs P.I	-0.814*	4.874*	72
Relative humidity Vs P.I	0.338 NS	-0.974 NS	51
Rainfall Vs P.I	-0.529 NS	0.524*	92

*Significant at P=5%
NS=Not significant
P.I.=Percentage of infection

Table:-10. Multiple linear regressions - interaction of leaffolder infection percentage with weather parameters in Anaikarai during 2013-2014.

	x_1=Max. temperature	x_2=Min. temperature	x_3=Relative humidity	x_4=Rainfall
Y=23.62	- 0.343 NS (x1)	34.726 NS (x2)	-17.631* (x3)	0.648*(x4)
Tb	-1.432	-2.334	-0.749	-0.349

$R^{2=}$ 0.865

Among the different weather parameters during 2013-2014, maximum temperature (-0.343) and relative humidity (-17.631) were negatively correlated whereas minimum temperature (34.726) and rainfall (0.648) showed positive correlation with percentage of infection.

Evaluation of Potential Entomofungal pathogens in selected rice fields

Maximum numbers of rice leaffolder cadavers infected with Z.radicans were collected when compared to other cadavers and it was found that Z.*radicans* was found as a dominant fungal pathogen in controlling rice leaffolder in coastal districts of Tamilnadu.

3. Pathogenicity of selected isolates of *Z. radicans* to rice leaffolder

Among the twelve isolates of *Z. radicans* gathered from various places mentioned earlier, five isolates were selected based on its infection percentage in the field and they were subjected to pathogenicity test (Table 11). Of the five isolates evaluated, Annamalainagar isolate was found to be highly pathogenic (57.72%) to the test insect followed by other isolates from B.Mutlur, Periyapattu, Bhuvanagiri and Sethiathoppu. Hence, Annamalainagar isolate was utilized for further studies.

The Combined effect between two concentrations of the fungus, *Z. radicans of* two doses of gamma radiation on the larval mortality of the rice leaffolder ,*Cnapahalocrocis medinalis.*

Biological aspects determinations due to the pathogenicity of different concentrations of the fungus *Z. radicans*, combined with gamma irradiation against the rice leaffolder, *Cnaphalocrocis medinalis*.

4. Genetic improvement of strains to produce virulent and resistant spores

The combined effect between fungal concentrations and gamma irradiation doses gave better results in increasing the larval mortality percentage than each treatment applied separately (Table 12 &13). It could be concluded that there was a positive correlation between the larval mortality percentages and the fungal concentrations and gamma irradiation doses.

Table:-11.Pathogenicty of different isolates of *Z. radicans* on rice leaffolder, *C. medinalis*

S.No.	Isolates	Larval mortality percentage
1	Annamalainagar	57.72 [a] (53.10)
2	B.Mutlur	52.34 [b] (48.70)
3	Periyapattu	48.56 [c] (46.10)
4	Bhuvanagiri	31.27 [d] (44.40)
5	Sethiathoppu	22.79 [e] (34.10)
6	Control	11.73 [f] (19.90)
	CD (P=0.05%)	2.07
	SEd	0.95

Each value is mean of four replications
Figures in parenthesis are arc sintransformed values
In a column means followed by common letter are not significantly different

Table 12.The Combined effect between two concentrations of the fungus, *Z. radicans* of two doses of gamma radiation on the larval mortality of the rice leaffolder, *Cnapahalocrocis medinalis.*

Periods (h)	**Average larval mortality (%)**			
concentrations (spores ml 1)	24	48	72	96
Fungal treatment only				
0	0.00	0.00	0.00	3.33
10^4	3.33	16.67	33.33	44.83
10^8	6.67	33.33	50.00	75.87
Fungi + gamma irradiation with 50 Gy				
0	0.00	3.33	6.67	13.34
10^4	6.67	20.00	40.00	51.72
10^8	10.00	43.33	60.00	86.21
Fungi + gamma irradiation with 100 Gy				
0	13.33	20.00	26.67	34.49
10^4	16.67	26.67	46.67	58.62
10^8	23.33	50.00	73.33	89.66
LSD 0.05	7.20	7.80	10.00	12.20
0.01	9.40	9.80	14.30	16.10

Each value is mean of four replications

Table 13. Biological aspects determinations due to the pathogenicity of different concentrations of the fungus *Z. radicans*, combined with gamma

Fungal	Biological aspects

irradiation against the rice leaffolder, *Cnaphalocrocis medinalis.*

	Survived larvae (%)			Pupation (%)			Emergence (%)			Sex ratio (%)					
										(Male)			(Female)		
	0	10^4	10^8	0	10^4	10^8	0	10^4	10^8	0	10^4	10^8	0	10^4	10^8
0	96.67	55.17	24.13	96.55	50.00	20.00	96.55	40.00	13.33	57.14	58.33	75.00	42.86	41.67	25.00
50	86.66	48.28	13.79	86.66	43.33	10.00	60.00	30.00	0.00	55.00	66.67	-	45.00	33.33	-
100	65.51	41.38	10.34	56.17	36.67	6.67	40.00	23.33	0.00	66.67	71.43	-	28.57	-	-
LSD 0.005 0.01	12.30 16.70			9.20 14.10			7.80 9.70								

The highest score of larval mortality was obtained when the fungal concentration was 10^8 spores per ml and combined with 100 Gy dose after 96 h exposure time which was 89.66% followed by the treatment of fungal concentration 104 spores ml per ml and combined with 100 Gy dose after 96 h exposure time being 58.62% as compared with 3.33% larval mortality percentage in untreated control. These findings are in agreement with those found by Brinkman *et al*. El-Sinary and Quesada-Moraga *et al*. which explained that the efficiency of the entomopathogenic fungi began clearly after 48 h from inoculation and the hyphae penetrated the integument inside the trachea and the epithelial and epidermal cells, after 72 fours the fat tissues were damaged and lethality may release to 100% after 96 h. (Table 12).

There was a great reduction in the viability of different stages of rice leaf folder, *C.medinlais* especially when fungal concentrations were combined with gamma irradiation. Highly significant reductions were reported when *C.medinlais* was treated with 10^8 spores ml^{-1} 1 of fungi and 100 Gy dose of gamma irradiation where results were, 10.34, 6.67 and 0.00% for survived larvae, pupation and emergence percentages, respectively. The previous obtained results are in harmony with results gained by Klingen *et al*. and El-Sinary who stated that the greatest the fungal concentration the highest the reduction in viability of tested insect. Also Prasad and El-Sinary stated that males are more tolerant than females for the effect of gamma irradiation.(Table 13).

5. Evaluation of cheaper and coarse food grains and Agrowastes for mass production of different fungal pathogens

The crushed grains of sorghum**,** Bajra, Maize, Ragi, Broken rice and Wheat with 1 per cent yeast extract were assessed for their suitability as substrates for mass production of individual fungus. In addition to grains, agro waste *viz.*, Crushed maize cobs, Wheat bran, Rice bran, Baggase and Press mud with and without molasses were also tested. To each of these substrates, sterile distilled water was added in order to bring the moisture content to 50 per cent. After thorough mixing, the bottles were plugged with cotton and autoclaved at 15 psi and

Table 14.Effect of various grain media for the growth characteristics of *Z. radicans*

S.No.	Media	Biomass (mg)*	Radial growth (mm)*	Spore Germination (%) **	Infectivity (%) **
1.	Sorghum	498.2 (22.30)^{ab}	71.27 (8.40)^{a}	93.27 (75.90)^{b}	65.00 (54.10)^{b}
2.	Maize	439.80 (21.00)^{b}	70.56 (8.40)^{ab}	96.57 (78.57)^{a}	58.43 (49.50)^{c}
3.	Cumbu(Bajra)	410.57 (20.30)^{bc}	45.75 (6.80)^{d}	79.03 (62.90)^{e}	45.50 (42.10)^{d}
4.	Ragi	327.34 (18.10)^{d}	40.66 (6.40)^{de}	86.38 (68.40)^{d}	41.25 (39.80)^{e}
5.	PDA(Control)	508.90 (22.60)^{a}	70.35 (8.40)^{ac}	90.40 (71.80)^{c}	67.50 (55.10)^{a}
	CD ($p_{=0.05)}$	0.290	0.170	3.160	1.740
	SE	0.100	0.060	1.110	0.640

Each value is mean of four replications

Figures in parenthesis are square root* /arc sin** transformed values

In a column means followed by common letter are not significantly different

121°C for 30 minutes. Circular agar discs of 5 mm diameter were taken from the eight day old fungal culture grown on SMAY plates. One disc was inoculated to each bottle and mixed with it to disperse the inoculum. The bottles were incubated in BOD incubator at 25±1°C. Four replications were maintained for each treatment. The spores were harvested from sixth day onwards at definite intervals of up to 25 days by sampling 1 g of the digested material. The spore suspension of each sample was made by dispersing the inoculum in 10 ml sterile water blank with one drop of 0.02 per cent Tween-80, serially diluted and the spore count estimated using a haemo cytometer.

5.1. Effect of prawn exoskeleton supplemented SMAY on cultural characteristics of

Z. radicans

Prawn waste showed varying response on biomass production by *Z. radicans*(Table14). Among the different concentrations of the prawn waste tested in the standard media, 60%, 80% and 100% doses were found to be better (722.49 mg, 710.67 mg and 698.31 mg) in brought out the biomass production which was significantly higher than that produced from 20% (611.50 mg), 40% (644.17 mg), whereas the control (SMAY) recorded biomass of 597.63 mg and it was less than that of 20 and 40 percent dosages in the standard medium.

Among the various concentrations of prawn waste tested in the synthetic media, 60 per cent prawn waste has influenced the highest radial growth (84.88 mm) of the fungus followed by 80 per cent prawn waste (78.22 mm) and 100 per cent (76.65 mm). The control with synthetic media without prawn waste recorded (74.25 mm) radial growth and it was on par with the synthetic medium with 20 and 40 per cent (74.34 mm and 74.19 mm) respectively

Synthetic medium containing 60 per cent prawn waste resulted in the maximum spore germination (91.64%) followed by 80 and 100 per cent prawn waste (91.15 and 90.50%). The synthetic medium with 20 per cent prawn waste had lowest germination (81.06%) and on par with the control.

Highest per cent mortality (74.70%) of the leaf folder larvae was obtained with synthetic medium having 60 per cent fish scale waste followed by 80 and 100 per cent fish scale waste with 70.90 and 69.74% mortality. However the doses of prawn waste in the synthetic medium excelled the control medium (Synthetic media alone) in respect of infectivity of the fungus. The above studies conducted so far revealed among various fungal pathogens, *Z.radicans* is the most potential fungal pathogen which can be mass cultured economically on wastes of prawn scale powder. However further confirmation studies are to be done to make it effective commercial myco insecticide.

6. Influence of certain natural substrates and abiotic factors on the cultural characteristics of entomopathogenic fungus *Z. radicans*

6.1. Role of fish scale waste and prawn exoskeleton supplemented SMAY medium on the cultural characteristics of *Z. radicans.*

Results of role of fish scale waste supplemented with SMAY medium on the biomass, radial growth, spore germination and infectivity of the fungus illustrated (Table 15). Fish scale waste showed varying response on biomass production of Z. *radicans.* Among the different concentrations of the fish scale waste tested in the standard media, 100 % dose was found to be highest (620 mg) in bringing out the biomass production which was significantly higher than that produced from 80% (608 mg), 60% (558 mg), 40% (471.60 mg) and 20% (342.50 mg), whereas the control treatment (SMAY) recorded biomass of 306.28 mg and it was less than that of 20 and 40 per cent dosages in the standard medium.

Among various concentrations of fish scale tested in the synthetic media, 100 per cent fish scale waste resulted the highest radial growth (84.10 mm) of the fungus followed by 80 per cent fish scale waste (80.65 mm) whereas the other doses 20 ,40·and 60 per cent fish scale waste recorded 64.15, 70.30 and 74.15 mm growth respectively. The control with synthetic media without fish scale recorded 67.59 mm radial growth.

Table 15.Effect of prawn exoskeleton supplemented SMAY on cultural characterist Z. *radicans*

S.No.	Treatments	Biomass (mg)*	Radial growth (mm)*	Spore Germination (%)**	Infectivity (%)**
1.	SMAY+20% PE	611.50 (24.70)^{c}	74.34 (8.60)^{be}	81.06 (64.20)^{ef}	66.57 (55.10)^{de}
2	SMAY+40% PE	644.17 (25.40)^{b}	74.19 (8.60)^{bf}	86.23 (68.40)^{d}	67.26 (55.10)^{d}
3.	SMAY+60% PE	722.49 (26.90)^{a}	84.88 (9.20)^{a}	91.64 (73.70)^{a}	74.70 (59.30)^{a}
4	SMAY+80% PE	710.67 (26.70)^{ab}	78.22 (8.80)^{b}	91.15 (71.80)^{b}	70.91 (57.10)^{b}
5	SMAY+100% PE	698.31 (26.40)^{ac}	76.65 (8.80)^{bc}	90.50 (71.80)^{bc}	69.74 (57.10)^{bc}
6	SMAY (Control)	597.63 (24.40)^{cd}	74.24 (8.60)^{bd}	80.52 (64.20)^{e}	60.98 (51.30)^{f}
	CD($p_{=0.05}$)	0.006	0.0047	0.2193	0.4184
	SEd	0.0021	0.0016	0.0762	0.1423

Each value is mean of four replications
Figures in parenthesis are square root* /arc sin** transformed values
In a column means followed by common letter are not significantly different

Synthetic medium containing 100 per cent fish scale powder had the maximum spore germination (91.15%) followed by 80 per cent fish scale waste (84.50%). The synthetic medium with 20 per cent fish scale waste contributed lowest germination (72.36 %) and it was lower than the control. Highest per cent mortality (70.91 %) of the leaf folder larvae was obtained with synthetic medium having 100 per cent fish scale waste followed by 80 per cent fish scale waste with 69.74% mortality. However the doses of fish scale powder in the synthetic medium excelled in growth characteristics and infectivity over the control medium (synthetic media alone) except synthetic media with 20 per cent fish scale waste in respect of infectivity of the fungus.

The results obtained showed that 100% fish scale waste in the synthetic medium SMAY was rated as the superior in enhancing the growth parameters and infectivity of the fungus Z.*radicans* when compared to the control (Synthetic medium SMAY alone).

6.1. Effect of temperature on growth characteristics of *Z. radicans*

Results of effect of temperature on biomass, radial growth, spore germination and infectivity of the fungus are illustrated in Table 16). It was found that temperature level of 30°C favoured the maximum biomass harvest of Z. *radicans* (497.00 mg), while ranges below and above 30°C was found to be detrimental to fungal biomass production. 30°C was observed as appropriate and condusive to obtain maximum radial growth (80.18 mm) followed by 25°C and 20°C with 70.84 mm and 38.94 mm respectively. Decrease and increase in temperature coincided with decline in the radial growth of the fungus. Maximum spore germination of 83.63% was evidenced at 30°C to be succeeded by 25°C and 20°C respectively with 82.13 mm

and 68.79 mm, whereas 35°C had adversely affected germination of spores. From the various levels of temperature tested, 30°C was considered as ideal to mycose maximum number of leaf folder larvae (65.09%). Whereas 61.39%, 41.47%, 32.50% infectivity was observed with 25°C, 20°C and 15°C respectively.

Table 16. Effect of temperature on growth characteristics of *Z. radicans*

S.No.	Temperature (^{o}C)	Biomass (mg)*	Radial growth (mm)*	Spore Germination (%)**	Infectivity (%)**
1.	10	307.50 ^{f} (17.50)	15.00^{f} (3.90)	40.55 ^{e} (39.80)	26.5 ^{e} (31.30)
2.	15	321.00^{e} (17.90)	20.75^{e} (4.60)	57.86^{c} (49.50)	32.50^{d} (34.80)
3.	20	398.56^{c} (20.00)	38.94^{d} (6.20)	68.79^{b} (56.10)	41.47^{c} (39.80)
4.	25	471.67^{b} (21.70)	70.84^{b} (8.40)	82.13^{a} (65.50)	61.39^{b} (51.30)
5.	30	497.00^{a} (22.30)	80.18^{a} (9.00)	83.63^{a} (65.50)	65.09^{a} (54.10)
6.	35	361.48^{d} (19.00)	60.58^{c} (7.80)	60.25^{c} (51.30)	41.25^{c} (39.80)
CD (P=0.05%)		0.21	0.04	2.78	1.53
Sed		0.07	0.01	0.98	0.54

Each value is mean of four replications
Figures in parenthesis are square root* /arc sin** transformed values
In a column means followed by common letter are not significantly different

Table 17. Effect of Relative humidity on growth characteristics of *Z. radicans*

S. No.	Relative humidity (%)	Radial growth (mm)	Sporulation*
1.	75	58.00	+
2.	80	*57.00 .I*	+
3.	85	74.25	++
4.	90	79.25	+++
5.	95	74.25	+++
6.	100	58.00	+

Each value is mean of four replications

Symbol	Number of spores	Remarks
+	Less than 10 spores	Very poor sporulation

++	Only very few spores	More than 10 spore sporulation
+++	Spores too numerous	Abundant sporulation

*Scale of sporulation (Gustafsson, 1965)

6.3. Effect of different levels of relative humidity on growth and sporulation of *Z.radicans*

Results of varying levels of relative humidity on the growth and sporulation of the fungus showed (Table 17) that two levels of higher relative humidity *i.e*, 85% and 90% were observed ideal for the growth of fungus. It is noted that 75% and 100% RH found not only least infective for growth of the fungus but also detrimental to its spore germination. 85 and 90 per cent RH favoured growth of *Z. Iadicans* to the maximum of 79.25 mm and 74.25 mm respectively. 75 and 100% RH effected poor growth of 58 mm and 58 mm respectively. It is thus clear that RH levels like 85 and 90% were optimum for growth and sporulation of the test fungus.

6.4. Survival of Z. *radicans* in rice soil and its infectivity under different periods of storage

Results pertaining to the survival period of Z. *radicans* in rice soil based on the infectivity under different periods of storage are presented in (Table 18). The potency of Z. *radicans* to mycose the rice leaf folder was comparatively equal to that of original level (65%) after the first month of storage. There was a mortality of 57.50,56.25 and 52.50% observed in second, third, fourth month after storage. Mortality (41.25%) decreased rapidly in fifth month after storage. From the results it was clear that soil storage alone lead to loss of infectivity of Z. *radicans.* It is concluded that maximum survival period of the fungi in rice soil was six months with adequate

levels of infectivity to cause mortality of the test.

Effect of supplemented medium on the growth characteristics of *Z. radicans*

Results of suitability of various supplemented media for Biomass, radial growth, spore germination and infectivity of the fungus are illustrated in the Table 31, Figure 10 and Plate 18. Of the media evaluated, highest yield of biomass (687.48 mg) was obtained with SEMA medium followed by EYSDA and EYM (641.23 mg and 547.50 mg). The standard synthetic medium (SDA Y) used as control- I and coagulated egg as control-II accounted for poor biomass production. These results indicated varied performance due to the addition of supplemented media in the radial growth of fungus. The SEMA medium fared well with

Table 18. Survival of Z. *radicans* in rice soil and its infectivity under different periods of storage

S. No.	Storage Period (months)	Percent Mortality(**)	Percentage Infectivity lost due to storage
1.	I	65.00[a] (53.72)	0.06
2.	II	57.50[h] (49.31)	11.60
3.	III	56.25[b] (48.59)	13.94
4.	IV	52.50[c] (46.43)	19.68
5.	V	41.25[d] (39.96)	36.89
	VI	54.05 (47.27)[bc]	16.43
CD (P=0.05%)		1.53	3.04
Sed		0.54	1.06

Each value is mean of four replications
Figures in parenthesis are arc sin ** transformed values
Column means followed by common letter are not significantly different

Table 19. Effect of supplemented medium on the growth characteristics of Z. *radicans*

S.No.	Media	Biomass (mg)*	Radial growth (mm)*	Spore Germination (%) **	Infectivity (%) **
l.	Eggyolk- Sabouraud Maltose Agar (EYSDA)	641.23[b] (25.30)	74.20[a] (8.60)	85.11[a] (66.90)	64.45[a] (53.10)
2	Sabouraud -Egg-Milk-Agar (SEMA)	687.48[a] (26.20)	76.00[a] (8.70)	87.60[a] (70.10)	66.l0[a] (54.10)
3.	Coagulated Egg yolk-Milk (EYM)	547.50[c] (23.40)	71.65[b] (8.50)	74.02[b] (59.30)	62.28[b] (52.10)
4.	Sabouraud Dextrose Agar -Yeast extract (SDAY) (Control 1)	434.60[d] (20.80)	65.34[c] (8.10)	64.44[c] (53.10)	61.28[b] (51.30)
5	Coagulated Egg Yolk (CEY) (Control -2)	243.26[e] (15.60)	35.90[d] (6.0)	31.62[d] (34.10)	31.50[c] (34.10)
	CD(P=0.05)	0.50	0.11	3.10	0.65
	SEd	0.16	0.03	0.55	0.22

Each value is mean of four replications
Figures in parenthesis are square root* farc sin** transformed values
In a column means followed by common letter are not significantly different

highest radial growth of 76 mrn followed by those of EYSDA, EYM, SDA Y and CEY media. In general, the SEMA medium was influenced in maximum spore germination of 87.60 % compared to the other media and the control treatments I and II. The SEMA medium was found superior as evidenced by the maximum per cent mortality of 66.10% of the leaf folder larvae followed by EYSDA, EYM and control. It is inferred that among the various supplemented media tested, SEMA alone was found efficient in all the growth characteristics and infectivity of the fungus.

8.In vitro production of resting spores of the fungus*, Z. radicans*

8.1. Influence of various carbon and nitrogen sources on Azygospores production by *Z. radicans* in liquid shake culture.

Results showed that there was a drastic rise in the sporulation ($2.3x\ 10^5$ to $6.37x10^5$ spores/ml) rate with yeast extract as nitrogen source and sunflower oil as the carbon source in the sporulation medium with increase in the N:C ratio variable from 1:2 to 4:8. Downward trend of the sporulation was evident with peptone (Nitrogen source) and sunflower oil (Carbon source) in the sporulation medium with increase in the N: C ratio of 1:2 to 4:8 combinations. A gradual increase in the sporulation of the fungus was evident with dextrose as carbon and yeast extract as nitrogen sources except at the N:C range of 2:8. The carbon source dextrose resulted minimum sporulation with peptone and no sporulation was found at all the N: C ratios of2:4 to 4:8. (Table 20).

Results evidenced that 2:8 and 4:8 per cent combinations of yeast extract and sunflower oil as well as 1:2 and 1:4 per cent combination of peptone with sunflower oil exerted Z. *radicans* to

sporulate tremendously. However there was an increasing trend .in the sporulation of Z. *radicans* with yeast extract and sunflower oil from 1:2 to 4:8 combinations. There was a gradual decrease in the sporulation with peptone and sunflower oil from 1:2 to 4:8 combinations, while nitrogen source peptone and carbon source dextrose were seen less supporting for sporulation of the test fungus. The sporulation was maximum with nitrogen sources namely yeast extract and peptone and carbon sources sunflower oil (> 1- 5x 10^5 spores/mI). The sporulation was poor with dextrose as carbon source and peptone as nitrogen source (less than 1 x 10^5 spores /ml).

In vitro* evaluation of *Z.* *radicans* against rice leaf folder, *C. medinalis

Different treatments like mycelium, conidia, resting spores, combination of mycelium conidia and resting spores, mycelium and resting spores, mycelium and conidia, control were tested in the current study. Treatment with conidia alone caused mortality of 47.15% and resting spores alone caused mortality of 38%. But treatment with both conidia and resting spores of Z. *radicans* brought out highest mortality of rice leaf folder larvae (54.28 %) in lab studies. Treatment with mycelium caused mortality of29.68 and it was observed to be better when compared to untreated check.

Table 20. Influence of various carbon and nitrogen sources on a zygospore production of *Z. radicans* in liquid shake culture.

Carbon sources	Nitrogen Sources	N:C Ratio	No of resting Spores (x 10^5/ml)
		1:2	2.35^{e} (1.53)
		1:4	3.75^{d} (1.93)
Yeast extract	Sunflower Oil	2:4	4.62^{c} (2.l4)
		2:8	4.68^{b} (2.16)
		4:8	6.37^{a} (2.52)
		1:2	0.12^{n} (0.34)
		1:4	0.17^{m} (0.41)
Yeast extract	Dextrose	2:4	0.30^{i} (0.54)
		2:8	0.50^{j} (0.70)
		4:8	0.37^{k} (0.60)
		1:2	2.32^{f} (1.51)
		1:4	2.20^{g} (1.48)
Peptone	Sunflower Oil	2:4	1.94^{h} (1.37)
		2:8	0.32^{l} (0.77)
		4:8	0.11^{o} (0.31)
		1 :2	0.05^{p} (0.22)
		1:4	0.02^{q} (0.14)
Peptone	Dextrose	2:4	0.00^{r} (0.00)
		2:8	0.00^{r} (0.00)
		4:8	0.00^{r} (0.00)
		CD(p=0.05)	0.25
		SEd	0.12

Each value is a mean of four replications

Figures in parenthesis are square root transformed

In a column means followed by a common letter are not significantly different (P=0.05) by DMRT

Annexure

Work to be continued

- Mass production of best fungal pathogen *(Z. radicans)* isolated for preparation of oil and wettable formulation.
- Evaluation of various formulations of (*Z. radicans)* against major pests of rice under laboratory and field condition.
- Safely studies on natural enemies
- Lab and field studies and confirmative trial
- Data collection, analysis, documentation and report preparation

SUMMARY

- Preliminary studies were carried out in different places covering three major rice growing districts during samba season of Tamilnadu to identify major pests on rice revealed that Rice leaffolder *Cnaphalocrocis medinalis* .is the major pest in this coastal tract of Cuddalore district.

- Among the different places in Cuddalore district, Annamalainagar recorded maximum fungal infected larvae and cadavers in the survey.

- Among different months, incidence of fungal infection was highest during December.

- Very high relative humidity was recorded at Annamalainagar in Cuddalore district

when compared to remaining surveyed areas as it is near to the sea coast.

- Among weather parameters RH and minimum temperature exerted positive correlation with incidence of entomopathogenic fungi.
- Mutagenesis studies showed gamma rays exposures have significant influence and genetic improvement of strains to produce and resistant spores.
- Among the evaluated isolates of *Z. radicans*, Annamalainagar isolate exhibited maximum pathogenicity on the leaffolder.
- The maize grain medium followed by sorghum medium was efficient and ideal for culturing *Z. radicans*.
- There was remarkable enhancement of the growth and infectivity of *Z. radicans* with the addition of 100 per cent fish scale powder.
- There was appreciable increase of the growth and infectivity of *Z. radicans* with addition of prawn waste @ 60 percent.

Plate 1. Plates related to research activites

Leaffolder Larvae

Pupa

Adult

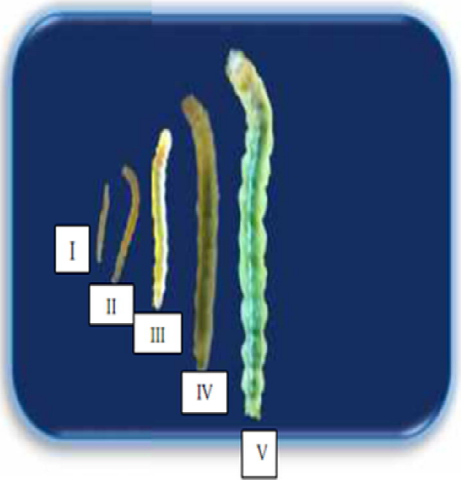
Different Instars of rice leaffolder

Collection of pests and cadavers Leaffolder cadaver in rice field

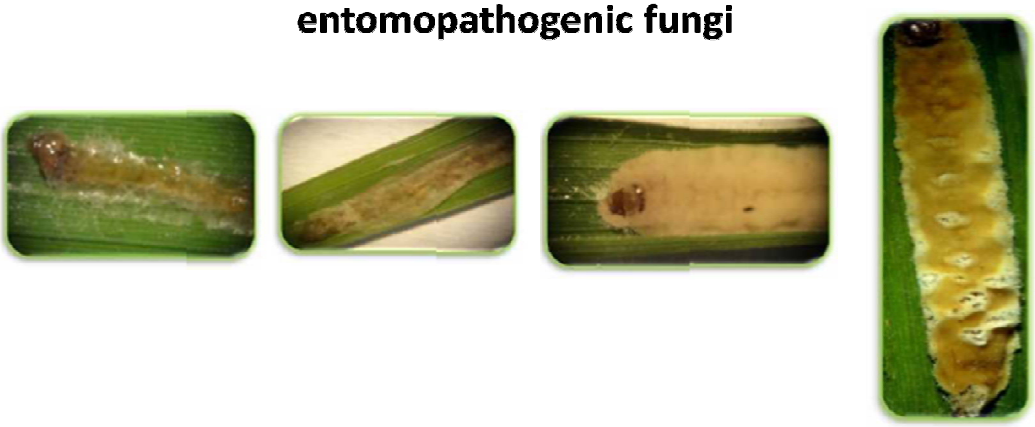
Plate 2. Collection and observation leaf folder cadaver

Cadaver collection in the rice field

Different instars of rice leaffolder



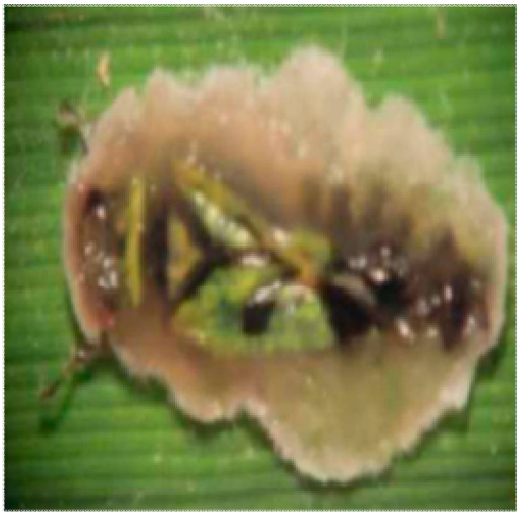
Different instars of rice leaffolder infected with entomopathogenic fungi

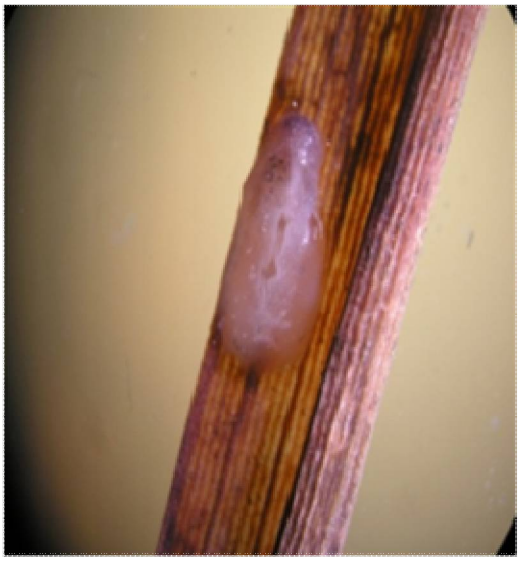


Leaffolder adult - Male

Female

Plate 3. Different rice pests infested with entomopathogenic fungai





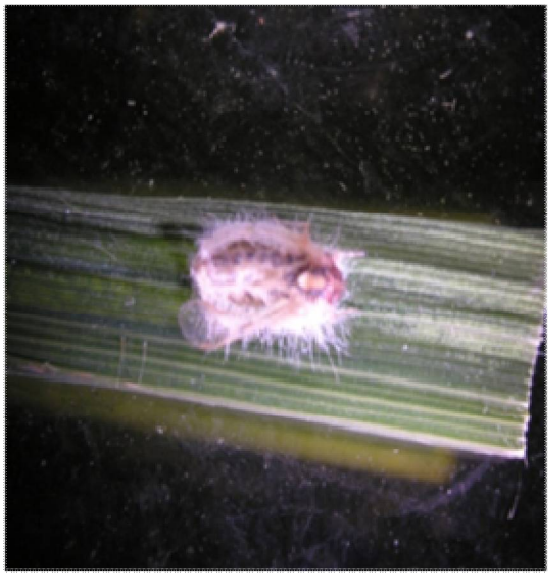
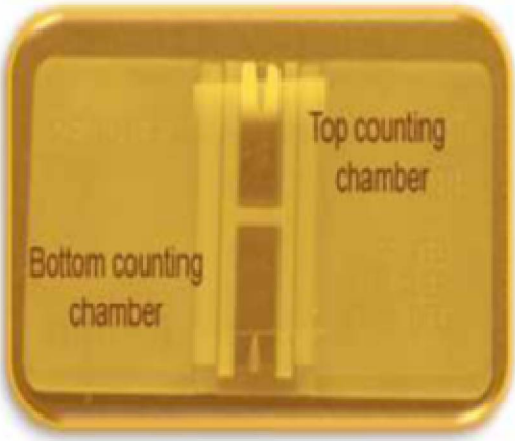


Plate 4. Equipments used for culturing Entomopathogenic fungus


Top counting chamber
Bottom counting chamber

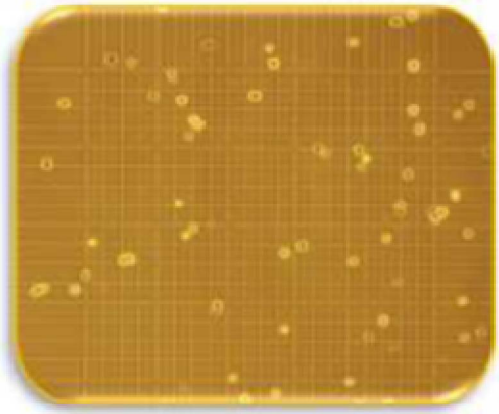
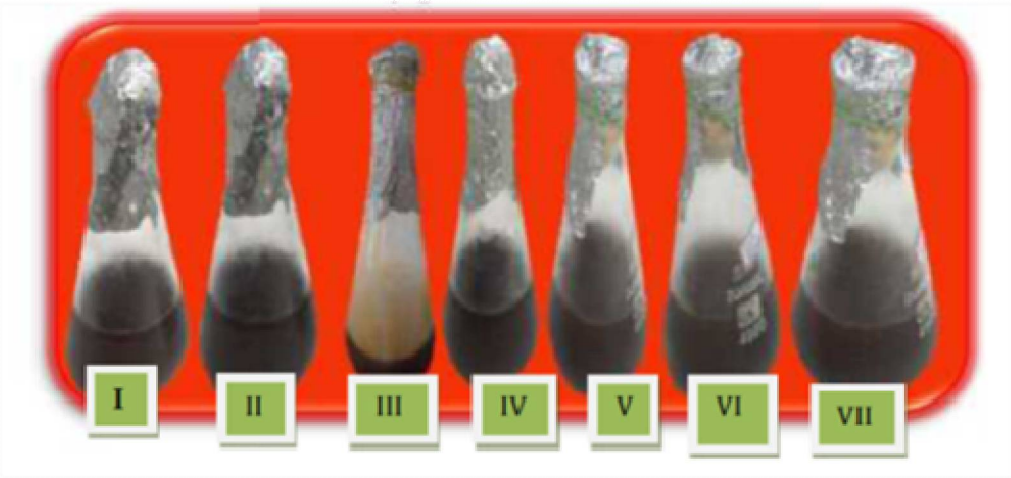
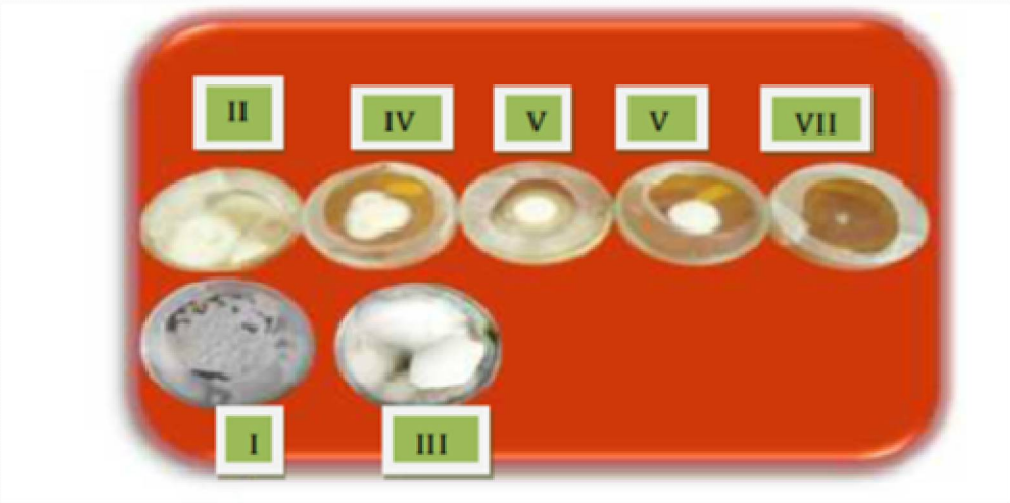


Plate 5. Exposure of entomopathogenic fungi to different levels of gamma rays


CARRT
CARRT

Plate 6. Growth of entomopathogenic fungai in different natural media





Bioassay studies

Plate 7. Effect of prawn exoskeleton supplemented SMAY on cultural characteristic of *Z. radicans*

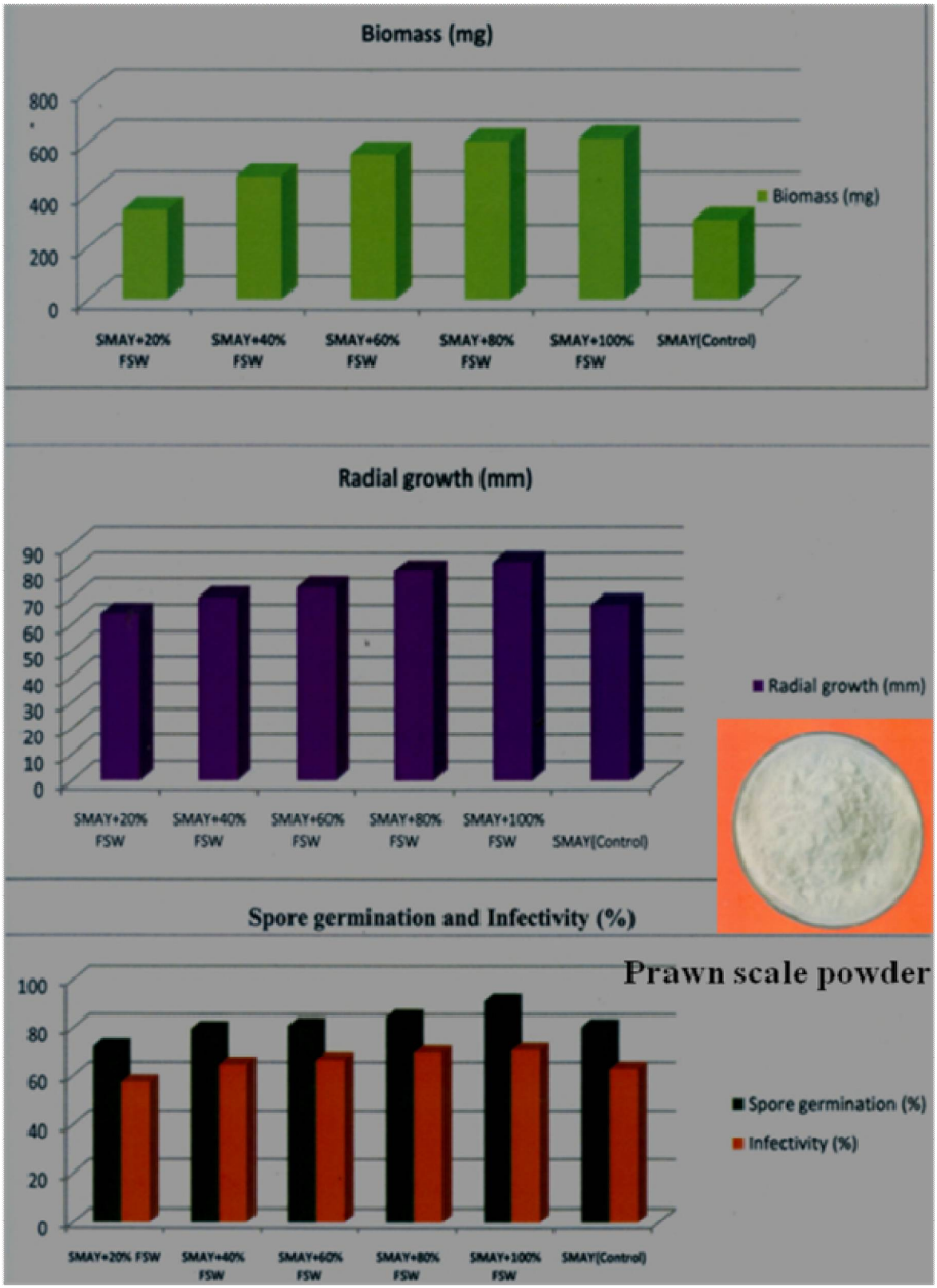
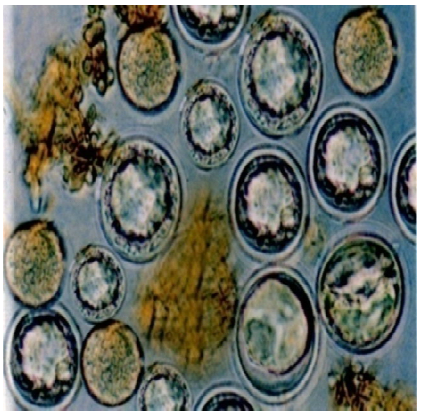


Plate 8. Mass culturing of *Z. Radicans* and pathogenicity

***Zoophthora* Culture in the petriplate**

Mass of resting spores under the microscope(40x)



Raising seedlings for pathogenicity

Mass rearing of rice leaffolder

Objectives for Second and third year

5. (Confirmation studies).
6. Completed in first year
7. Continued in 2^{nd} and 3^{rd} year
8. To evaluate various natural substrates *i.e.* Prawn & fish scales and other agro wastes for mass production of major potential fungal pathogens
9. To formulate and study the virulence and efficacy of selected entomopathogenic fungi under lab and field conditions
10. To test the safety levels of fungal formulations on non target organisms
11. To develop cheaper and eco friendly myco insecticide for management of major rice pests to minimize the losses (continued in second and third year).

12. Data collection, analysis, documentation and report preparation

List of experiments

1. Lab and field studies and confirmative trial, Survey of entomofungal pathogens of rice leaf folder, stemborer and brown planthopper in rice ecosystem of Cuddalore District (Confirmation studies).
2. Identify promising Entomofungal pathogen –Continued, (Mutagenesis studies - Completed in first year)
3. Pathogenicity studies -Confirmation studies (Continued in 2^{nd} and 3^{rd} year)
4. Evaluation of cheaper and coarse food grains, agro waste and agro product for mass production of fungal pathogens
5. Mass production of potential fungal pathogens
    a. Mass production of best fungal pathogen *(Z. radicans)* isolated for preparation of oil and wettable formulation.

6. Preparation of different formulations for lab and filed studies
    a. Evaluation of various formulations of (*Z. radicans)* against major pests of rice under laboratory and field condition.
7. Safety level of fungal formulation on non target organism-Compatibility studies

8. Effect of sub culturing on various growth characteristics and infectivity of *Z. radicans*

Part II

Methodology (Confirmation studies – second year)

EXPERIMENT- I & III

1.1 Survey of entomofungal pathogens of rice leaf folder, stemborer and brown planthopper in rice ecosystem of Cuddalore District.

In the confirmation studies to document the dominant fungal pathogen available in this rice ecosystem, surveys were undertaken to collect naturally occurring entomopathogenic fungi on rice leaf folder, stem borer and brown plant hopper from rice fields. Samba (Oct to Jan) is the main rice growing season in this coastal part of Tamilnadu and also for the incidence for major pests of rice. Just like in the previous season, the hot spot location was confirmed by conducting surveys in five different localities viz., Annamalainagar, B.Mutlur, Kattumannarkoil, Periapattu and Sivapuri in Cuddalore district of Tamil Nadu. As the natural incidence of the entomopathogenic fungi was aided by the humid climate coinciding with the winter season of the year, hence field visits were effective during samba season of 2014-2015 in all the above places.

Based on the infection percentage of entomopathogenic fungi from different places, one hot spot was identified and correlated with weather parameters. Five places of Cuddalore district were surveyed for incidence of major pests at fortnight intervals during samba season of 2014 - 2015. Diseased cadavers were gathered during early morning hours between 6.00 and 8.00 am, preserved in sterile Petri plates and glass vials, brought to laboratory and data like stage of the date and place of collection were recorded. Fungi isolated were subjected to pathogenicity test (Plate 6).

Isolation

The mycosed leaffolder larvae, stemborer and brown planthoppers collected from the rice fields were surface sterilized in 70 % ethanol for 10 seconds followed by 2% sodium

hypochlorite solution for two minutes and two washed twice with sterile distilled water for two minutes each. Then the cadavers were transferred to synthetic medium, Sabouraud Dextrose Aagar with Yeast extract (Keller, 1994). As the cadavers found on stemborer were minimum further studies conducted only on rice leaffolder and brownplant hopper.

Identification and storage: The fungi isolated were subjected to identification as per the standard keys followed during previous season Parthasarathry (1997), Udayaprabhakar (1995).

Mass rearing of Leaffolder *C. medinalis* and brown planthopper *Nilaparvata lugens*

The rearing technique developed at IRRI by Waldbaurer and Marciano (1979) was followed with slight modifications for rearing Leaffolder *C. medinalis* and brown planthopper *Nilaparvata lugens* suitable for high humid conditions (Plate 5).

Observations recorded

Spore suspension for each isolate was prepared. The appropriate spore concentration was estimated with haemocytometer. The per cent mycosis of the test insect over control was computed. The isolate showing highest percentage mycosis was resolute in pure culture from the diseases cadavers showing typical mycosis while confirming the pathogenicity. Reconfirmation of the incidence of *Z. radicans* and *B. bassiana* among the five places surveyed helps in development of techniques to identify and evaluate on the incidence of the hot spot of leaffolder & brown plant hopper and *Z .radicans* & *B.bassiana* The correlation studies provide the significance of weather factors especially temperature, relative humidity and rainfall on the incidence major pests i.e., leaf folder and brown plant hopper and its entomofungal pathogen multiplication in a particular location (Plate 4).

EXPERIMENT IV

4.1. Evaluation of cheaper and coarse food grains, agro waste and agro product for mass production of fungal pathogens

To evaluate suitable natural media cheap millets on test fungus *B. bassiana* and *Z. radicans,* selected millets like Bajra, Broom Corn {Proso(*Panicum milaceum*) millet}, Maize, Ragi, Sorghum, Soya bean and Cereals Rice, Wheat were used for mass production of spores. Similarly to identify and explore the possibility and potential of agro wastes like Farm yard manure, pressmud, sugarcane baggase, Fish scale, Prawn scales were tested for mass culturing entomofungal pathogens. However other important factors like availability of raw materials for large scale production cost of the material, quality, shelf life, perish ability and feasibility are required to be further tested to identify and develop most cheaply viable technology for mass cultivation. Further to produce most effective entomopathogen in large scale, to standardize the other procedures like dose, quantity and quality further intensive studies are to be conducted. Cost of production studies confirmed the expenditure involved in unit production of spores. Among most promising suitable media, the evaluation test was conducted to know the cost of production of the spores, with different media (Plate 3).

Hence most suitable entomopathogen was selected for further studies.

4.2. Preparation of natural media

Entomopathgens are found in plenty during winter season in this part of ecosystem and cheap to mass produce, easy to store and effective over a wide range of temperature and humidity levels. Hence this study was designed to mass culture the entomopathogenus fungi on

locally available agricultural and industrial wastes for a cheap and suitable substrates for the low cost production of entomopathogens of virulent spores i.e., liquid and solid media.

For evaluating biomass and spores, different media like cheaper and coarse food grains Broom corn millet, bajra, maize, sorghum, cereals, rice and wheat, agro wastes**,** Farm yard manure, Press mud Sugarcane, baggase, Fish scale, Prawn scales, Cornmeal, Rice bran were tested with a synthetic diet namely, PDA and SDA medium as standard check. Vegetable like Potato, soybean, and carrot were also evaluated. Millet grains were selected because there cost of production is cheap, easy to handle and above all rich in nutrients. Whereas Broom corn (Proso millet) pani varagu in tamil is the richest sources of protein (12.5 % & 364 energy k cal/ 100g) (Plate 2).

4.3. Preparation of substrate for Solid media

Different natural substrates viz Rice grain, wheat grain, Bajra, Broom Corn, Maize, Ragi, Sorghum, Soyabeen and also vegetables Potato, Carrot, green banana were evaluated the growth and sporulation of *B. bassiana and Z. radicans*. 100 g of each test (media) grain was washed well and soaked in water overnight except rice which was soaked for 2 - 3 h prior to starting the experiments. The excess water was drained by decanting and shade drying it for half an hour to further remove the excess moisture. Three replications were maintained for each substrate.

The substrates were packed separately in individual 250 ml bottle for *B. bassiana* and *Z. radicans*. They were plugged with cotton wool and auto calved at 15 psi for 1h. After cooling, 1 ml of the spore suspension of fungal pathogen was inoculated into each bottle, separately. All these procedures were done under laminar air flow chamber. They were incubated in BOD

incubator at 28^0 C for 15 days. After 15 days of incubation, 10 g of homogenous substrate sample drawn from each replicate uniformly sporulating flasks were transferred to 100 ml sterilized distilled water containing Tween 80 (0.05%) solutions in 250 ml conical flasks. The flasks were shaken in mechanical shaker for 10 min. The suspension was filtered through double layered muslin cloth. Counting of spores were made after the serial dilution of the suspension using double ruled Neubauer haemocytometer for determining the number of conidia in 1 g of the substrates.

However, steamed millet grains Broomcorn millet was inoculated by mixing 15 g Sabouraud dextrose broth are incubated at 15^0 C for up to 24 days.

Liquid media; All the test materials used for solid media were also used in liquid media and evaluated for the growth and sporulation of two tested fungi. 100 ml of each medium was poured in 250 ml capacity conical flasks and autoclaved at 15 psi pressure for 20 min. Each flask was inoculated with 1 ml of spore suspension of each fungus separately and incubated at 28^oC for 15 days. The spore suspension was subjected to spore counting and it was carried out as described in whole solid media. Sabourad's Dextrose Medium was used as control.

Aqueous extract of Potato and Carrot were used in this study. The extract was prepared, at a final concentration of 400g peeled tubers/lit., as follows: Tubers were peeled, minced into three quarters of the final quantity of water and boiled with constant stirring for 5 – 15 minutes. The mixture was frozen at -10^0C overnight, after thawing, was filtered through muslin cloth. The filtrate was stored in conical flask at -10^0C. When required, portions were thawed and filtered (Whatman No. 5 filter paper) to give a faintly opalescent extract (Beever and Bollard, 1970).

The required samples were completely ground into fine powder by electric blender with the use of sieve. Then the powdered samples were kept in air tight containers until its use. Two different solid media were prepared as follows. Specific amount of samples were taken and mixed with (1.5 – 6g) of agar dissolved in 100ml of distilled water with Cornmeal 1.7g/100ml water of each sample powder. The medium pH was adjusted to pH 6.4 before autoclaving. Then the dissolved media were sterilized by an autoclave at 121^0C for 20 min under the pressure of 15 lbs/inch^2 and they were poured into sterile petri dishes separately (Mekala *et al.*, 2016).

Fungal culture of 10 mm discs of the *Z. radicans* grown in SMAY medium were taken from Petri plate and inoculated in the different natural test materials grains of cereals, millets were separately incubated for 12 days at 25^0 C. The mycelial mats were collected by suction filtering on pre-weighed filter paper (Whatman No.1) dried in hot air oven at 105^0C for 24 hrs and weighed again. The difference in the weight denoted the biomass produced (Hall and Bell, 1961).

Flame sterilized cork borer of 10 mm diameter were used to core out discs of the fungal cultures grown on respective mycological medium in the petri plate and the discs were inoculated into the respective natural media like Potato, Carrot and Cornmeal. The radial growth was recorded on 12^{th} day and expressed in mm.

The spore suspension of each natural media was prepared at a concentration of 10^7 Spores/ml. For germination the spore suspension of drop was placed on 2mm agar discs of the respective media, which provided nourishment, taken on a series of six sterile cavity glass slides. The slides were incubated at 25^0C in moist chambers with around 100% relative humidity and per cent germination was determined after 24 hrs as suggested by Walstad *et al*. (1970). The

criterion for spore germination was the development of a germ tube equal to the diameter of the spore.

The spore suspensions of *Z. radicans* culture plate with a spore load of 10^7 spore/ml prepared from the different medium was given as a fine mist spray on the leaf & larvae confined in the mylar film cages. Five numbers of third instar larvae were tested in each replication and mortality due to mycosis was recorded after four days of treatment. All the treatments were replicated three times. Synthetic SMAY plates served as control.

To prepare granular culture of *Z. radicans,* 20 gms of / per 100 mlflask was soaked in 30m min. in water. After rinsing to remove the dust, the grains were autoclaved for 15 min. at 120 psi and cooled at ambient temperature. Each flask of the autoclaved grains were inoculated with a half plate colony homogenized in 3ml Sabouraud dextrose broth supplemented with 0.5 mustard oil and 0.1% sucrose fatty acid esters. After plugging all flasks were incubated to 24 days. This millet based technology is easy, inexpensive and highly efficient and not required any special additives, drying, freezing and milling.

The use of different agricultural wastes is economical and also helped in their efficient utilization. For a successful integrated pest management programme, the agents like entomopathogenic fungi should be amenable to easy and cheap for mass multiplication. However based on the practical conditions like availability of test media, shelf life, cost , quality and its response in producing more number of viable and effective spores different media available in different sources were evaluated to identify most promising, potential media for mass culturing entomofungal pathogens.

EXPERIMENT V

Mass production of potential fungal pathogens

5.0. Suitability of various supplemented media for biomass, radial growth, spore germination, infectivity of the fungus

To select the appropriate medium for growing the test fungus, an experiment was conducted with different media *viz.,* Coagulated Egg Yolk (CEY), Egg Yolk– Sabouraud Dextrose Agar (EYSDA), Sabouraud-Egg-Milk Agar (SEMA) and Coagulated Egg and Milk (EYM).

5.1. Preparation of media

5.1.1. Coagulated Egg Yolk

Washed hen's eggs were soaked in 50 % ethanol for 30 minutes and aseptically separated the yolks from whites, inside the laminar air flow chamber. The yolk membranes were broken and dispensed into glass petri dishes along with antibacterial antibiotic. The medium was coagulated at 80°C for 6-15 minutes in moist heat (streaming steam) in an autoclave with an isothermal setting. The plates were sealed with parafilm membrane after coagulation (Gustafsson, 1965).

5.1.2. Egg Yolk – Sabouraud Dextrose Agar

Egg yolk collected aseptically as explained in the former was broken in sterile beaker. To each five yolks (100 ml), 30 ml of sterile molten Sabouraud Dextrose Agar with Yeast extract (SDAY) was added and mixed thoroughly, then dispensed in petri dishes along with antibiotic. (Soper *et al.*, 1975).

5.1.3. Coagulated Egg and Milk

The coagulated egg and milk mixture was prepared as explained earlier and dispensed directly on petri plate. Care was taken to secure the plates with parafilm. The media were kept under dark for longer duration without loss of its quality (Keller, 1997).

5.1.4. Sabouraud - Egg - Milk Agar (SEMA)

Five egg yolks were collected aseptically in a graduated cylinder to which 85 ml of sterile skimmed milk was added. Molten sterile Sabouraud Dextrose Agar with Yeast (SDAY) extract (800 ml) was prepared separately.

The egg yolk-milk mixture was mixed with molten synthetic media and dispensed in petri plate. Care was taken in not allowing the milk to turn brown while autoclaving (120°C for 30 min.) (Milner and Soper, 1981).

5.2. Preparation of the fungal culture

The fungal culture in the Petri plates containing SDAY media was incubated for 10 days at 25°C and maintained under refrigerated conditions till use. The fungal inoculum sub cultured not more than four times was used. Dishes showing good fungal growth were selected for the experiment.

5.2.1. Biomass production

Test broth for each medium was prepared in 250 ml Erlenmeyer flasks with egg yolks added in cold broth after autoclaving to avoid coagulation. Flame sterilized cork borers of 10 mm diameter were used to core out discs of the fungal cultures grown on respective mycological medium in the petri plates and the discs were inoculated into the respective test media broth. Four replications were maintained in each medium. The entire set up was incubated for 10 days

at 25°C to attain maximum growth and sporulation. The mycelial mats were collected by suction filtering on pre-weighed filter paper (Whatman No.1) dried in hot air oven at 105°C for 24 hrs and weighed again. The difference in the weight showed the biomass produced (Hall and Bell, 1961).

5.2.2. Radial growth

The solid media of each medium was used to study radial growth of the test fungus. They were prepared and poured into sterile petri dishes. A mycelial disc of 10 mm diameter taken from the edges of an actively growing colony of the test fungus with a sterilized cork borer was seeded in the centre of each agar medium. The inoculated dishes were incubated at 25°C for 10 days. In each medium four replications were maintained. The diameter of the growth circle of the fungal colony was measured as suggested by Daggupati Komala (1988).

5.2.3. Spore germination

The spore suspension was prepared at a concentration of 10^7 spores ml^{-1}. For germination, the spore suspension drop was placed on 2 mm agar discs of the respective media, which provided nourishment, taken on a series of six sterile cavity glass slides. The slides were incubated at 25°C in moist chambers with around 100% RH and the per cent germination was determined after 24 hours as suggested by Walstad *et al.* (1970). The procedure for spore germination was the development of a germ tube equal to the diameter of the spore.

5.2.4. Infectivity

The spore suspensions were prepared from the cultures grown in different supplemented media were tested against third instar leaf folder larvae at a spore load of 10^7 spores ml^{-1} at 25 ± 2°C. For each medium four replications were maintained. Twenty leaf folder larvae were tested

in each replication and mortality due to mycosis was recorded four days after treatment. Mortality in the control was also recorded and the experiment was conducted in completely randomized block design.

5.3. *In vitro* production of resting spores of the fungus, *Z. radicans*

5.3.1. Effect of various levels of different carbon and nitrogen sources on resting spore production in liquid shake culture

In an attempt to detect an appropriate *in vitro* sporulation medium for *Z. radicans*, an experiment was conducted with twenty combinations between two nitrogen (N) and two carbon sources (C) in liquid medium. Two each of carbon sources and nitrogen sources utilized in experiment were sunflower oil, dextrose and yeast extract, peptone respectively. The five different proportions of the nutrients tested for sporulation were 1 % N + 2 % C, 1 % N + 4 % C, 2 % N + 4 % C, 2 % N +8 % C and 4 % N + 8 % C. Four replicates were used in each treatment.

5.3.2. Preparation of sporulation medium

Each semi-defined medium was prepared with a concentration of 10g per 1000 ml of medium at one per cent of the corresponding carbon or nitrogen source utilized. The sunflower oil was added at the rate of 30 ml for 1000 ml of the medium. In each replicate, 30 ml of the medium was taken in 250 ml. Erlenmeyer flask and pH was adjusted to 6.5 before autoclaving at 1.15 kg/cm^2 for 15 min.

5.3.3. Preparation of the fungal pathogen

The flasks with semi defined medium were inoculated with 25 ml of the *Z. radicans* culture grown in Sabouraud-Egg-Milk-Agar (SEMA) broth at 25°C for five days showing good growth and not sub cultured more than four times. The experimental cultures were grown at 25 ± 2°C on a reciprocating shaker with 100 oscillations per minute.

5.3.4. Quantification of the resting spores

Quantification of the resting spores was done for each replicate at tenth day after initial day of inoculation. The resting spores formed were recovered by filtration and washing with sterile distilled water through the top of stack of sieves (123, 63 and 20 mm sieve openings) to discard the mycelial debris. The fraction collected over 20 mm sieve was resuspended in distilled water and used for the estimation of sporulation rate through determining the number of spores per ml with an improved Neubauer's haemocytometer. Number of cells was counted as resting spores and was detected as double walled and contained oil globules (Kogan and Hajek, 2000).

5.4. Germination of *Z. radicans* resting spores

5.4.1. Effect of temperature on germination

The resting spore suspension was washed four times with sterile distilled water through 63 mm sieve into 20 mm sieve, plated on 100 mm dishes with sterile water agar 1.5 % (w/v). 100 % RH was maintained and 25 µL of 50 mg/ml gentamycin sulphate was added and observations were recorded for 24 hrs. The cumulative per cent germination of the resting spores was estimated by recording the developmental stages of 10 resting spores in each of 10 microscopic fields at 450 x magnification in Nikon microscope at various time intervals *viz.,* 5^{th}, 15^{th}, 25^{th} & and 35^{th} day from the initial day of the experiment. Four replications were maintained for temperature levels *viz.,* 10°C, 15°C, 20°C and 25°C respectively (Perry *et al.,* 1980).

5.5. Effect of *Z. radicans* on the growth of larvae and pupa of *C. medinalis*

5.5.1. Collection and rearing of pest.

The larvae of *C. medinalis* were collected from infested rice plants from Annamalainagar in Cuddalore district. The larvae collected were maintained in the laboratory at 22 ± 2°C and 70 –75 % relative humidity. The larvae were reared on potted rice plants.

5.5.2. Bioassay

Spore suspension was prepared from 15 days old culture of *Z. radicans* on SMA medium. The fungal surface was scraped using a sterile loop with 10 ml of sterile distilled water having 0.02% Tween 80 as a wetting agent (Rombach *et al.,* 1986). The suspension was then filtered through sterile muslin cloth to eliminate the medium (Sasidharan and Varma, 2005). Spore concentration of the filtrate was determined using a Neubauer's haemocytometer. This served as the stock suspension. Different spore concentration was prepared by adding sterile 0.02% Tween 80 in distilled water. Spore suspension of *Z. radicans* at four different concentrations, 2.4×10^7, 2.4×10^6, 2.4×10^5 and 2.4×10^4 spores/ml was prepared and tested for its efficacy on third instar larvae, pupae and adults of *Z radicans.*

5.5.3. Growth inhibition of larvae

For bioassay, spraying method was adopted. Nine ml of different spore concentrations of *Z.radicans* was sprayed against rice leaf folder larvae. Ten larvae were used per replication. The larvae were treated with sterile distilled water and 0.006 % (v/v) of neem product and cypermethrin. These three served as positive control. After treatment, the larvae were allowed to feed on rice leaves. Each treatment was replicated thrice. Growth parameters namely larval duration (days), larval length, larval weight and pupation (%) were recorded. (Hafez *et al.,* 1994).

5.5.4. Growth inhibition of pupae

Four different spore concentrations of *Z. radicans* with three replications each were used for infecting the pupa of *C. medinalis.* The pupae were sprayed with 10 ml of respective fungal spore suspensions using hand atomizer. The pupae were treated with sterile distilled water and 0.006 % (v/v) neem product and cypermethrin. These three served as positive control. The

growth of surviving pupa was recorded upto adult emergence for the parameters, such as pupal duration (days), pupal weight (mg), pupal length (cm) and adult emergence (%). (Hafez *et al.*, 1994). All the treatments were replicated four times and the experiment was conducted in Complete Randomized Block Design.

5.6. Production of mycelial powder, conidial substrate and resting spores for *in vitro* studies

5.6.1. Production of mycelial powder

The mycelia were harvested from the petri plates along with medium. The mycelia were then suspended in an equal volume of water. The water was drained to remove the spores. The mycelial mat (mycelia with solid medium) was then sprayed with 10% w/v aqueous solution of glucose. The sprayed mats were shade dried at a room temperature of about 28°C.The mats were dried to crispness in about 6 to 8 hours. The dried mats were removed from the racks and ground and sieved through a 20-mesh screen. The powder was sealed in a container and stored at 4°C for *in vitro* studies (Plate 7).

5.6.2. Development of conidial substrate for spraying

Synthesis of 70% wettable powder formulation of *Z. radicans* using different millet and selected test grain medium were attempted and evaluated in the field.

Five hundred ml Erlenmeyer flasks were used for mass production of the fungus. Cheaper and highly suitable test grains and other materials were crushed in the house hold mixer well boiled, cooled, air-dried, and calcified for easier penetration of the fungus between the grains. Twenty gram of calcium carbonate was added to one kg of boiled millet grains. The calcified grains were stuffed in the conical flasks and double sterilized in autoclave for 15 min, at 1.15 kg/cm^2 pressure. These flasks were cooled and later inoculated with the fungus and incubated for

12 days to attain maximum sporulation. The fungal mass developed on the grains were harvested along with sorghum grain carrier. Concentration of spores per ml was standardized through haemocytometer (Udayaprabhakar, 1995).

5.6.3. Production of resting spores

The methodology which was described earlier for *in vitro* production of resting spores of the fungus, *Z. radicans* was followed for evaluation in the pot culture studies.

5.7. Evaluation of certain forms of *Z. radicans* against rice leaf folder under lab conditions.

The rice plants maintained in potted cages (CR 1009) were used to evaluate Z. *radicans.* Totally seven treatments were fixed and replicated three times. A control without spraying was maintained.

Twenty insects (third-fourth instar larvae) were released per cage. The fungal spray was given as a fine mist using hand glass atomizer directly on the larvae and on the plants confined in mylar film cages. The percentage mycosis of the test insect over the control was recorded from fourth day after treatment onwards.

EXPERIMENT VI

Methodology - (Second year, second half)

6.0. Preparation of different formulations for lab and filed studies

6.1. Preparation of powder and oil formulation of *Z. radicans*

Fresh potato of 250 g skin was peeled off and sliced in to small pieces. 500 ml of water was added and boiled for 20 minutes in a pressure cooker. Simultaneously 20g agar was mixed with 500 ml of water and boiled in a cooker for 30 minutes. The potato extract was collected by filtered through muslin cloth. Dextrose 20 g was added to the potato extract. The molten agar was mixed thoroughly with the potato-agar mixture and 1 litre volume was made with distilled water. The pH of the medium was retested using pH papers. *Z. radicans* employed in this study was isolated from infected rice leaf folder collected from the rice field of Annamalai nagar. *Z.radicans* was grown separately on PDA broth to develop the fungal mat. The blended fungal mat (350 gm) was grinded with talcum powder (600 gm) and gum (100 gm) for powder formulation. The blended fungal mat (350 gm) was grinded with corn oil (500 gms), gum (100gm) and glycerine (100 gms) for oil formulation (Agarwal *et al*., 2012).

6.2. Evaluation of powder and oil formulation of *Z. radicans* against biology of *C. medinalis*

Newly moulted second instar larvae of *C. medinalis* were bioassay for their susceptibility to fungal pathogen. The larvae were air-dried by keeping them in laminar air flow for 5 minutes and carefully transferred to individual clean sterile petriplate. Different concentrations of powder and oil formulations like 2 %, 4%, 6% and 8% and control were directly sprayed on the larvae using a hand atomizer. Four replicates of four larvae were used in

each treatment. The larval mortality, pupal mortality, adult percentage and adult mortality percentage due to mycosis were calculated and the results were tabulated (Sivasundaram *et al.*, 2007).

6.3. *In vitro* evaluation of powder and oil formulation of *Z. radicans* against mortality of *C.medinalis* .

In pot culture experiments *Z. radicans* were evaluated against third instar larvae. The larvae were released in pots pre- planted with one month rice plants and allowed to settle for 24 hours. In the test against larvae, powder and oil formulations carrying the different concentrations were applied to pots in replicates of ten larvae each. The pots were watered immediately. Larvae were examined for infection due to *Z .radians* at regular intervals and results were tabulated (Agarwal *et al*., 2012).

EXPERIMENT VII

Safety studies on natural enemies

7.0. Saftey level of fungal formulation on non target organism-Compatibility studies

7.1 Evaluation of *Z. radicans* and *B. bassiana* against parasitoids

Three isolates of *Z. radicans* were evaluated against the adult stage of the parasitoids. Parasitoids were placed in a plastic cup for 24 hours with the larvae *C. medinalis*. Then sprayed with test concentrations of entomopathogenic fungi, *Z. radicans*. Other group of parasitoid adults was sprayed with water only as a control. Two days later, each of the parasitoid adult was transferred to new plastic cup with fresh larval host and incubated at 25 ± 2^0C, 18L: 6D and 65 ± 5^0C RH.

The cups were check daily, for adding fresh hosts and/or removing the dead parasitoids adult. The cadavers were removed, then surface sterilized in 5% Sodium hypochlorite and 75% ethanol solution and rinsed in plenty of sterile distilled water, then left to dry for 48 hours (Dourou – Kpindou *et al.*, 1995). After drying, they were kept in humid condition in clean desiccators at room temperature to examine whether they died because of fungus infection or not according to Luz and Fargues (1998).

7.2. Impact of the entomopathogenic fungus, *Z. radicans* on the honey bees, *Apis cerana indica* (Hymenoptera: Apidae)

Three isolates of *Z. radicans* were evaluated for virulence against adult worker Indian bees. The isolates were cultured on Sabouraud's maltose agar for two weeks in the dark at 24 – 25^0C. Four concentrations (1×10^6, 1×10^7, 1×10^8 and 1×10^9 CFU g^{-1}) from each isolates were prepared by mixing dry conidia with corn flour.

Young honey bee workers were collected from brood chamber of honeybee hives by brushing into ventilated cages with approximately 5 bees in each cage. Bees were immobilized using co_2 gas and treated by the inoculation mixture using a pollen applicator. Each cage received 1.0 g of the inoculums mixture. Then three cages for each concentration. Caged bees were kept at 24 ± 1^0C and a 16 hrs photoperiod. Honey bee mortality was recorded daily for 5 DAT. Dead bees were removed and placed in petriplates lined with moist filter paper to favor external growth and sporulation of the fungus. Then to determine the infection of *Z. radicans* on treated bees produce an external growth and spores (Al Mazra' awi, 2007).

EXPERIMENT VIII

Confirmation studies of mass production

Effect of sub culturing on various growth characteristics and infectivity of *Z. radicans*

8.1. Different formulation of entomopathogenic fungi

8.1.1. Preparation of oil based bio-formulations

Potato Dextrose Agar (PDA) with conidial concentration of 1×10^6cfu/ml was prepared. Ten ml of glycerol as adjuvant and one ml of oils like sunflower and mustard were added at different combination to the broth medium containing culture of *Z. radicans* (Boruah *et al.*, 2015) (Plate 8).

8.1.1.1. *In vitro* evaluation of oil formulation of *Zoophthora radicans* against biology of rice leaf folder, *C. medinalis*

Newly moulted second instar larvae of *C. medinalis* were bioassay for their susceptibility to fungal pathogens. The larvae were air–dried by keeping them in laminar air flow for 5 minutes and carefully transferred the larvae to individual clean sterile petriplate which contains the moistened tissue paper. The different treatments of oil formulation were directly sprayed on the larvae using a hand atomizer. Water sprayed on the larvae as a control. Three replications and five larvae were used in each replication (Plate).

The larval mortality, pupal per cent, pupal mortality, adult emergence and adult mortality per cent due to mycosis were calculated and the results were tabulated (Sivasundaram *et al.*, 2007).

8.1.2. Preparation of granular formulation

Shelled broomcorn millet *Panicum miliceum* (Panivaragu) grains were used as solid substrate to prepare granular cultures of *Z. radicans*. The following procedure was described

(Feng and Liang 2003, Hua and Feng 2003) the millet grains (15g per 100ml flask) were soaked in water for 30 min at 80^0C. Then after rinsing to remove the excess water and dust, the grains were autoclaved for 15 min at 121^0C and cooled to room temperature. Then each flask of the autoclaved grains was inoculated with half a plate colony homogenized in 3ml Sabouraud dextrose broth supplemented with 0.5% (v/v) mustard oil and 0.1% sucrose fatty and esters. After plugging with vent stoppers, all flasks were incubated for up to 24 days at 15^0C and Light: Dark 12: 12. No agitation measures for aeration were taken during the incubation period.

8.1.2.1 *In vitro* evaluation of granular formulation of *Z. radicans* against biology of rice leaf folder, *C. medinalis*

Millet grains cultured with *Z. radicans* for 21 days were uniformly distributed on 2% agar in a 90 mm diameter Petri dish and incubated at 15^0C and Light/Dark 12/12 hrs for 24 – 48hrs for abundant sporulation. The larvae of *C.medinalis* were taken in the Petri plates containing millet grain culture (*Z. radicans*). For inoculation, second instars *C. medinalis* larvae on detached rice leaves in Petri dishes were exposed to spore showers containing different concentrations (12.2±2.5, 34.6±3.1, 54.3±3.5 and 81.6±4.8 Spores/mm^{-2}). Different concentrations of *Z. radicans* in the grain pre assessed using haemocytometer. Each spore concentration included three replications (5 larvae/ replicate). Larvae in three Petri dishes unexposed to spore showers were included as blank control (Hua and Feng, 2005).

8.2. Semi-field evaluation of oil and granular formulation of *Z. radicans* against mortality of *C. medinalis*

In pot culture conditions *Z. radicans* was evaluated against third instar larvae. The larvae were released in pots pre-planted with one month rice plants and allowed to settle for 24 hrs. In

the test against larvae, the different treatments of oil formulation were directly sprayed on the larvae using a hand atomizer. Water sprayed on the larvae as a control. (Agarwal *et al.*, 2012). The different doses of granular formulation were applied at 10, 20, 30 and 40g in each pot. Before applying the granules is thoroughly mixing the product of fine sandy soils, and then evenly distributing the pots. Soil was moist at the time of application (Erler and Ates, 2015). Only soil placed in the pot as a control treatment. Three replications and 10 larvae were used in each replication. The pots were watered immediately. Larvae were examined for infection due to *Z. radicans* at regular intervals and results were tabulated.

8.3. Field evaluation of oil and granular formulation of *Z. radicans* against mortality of *C. medinalis*

A field trial was conducted during August 2016 to January 2017 to evaluate the mycological suppression of rice leaf folder in two different formulations like oil based bio-formulation and granular formulation of *Z. radicans* in the Experimental Farm of Faculty of Agriculture, Annamalai University.

The field trial was laid out in a randomized block design (RBD) in oil formulation with seven treatments and granular formulation with five treatments, replicated three times in plot size 5×4 m each, with the variety CR – 1009.

The treatment details were given below.

1. Oil formulations

T_1 - *Z. radicans* alone, T_2 - *Z. radicans* + Glycerol 10%

T_3 -*Z. radicans* + Sunflower oil 1%, T_4 - *Z. radicans* + Sunflower oil 1%+ Glycerol 10%,

T_5- *Z. radicans* + Mustard oil 1%, T_6 - *Z. radicans* + Mustard oil 1% + Glycerol 10%

T_7 - Control (Water)

2. Granular formulation

T_1 - *Z. radicans* 100 g/1 kg of soil, T_2 - *Z. radicans* 200 g/2 kg of soil

T_3 - *Z. radicans* 300 g/3 kg of soil, T_4 - *Z. radicans* 400g/4 kg of soil

T_5 - Control (Soil).

Liquid formulation of *Z. radicans* amended with best concentrations of adjuvant and vegetable oils *viz., Z. radicans* + Sunflower oil 1% + Glycerol 10% at 1×10^6 spores/ml was sprayed thoroughly with the help of knapsack sprayer. Only water spray used as a control. Three replications maintained in each treatment (Boruah *et al.,* 2015).

The concentration of conidia on the granules (*Z. radicans*) was $2\text{-}3\times10^5$ viable conidia/granule. The granule (*Z. radicans*) was applied at 100, 200, 300 and 400 g/m^3 of moist soil medium for the granular formulation. The granular formulation of *Z. radicans* was applied by thoroughly mixing for fine sandy soil, and then the granules are evenly distributed in the soil. Every 10g of granules are mixed with 100g of fine sandy soils. The formulation was applied by broadcasting the granules as uniformly possible over each plot (Erler and Ates, 2015). Observation on mortality of leaf folder was recorded by counting dead cadavers. Leaf folder mortality was recorded at 5, 7 and 10 days after spraying (DAS).

8.4. Statistical Analysis

Statistical analysis was carried out using the procedure described by Panse and Sukhatme (1961) and Gomez and Gomez (1972) and the various treatments were ranked according to Duncans Multiple Range Test (DMRT) with the help of the computer generated IRRISTAT analysis package. All the studies were conducted with completely randomized block design.

RESULTS

EXPERIMENT- I, II & III

1. Survey of entomofungal pathogens of rice leaf folder and brown planthopper in rice ecosystem of Cuddalore District.

Confirmation studies during second survey showed that highest infection of brown planthopper in rice was noticed in during first fortnight of January 90.00 % and 92.00 % of rice leaf folder during second fortnight of January. However stem borer cadavers were minimum (12.50 %) (Table 22). Hence studies were initiated only on BPH and leaffolder which were available in significant number.

Among the five places surveyed in the Cuddalore district, overall infection percentage of rice leaf folder was maximum at Annamalainagar (63.08 %) followed by Periyapattu (49.08 %), B.mutlur (47.25), Sivapuri (41.25) and Kattumannarkoil (41.00 %) respectively. Whereas, overall infection percentage of rice stem borer and brown planthopper was maximum at Annamalainagar (12.20) & (55.50 %) followed by Periyapattu (10.65 %) & (42.91 %), B. Mutlur (13.70 %) & (42.50 %), Sivapuri (11.42 %) & (37.66 %) and Kattumannarkoil (9.37 %) & (36.83 %) respectively. Weather parameters of Annamalainagar were correlated with incidence of entomopathogenic fungi infection on rice leaffolder and brown planthopper.

Table 22. Survey of entomofungal pathogens of BPH and rice leaf folder in Cuddalore district during samba season of 2014-2015

S.No	Month @ fortnight Intervals	Fungal infection percentage									
		Annamalainagar		Sivapuri		B.mutlur		Periyapattu		Kattumannarkoil	
		BPH	Leaf folder	BPH	Leaf folder	BPH	Leaf folder	BPH	Leaf folder	BPH	Leaf folder
1	September-I	25.00	30.00	14.00	26.00	17.00	23.00	20.00	27.00	19.00	25.00
2	September-II	32.00	28.00	19.00	25.00	22.00	20.00	24.00	25.00	23.00	21.00
3	October-I	35.00	48.00	24.00	24.00	27.00	22.00	29.00	30.00	26.00	28.00
4	October-II	44.00	50.00	28.00	26.00	31.00	24.00	32.00	35.00	29.00	39.00
5	November-I	51.00	55.00	32.00	23.00	40.00	26.00	38.00	44.00	31.00	67.00
6	November-II	56.00	60.00	37.00	28.00	43.00	35.00	45.00	64.00	36.00	69.00
7	December-I	60.00	62.00	45.00	39.00	51.00	55.00	52.00	68.00	40.00	64.00
8	December-II	62.00	92.00	52.00	68.00	56.00	74.00	55.00	79.00	48.00	45.00
9	January-I	90.00	91.00	64.00	65.00	72.00	86.00	66.00	74.00	58.00	38.00
10	January-II	86.00	92.00	58.00	70.00	64.00	79.00	68.00	49.00	57.00	33.00
11	February I	71.00	71.00	47.00	56.00	49.00	69.00	53.00	52.00	47.00	27.00
12	February-II	54.00	78.00	32.00	58.00	38.00	54.00	33.00	42.00	28.00	36.00
Average		55.50	63.08	37.66	41.25	42.50	47.25	42.91	49.08	36.83	41.00

I-Fortnight

II-Fortnight

2. Multiple correlations between incidence of entomopathogenic fungi on brown plant hopper and weather parameters in Annamalainagar during 2014-15

Multiple correlations between minimum temperature and infection percentage of brown plant hopper in Annamalainagar during 2014-2015 were highly significant (-0.571) followed by relative humidity (0.293) that these factors exhibited definite and appreciable influence on the infection. However correlation with rainfall (-0.057) and maximum temperature (-0.684) were not significant. But heavy rainfall in November delayed the growth of fungus. Maximum temperature also had no influence on infection (Table.24) .

With a view to bring out relationship among incidence of fungi on rice leaf folder and abiotic factors, multiple regressions were worked out. The fitted equation for the year 2014-2015 was

$Y= 65.25+ (X_1)\ 32.70^{**} + (X_2)\ -25.72^{*}+ (X_3)\ 79.98^{**} + (X_4)\ -0.645$

X_1=Maximum Temperature X_2 =Minimum Temperature

X_3 =Relative Humidity X_4= Rainfall

Among the different weather parameters (Table.25) during 2014-2015 maximum temperature (-32.70) and rainfall (-0.645) were negatively correlated and whereas, minimum temperature (25.72) and relative humidity (79.98) showed positive correlation with incidence of entomopathogenic fungi.

2.1. Multiple correlations between incidence of entomopathogenic fungi on leaffolder and weather parameters in Annamalainagar during 2014-2015

Multiple correlations between minimum temperature and infection percentage in Annamalainagar during 2014-2015 were highly significant (-0.642) followed by RH (0.213) indicating that these factors (Table.26) exhibited definite and appreciable influence on the

infection. However correlation with rainfall (-0.003) and maximum temperature (-0.714) were not significant. But heavy rainfall in November slowed down the growth of fungus. Maximum temperature also had no influence on infection. .

With a view to bring out relationship among incidence of fungi on rice leaf folder and abiotic factors, multiple regressions were worked out (Table 27). The fitted equation for the year 2014- 2015 was

$Y= 65.25+(X_1)33.36^{*}+ (X_2)-26.59^{*} + (X_3)80.41^{**} +(X_4)-0.645$

X_1=Maximum Temperature X_2 =Minimum Temperature

X_3 =Relative Humidity X_4= Rainfall

Among the different weather parameters during 2014-2015 maximum temperature (-3.36) and rainfall (-0.645) were negatively correlated and whereas, minimum temperature (26.59) and relative humidity (80.41) showed positive correlation with incidence of entomopathogenic fungi.

Table 23. Percentage of leaf folder and BPH infection in relation to weather parameters in Annamalainagar during 2014-2015.

Sl.No	Period	Weather Parameters					
		Maximum Temperature (°C)	Minimum Temperature (°C)	Relative Humidity (%)	Rainfall (mm)	BPH P.I (%)	L.F P.I (%)
1	September-I	30.10	23.40	80.00	007.7	25.00	30.00
2	September-II	34.00	29.10	90.00	099.4	32.00	28.00
3	October-I	33.80	24.70	83.00	029.8	35.00	48.00
4	October-II	29.30	23.30	86.00	356.9	44.00	50.00
5	November I	31.20	23.30	82.00	099.0	51.00	55.00
6	November II	29.10	24. 00	84.00	202.6	56.00	60.00
7	December I	30.20	23.45	86.00	078.6	60.00	62.00
8	December II	26.90	21.60	88.50	392.1	62.00	92.00
9	January I	28.90	20.12	90.00	7.6	90.00	91.00
10	January II	28.45	20.75	89.50	000.0	86.00	92.00
11	February I	29.60	21.80	73.00	000.0	71.00	71.00
12	February II	29.43	22.30	71.00	000.0	54.00	78.00

Table 24. Over all correlation and regression between percentage of BPH infestation and weather parameters in Annamalai Nagar during 2014-2015

Weather parameter	Correlation coefficient (r)	Regression coefficient (b)	Constant (a)
Maximum temperature Vs P.I	-0.684	0.414	46
Minimum temperature Vs P.I	-0.571	0.258	32
Relative humidity Vs P.I	0.293	-0.053	28
Rainfall Vs P.I	-0.057	-0.096	33

P. I - Percentage infection

Table 25. Multiple linear regression - interaction of BPH infestation percentage with weather parameters in Annamalainagar during 2014-2015

	x_1=Max. temperature	x_2=Min. temperature	x_3=Relative humidity	x_4=Rainfall
Y	-32.70**	25.72*	79.98**	-0.645
Tb	-0.051	-0.049	0.067	-0.292

Table 26. Over all correlation and regression between percentage of leaffolder infestation and weather parameters in Annamalainagar during 2014-2015

Weather parameter	Correlation coefficient (r)	Regression coefficient (b)	Constant (a)
Maximum temperature Vs P.I	-0.714	0.461	51
Minimum temperature Vs P.I	-0.642	0.354	41
Relative humidity Vs P.I	0.213	-0.049	45
Rainfall Vs P.I	-0.003	-0.009	13

P. I - Percentage infection

Table 27. Multiple linear regression - interaction of leaffolder infestation percentage with weather parameters in Annamalainagar during 2014-2015

	x_1=Max. temperature	x_2=Min. temperature	x_3=Relative humidity	x_4=Rainfall
Y	-33.36*	26.59*	80.41**	-0.645
Tb	-0.056	-0.058	0.051	-0.019

Table 28. Pathogenicity of different isolates of brown plant hopper *N. lugens* and leaf folder *Z. radicans* on rice.

S.No.	Isolates	Per cent of mortality	
		Brown plant hopper	Leaf folder
1	Annamalainagar	62.50 (53.43)[a]	65.62 (55.29)[a]
2	B.Mutlur	51.56 (45.93)[b]	62.50 (47.17)[b]
3	Sivapuri	46.86 (43.12)[c]	53.12 (46.87)[c]
4	Periyapattu	40.62 (30.00)[d]	39.06 (37.51)[d]
5	Kattumannarkoil	26.56 (29.09)[e]	23.43 (26.30)[e]
6	Control	0.00 (0.28)[f]	0.00 (0.28)[f]
CD (p=0.05)		11.7	12.87
SE.d		7.44	6.04

Each value is mean of four replication, Figures in parentheses are arc sine transformed values.
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT

3.1. Pathogenicity of selected isolates of *Z. radicans* to rice leaffolder and *Beauveria bassiana* to rice brown planthopper.

Five isolates of *B. bassiana* were selected based on its infection percentage in the field and they were subjected to pathogenicity test on BPH. Of the five isolates evaluated, Annamalainagar isolate was found to be highly pathogenic (62.50%) to the test insect followed by other isolates from B.Mutlur (51.56%), Periyapatu (40.62%), Kattumannarkoil (26.56%) Sivapuri (46.86%) respectively (Table 28).

Five isolates of evaluated, *Z. radicans* Annamalainagar isolate was found to be highly pathogenic (65.62%) to the leaf folder followed by other isolates from B.Mutlur (62.50%), Sivapuri (53.12%),Periyapattu (39.06%) and Kattumannarkoil (23.43%). Among the five places surveyed in the confirmation studies Annamalai nagar recoreded maximum mortality,of leaf folder and BPH. Hence, Annamalainagar isolate *Z. radicans and B. bassiana* were utilized for further studies.

EXPERIMENT IV

4.1. Effect of natural media on the cultural characteristics of *Z. radicans* and *B.bassiana*

4.1.1. Solid phase

Among nine different media evaluated (Table 29) for their suitability in mass production of spores, maximum concentration of spores 5.63 x 10^7 & 5.76 x 10^7 and 92.24 & 90.62 % were found on rice grains and closely followed by Proso (Broom corn) 4.61 x 10^7 & 5.62x 10^7 and concentration of spores 90.16 & 92.24 % were recorded on *B. bassina and Z. radicans*. The remaining test media were wheat, sorghum, bajra, maize, soya bean, ragi and rice.

4.1.1. Liquid phase

In the mass production of entomofungal pathogens, (Table 30) similar trend was observed with more number of concentration of spores 6.14 x 10^7 & 6.92 × 10^7 and percentage germination of 95.34 & 96.72 % were harvested with broomcorn millet grains in *B.bassina and Z. radicans*. It was followed by rice grains where 6.23 × 10^7 & 6.74 × 10^7 spore concentration and 94.26 & 96.32 % germination was recorded . Further the trend was similar to the solid phase but with higher number of spore concentration and percentage germination of spores in *B.bassina and Z. radicans*.

Mass Production of *Beauveria bassiana* and *Zoopthora radicans* on selected by products, agrowastes and waste products revealed (Table 31) that Farm yard manure was the favourable substrate with and 171.25and 162.52 spores on *B. bassina* and *Z. radicana.* It was followed by SMAY+ prawn scales and SMAY+ fish scales 121.53 & 128.41 and 117.36 & 114.61 respectively. It was followed by pressmud and sugarcane baggase. Similarly among the cereal and millet grains evaluated broom corn and rice grains stands first with production 257.42

& 271.40 and 245.16 & 256.32 respectively. Among the remaining substrates wheat, sorghum and maize were in descending order of production of spores on *B. bassiana* and *Z. radicana*

` Studies on cost of production (Table 32) of Resting spores of *Beauveria bassiana* and *Z. radicans* on selected agrowastes, agricultural by products and waste products revealed significant results. Among different agrowastes Farm yard manure was the most economical with least cost of production of Rs 0.52 & Rs 0.58 for one unit of spores on *B.bassina* and *Z. radicana* . It was followed by SMAY+ prawn scales and SMAY+ fish scales with 0.67 & 0.63 and Rs. 0.74 & 0.61 respectively. However on agricultural products like cereal and millet grains it was lowest on proso millet and rice with Rs 0.88 . & Rs 0.76 and 0.79 & 0.67 respectively, for production of spores of *B. bassiana* and *Z. radicans* .The trend was similar like in the previous table where sorghum, wheat and maize grains were in the ascending order of cost of production.

Table 29. Mass Production *Z. radicans* and *B.bassiana* on selected agricultural products through solid phase method

	B. bassisana		***Z. radicans***	
Treatments	**Concentration of spores x 10 ^{7}**	**Germination %**	**Concentration of spores x 10 ^{7}**	**Germination %**
Broom corn (Proso millet)	4.6 1 (2.37)^{b}	90.16 (72.13)^{a}	5.62 (2.57)^{a}	92.24 (74.02)^{a}
Maize	1.72 (1.65)^{e}	85.32 (67.51)^{b}	1.95 (1.72)^{d}	82.16 (65.03)^{bc}
Wheat	3.25 (2.06)^{d}	87.25 (69.32)^{b}	3.04 (2.01)^{bc}	86.53 (68.68)^{b}
Rice	5.63 (2.57)^{a}	90.62 (72.40)a	5.76 (2.60)^{a}	92.42 (74.38)a
Bajra	4.02 (2.24)^{c}	88.13 (69.92)^{ab}	3.81 (2.19)^{b}	85.31 (67.54)^{b}
Sorghum	2.91 (1.97)^{de}	86.53 (68.57)^{b}	2.63 (1.91)^{c}	89.45 (71.41)^{ab}
Soyabeen	1.45 (1.56)^{f}	82.00 (64.89)^{c}	1.73 (1.65)^{de}	80.16 (63.58)^{c}
Ragi	1.26 (1.50)^{fg}	79.00 (62.71)^{cd}	1.52 (1.58)^{e}	76.50 (61.02)^{cd}
Rice bran	1.12 (1.46)^{g}	73.00 (58.67)^{d}	1.24 (1.49)	69.27 (56.32)^{d}
Sed	0.031	2.799	0.032	2.885
CD (0.05)	0.066	5.984	0.068	6.168

Each value is mean of three replications.
Figures in parentheses are square root*/arc sine** transformed values.
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

Table 30. Mass Production of *Z. radicans* and *B. bassiana* spores on selected agricultural products through liquid phase method

	B. bassisana		***Z. radicans***	
Treatments	**Concentration of spores x 10 ^{7}**	**Germination %**	**Concentration of spores x 10 ^{7}**	**Germination %**
Broom corn (Proso millet)	6.14 (2.67)^{a}	95.34 (78.06)^{a}	6.92 (2.81)^{a}	96.72 (80.71)^{a}
Wheat(Broken grains)	3.46 (2.11)^{b}	90.62 (72.30)^{ab}	3.66 (2.16)^{b}	93.24 (75.18)^{ab}
Maize	2.02 (1.74)^{c}	82.15 (65.12)^{b}	2.13 (1.77)^{d}	83.61 (66.25)^{b}
Rice (Broken grains)	6.23 (2.69)^{a}	94.26 (76.77)^{a}	6.74 (2.78)^{ab}	96.32 (79.76)^{a}
Sorghum	3.15 (2.03)^{bc}	91.50 (73.34)^{ab}	3.24 (2.06)^{c}	95.40 (78.67)^{a}
Bajra (cumbu)	2.06 (1.75)^{c}	85.23 (67.57)^{b}	2.21 (1.79)^{d}	86.45 (68.62)^{b}
Soyabeen	1.05 (1.43)^{d}	81.00 (64.19)^{bc}	1.09 (1.45)^{e}	80.28 (63.67)^{bc}
Rice bran	0.93 (1.39)^{d}	75.00 (60.01)^{c}	0.96 (1.40)^{ef}	73.19 (58.79)^{c}
Ragi	0.89 (1.38)^{d}	72.00 (58.04)^{c}	0.84 (1.36)^{f}	69.53 (56.48)^{c}
Sed	0.030	3.161	0.031	3.587
CD (0.05)	0.064	6.758	0.067	7.669

Each value is mean of three replications.
Figures in parentheses are square root*/arc sine** transformed values.
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

Table 31. Mass Production of *Zoopthora radicans* and *Beauveria bassiana* on selected agricultural by products, agrowastes and waste products

Sl. No	Treatments	*Beauveria bassiana*	*Zoopthora radicans*
1	(SMAY)+Fish scale	117.36^{e}	114.61^{ef}
2	FYM	171.25^{d}	162.52^{de}
3	(SMAY) + Prawn scale	121.53^{e}	128.41^{e}
4	Press mud	236.32^{b}	225.35^{c}
5	Sugarcane baggase	217.24^{c}	243.19^{b}
6	Broom corn millet+ (SDB)	257.42^{a}	271.40^{a}
7	Maize grain	56.75^{f}	59.63^{g}
8	Sorghum grain	109.51^{e}	110.24^{f}
9	Rice(broken grains)	245.16^{ab}	256.32^{ab}
10	Wheat (broken grains)	185.40^{d}	176.38^{d}
	SE(d)	7.43	7.62
	CD (0.05%)	15.61	16.01

Each value is mean of three replications.

Figures in parentheses are square root*/arc sine** transformed values.

In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

Table 32. Cost of production for production Resting spores of *Z. radicans* and *B. bassiana* on selected agrowastes agricultural by products and waste products

Sl. No	Treatments	*B. bassiana* RS	*Z. radicans* RS
1	FYM	0.58[a]	0.52[a]
2	Fish scale $_{+(}$ SMAY)+	0.67[ab]	0.74[ab]
3	Prawn scale+ (SMAY)	0.61[ab]	0.63[ab]
4	Press mud	1.85[d]	1.73[f]
5	Sugarcane baggase	2.70[e]	2.54[g]
6	**Broom corn (Proso millet)**	0.88[b]	0.76[cd]
7	**Sorghum grain**	1.35[c]	1.48[e]
8	**Maize**	1.61[cd]	1.52[e]
9	**Rice broken**	0.79[ab]	0.67[bc]
10	**Wheat**	0.94[b]	0.87[d]
	SE(d)	0.061	0.138
	CD (0.05%)	0.128	0.291

Each value is mean of three replications.
Figures in parentheses are square root[*]/arc sine[**] transformed values.
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

4.2. Effect of natural media on the cultural characteristics of *Z. radicans*

Biomass and radial growth recorded on Corn meal (140.4 mg and 48.18 mm) and Beet root (164.7 mg and 57.21 mm) and green banana (273.5 mg and 60.08 mm) were very less and they were slightly supported the fungal growth. But Potato (847.5 mg and 78.04 mm) and carrot (364.6 mg and 69.30 mm) were highly supported for biomass production and radial growth of entomopathogenic fungi, *Z. radicans* but SMAY (control) was also recorded higher biomass and radial growth recorded in control medium (654.31 mg and 73.33 mm) (Table 33).

The lowest spore germination and infectivity were recorded in cornmeal (52.70 % and 39.30 %) and beetroot (57.62 % and 46.37 %). Higher spore germination and infectivity were recorded in Potato (81.26 % and 68.32 %), Carrot (68.04% and 52.03 %) and green banana (63.72 % and 48.04%) but SMAY was also higher in control medium (79.41 % and 65.60 %). The natural media like corn meal and green banana were slightly supporting the growth and infectivity of entomopathogenic fungi, *Z. radicans*.

Potato and SMAY were highly supporting for the growth of *Z. radicans* and well suited for the mass production of entomopathogenic fungi, *Z. radicans*.

Table 33. Effect of natural media on the cultural characteristics of *Z. radicans*

Sl. No	Natural media	Biomass (mg)*	Radial growth (mm)*	Spore germination (%)**	Infectivity (%)**
1	Potato	847.5 (29.13)[a]	78.04 (8.89)[a]	81.26 (64.36)[a]	68.32 (55.71)[a]
2	Carrot	364.6 (19.12)[c]	69.30 (8.38)[b]	68.04 (55.56)[b]	52.03 (46.15)[b]
3	Green Banana	273.5 (16.56)[d]	60.08 (7.81)[c]	63.72 (52.96)[bc]	48.04 (43.86)[bc]
4	Beetroot	164.7 (12.87)[e]	57.21 (7.63)[c]	57.62 (49.37)[cd]	46.37 (42.90)[c]
5	Cornmeal	140.4 (11.89)[f]	48.18 (7.01)[d]	52.70 (46.53)[d]	39.30 (38.80)[d]
6	Control (SMAY)	654.31 (25.59)[b]	73.33 (8.62)[ab]	79.41 (63.09)[a]	65.60 (54.09)[a]
C. D (p= 0.05)		0.89	0.35	3.93	2.85
SE(d)		0.40	0.16	1.78	1.29

Each value is mean of three replications.
Figures in parentheses are square root* /arc sine** transformed values.
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

4.3. Suitability of various grain media for the growth characteristics and infectivity of *Z. radicans*

Results on the suitability of various grain media on the biomass, radial growth, spore germination and infectivity of the fungus are illustrated in Table 34.

Among the various grain media tested for biomass production of *Z. radicans*, Proso millet (broom corn) medium (500.25 mg) resulted in the best performance followed by sorghum (451.40 mg), bajra (442.61 mg) and maize (336.55 mg), whereas in control, rice (503.40 mg) yielded maximum. With regard to radial growth of *Z. radicans*, control (rice) (74.20 mm) and broom corn (72.50 mm) and sorghum (67.30 mm) medium found better than the other medium like bajra and maize 63.25 mm and 60.65 mm respectively. The spores of *Z. radicans* germinated more in rice (91.10 %) and broom corn (90.30 %) with infectivity of 69.25 and 68.30 % respectively.In the remaining media sorghum, bajra and maize infectivity of the fungus was 60.15 %, 55.40 % and 52.75 % respectively.

From these results, it was clear that rice and broom corn were almost similar in performance and growth characteristics like production of biomass, radial growth, spore germination and infectivity of *Z. radicans*. The cost of broom corn was comparatively economical than rice and other millets in cost of production, and it is the richest source of proteins and other nutrients so it could be better utilized for the mass production of *Z. radicans.*

Large-scale availability of the pathogen is a primary requirement in the bio-control programme. Compare to cereals millets grains are cheap, easily available and act as best nutritive media for the mass multiplication of many micro and macro organisms.

Table 34. Effect of selected grain media on the growth characteristics of *Z. radicans*

S.No.	Media	Biomass (mg)*	Radial growth (mm)*	Spore Germination (%) **	Infectivity (%) **
1.	Broom corn(Proso millet)	500.25 (22.38)^{ab}	72.50 (8.57)^{b}	90.30 (72.08)^{a}	68.30 (55.73)^{a}
2.	Sorghum	451.40 (21.27)^{b}	67.30 (8.26)^{c}	82.25 (65.10)^{b}	60.15 (50.84)^{b}
3.	Bajra	442.61 (21.05)^{c}	63.25 (8.01)^{d}	80.60 (63.95)^{b}	55.40 (48.08)^{c}
4.	Maize	336.55 (18.37)^{d}	60.65 (7.85)^{e}	77.75 (61.87)^{c}	52.70 (46.53)^{c}
5.	Rice (control)	503.40 (22.45)^{a}	74.20 (8.67)^{a}	91.10 (72.91)^{a}	69.50 (56.48)^{a}
	CD ($p_{=0.05}$)	1.081	0.415	7.072	3.664
	SE	0.462	0.177	3.020	1.565

Each value is mean of four replications
Figures in parenthesis are square root***** /arc sin** transformed values
In a column means followed by common letter are not significantly different

Inference

The above studies revealed that the selected two entomopathogens can be cultured on a different wide range of agricultural products and byproducts both in solid and liquid state.

The use of different agricultural wastes is economical and also helps in efficient utilization. For a successful integrated pest management programme, the agents like entomopathogenic fungi should be amenable for culture, easy and cheap for mass multiplication. Though it was proved both the *B. bassiana* and *Z. radicans* can be mass cultured on various agro wastes, agricultural products, their consistent performance varies due to quality of the materials, purity, cost of production, shelf life differs and depends on the availability of the media condition. However it was found that compared to *B. bassiana*, *Z.radicans* was slightly better in performance, Similarly rice was better for mass culturing However when compared to production cost the coarse millets, broom corm (proso) millet consists of highest protein and rich in nutrients and favourable for mass culture. It was also cheaper and easy for production compared to rice, with latest technologies available for mass culturing *Z.radicans.* Further refined studies with more and other easily available nutrient sources, agrowastes FYM, pressmud, sugarcane baggassse waste food products, agricultural wastes will perhaps provide more information on the utility of different agro wastes for production of mycoinsecticide like *Z.radicans.* Hence coarse millets like bajra, sorghum, maize, broomcorn millet were used to test the suitability.

Previous studies confirmed that incidence of *Z. radicans* was more in this part of Tamil nadu where higher humidity and low temperature were ideal compared to *B.bassiana*. .Hence further studies were conducted on *Z. radicans* with other required tests to evaluate and confirm appropriate, economically cheap, effective and viable entomopathogen to develop suitable formulation against major rice pests

Large-scale availability of the pathogen is a primary requirement in the bio-control programme. Compare to cereals millets Grains are cheap, easily available and act as best nutritive media for the mass multiplication of many micro and macro organisms.

EXPERIMENT V

5.1. Effect of subculturing on various growth characteristics and infectivity of *Z. radicans*

Observations on the effect of subculturing on various growth characteristics and infectivity of the test fungus are tabulated (Table 35).

Biomass production of *Z. radicans* decreased from third subculturing onwards and there was a gradual diminishing trend in the biomass weight. The biomass weight was initially 481.0 mg in the initial subculture and 302.50 mg in the last subculture. The radial growth of *Z. radicans* was uniform upto third sub culturing (77.50 mm, 76.75 mm and 76.50 mm). From the fourth sub culturing onwards the growth was declined. While in the control radial growth was 78.24 mm there was a gradual reduction in the radial growth at the end of eighth subculturing.

The spore germination inhibited slowly as the subculturing increased, the spore germination potency was 90.75% at the initial stage and 78.75% at the last subculturing. The control (PDA) treatment also showed good spore germination potential (90.75%) noticed. In the first subculture 65% per cent mortality was recorded and afterwards the fungus diminished gradually. The per cent mortality of the target pest, leaf folder in the control (65.0%) was on par with first sub culturing. However, in the last subculture 41.25 per cent mortality was observed.

5.2. Germination of *Z. radicans* resting spores

5.2.1. Effect of temperature on germination of resting spores

Results pertaining to germination (germ tube formation) of azygospores on *Z. radicans* influenced by different levels of temperature are tabulated (Table 36). Among the various levels of temperature tested 20°C made the azygospores of the fungus to germinate at a maximum rate,

while the other temperature levels (20, 15 and 10°C) showed a downward trend. However, an upward trend in the germination of azygospores from initial fifth day of experiment upto the end (thirty fifth day) was observed. Both the extremes of the temperature range tested *viz.*, 10 and 25°C were unfavourable for the spore germination with mean of 27.11 and 21.89 % respectively. However, the results therefore showed that the optimum temperature range of 55.30 – 61.92 mean per cent needed for the germination of the azygospores of *Z. radicans* was 15-20°C.

5.3. Effect of *Z. radicans* and insecticides on the growth of larvae and pupa of *C. medinalis*

5.3.1. Larval growth

Among the different fungal concentrations, the least pupation was noticed in 2.4×10^7 and 2.4×10^4 (43.30 %) as against 100 % in untreated (Table 37).The variation between different treatments was significant. The larval mortality was observed after 3 -7 days of fungal treatment.

The *C. medinalis* length ranged from 2.70–3.90 cm in the different treatments. The least larval length (2.70 cm) was recorded in 2.4×10^5 spore concentration as against the highest in the untreated (3.90 cm). Similarly, among the fungal treatments, the larval weight was least (357.00 mg) in 2.4×10^5 treatment compared to untreated 396.20 mg. The larval duration was 7.30 days in 2.4×10^6 as against the untreated (7.90 days).

Table 35. Effect of subculturing on growth characteristics and infectivity of *Z. radicans*

S.No.	Subculture NO	Biomass (mg)*	Radial growth	Spore Germination (%) **	Infectivity (%) **
1	I	481.00 (21.95)^{b}	77.50 (8.86)^{b}	90.75 (72.43)^{a}	65.00 (53.72)^{a}
2	II	478.25 (21.89)^{c}	76.75 (8.81)^{c}	90.75 (72.43)^{a}	63.75 (52.96)^{a}
3	III	413.75 (20.34)^{d}	76.50 (8.80)^{c}	86.75 (68.88)^{ab}	62.50 (52.23)^{ab}
4	IV	390.25 (18.45)^{e}	71.00 (8.48)^{d}	84.50 (66.88)^{b}	56.25 (48.59)^{b}
5	V	339.50 (18.41)^{f}	71.00 (8.48)^{d}	84.50 (66.88)^{b}	56.25 (48.59)^{b}
6	VI	338.00 (18.45)^{fg}	71.25 (8.49)^{d}	81.00 (64.25)^{bc}	50.00 (44.98)^{c}
7	VII	315.25 (17.78)^{g}	70.25 (8.36)^{e}	80.00 (63.46)^{c}	50.00 (44.98)^{c}
8	VIII	302.50 (17.41)^{h}	69.00 (8.36)^{f}	78.75 (62.58)^{c}	41.25 (39.94)^{d}
9	PDA	508.75 (22.57)^{a}	78.24 (8.90)^{a}	90.75 (72.43)^{a}	65.00 (53.72)^{a}
CD($p_{=0.05}$)		0.841	0.369	5.83	2.806
SEd		0.394	0.173	2.73	1.312

Each value is mean of four replications
Figures in parenthesis are square root***** /arc sin** transformed values
In a column means followed by common letter are not significantly different

Table 36. Effect of temperature on germination of azygospores of *Z. radicans*

S.No.	Temperature (°C)	Cumulative per cent germinating spores				Mean Percentage
		5th day	15th day	25th day	35th day	
1	10	17.50 (24.80)[c]	29.50 (32.70)[c]	30.12 (33.40)[c]	31.34 (34.10)[c]	27.11 (31.30)[c]
2	15	10.20 (18.70)[d]	61.22 (51.30)[a]	71.11 (57.10)[b]	78.68 (62.90)[b]	55.30 (47.70)[b]
3	20	32.60 (34.80)[a]	59.46 (50.40)[b]	74.96 (60.50)[a]	80.68 (64.20)[a]	61.92 (52.22)[a]
4	25	20.34 (26.70)[b]	20.60 (26.70)[d]	24.90 (30.00)[d]	21.73 (28.00)[d]	21.89 (28.00)[d]
CD($p_{=0.05}$)		2.3	0.65	0.21	2.78	1.20
SEd		0.8	0.22	0.07	0.98	0.30

Each value is a mean of four replications
Figures in parenthesis are arcsin transformed
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT

Table 37. Effect of *Z. radicans* on the growth of larvae of *C. medinalis*

Treatments		Larval growth parameters (Third instar)			
		Larval length (cm)	**Larval weight (mg)**	**Larval duration (days)**	**Pupation percentage**
Control	Untreated	3.90	396.00	7.90	100.00 (90.00)[a]
	Cypermethrin	3.00	340.50	7.30	36.70 (37.22)[g]
	Neemplus	3.10	346.20	7.80	40.00 (39.19)[f]
Z. radicans (Spores/ml)	2.4×10^4	2.80	361.20	7.60	43.30 (41.14)[cd]
	2.4×10^5	2.70	357.00	7.70	46.70 (42.84)[c]
	2.4×10^6	2.90	366.90	7.30	50.00 (44.99)[b]
	2.4×10^7	3.00	367.50	7.50	42.90 (41.14)[ce]
CD($p_{=0.05}$)		0.29	3.16	0.17	1.74
SE		0.10	1.11	0.05	0.64

Each value is a mean of four replications
Figures in parenthesis are arcsin transformed
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT

5.3.2. Pupal growth

The pupal weight ranged from 163.50 to 205.10 mg (Table 38). Among the cypermethrin and neem plus tested, Neem plus recorded least pupal weight (163.50 mg) as against the untreated recording 205.10 mg. Among the different fungal treatments least pupal weight (184.10 mg) was recorded in 2.4 x 10^5 spore concentration/ml as against the untreated (205.10 mg). In general there was not much variation in the pupal length. It was least (1.30 ,1.32..1.39 & 1.35cm) in 2.4 x 10^5, 2.4 x 10^6 and 2.4 x 10^7 spore concentrations which was on par with cypermethrin (1.30 cm). Malformed pupal stages were observed in the fungal treatments. All the cypermethrin treated pupae are malformed when compared to the untreated (20.00%). Among the fungal concentrations, 2.4 $\times 10^4$ spores/ml caused more malformed pupae (86.60%). Healthy adult emergence percentage from pupae was very high (80.00%) in untreated and no adults emergence was observed in cypermethrin treatment. Among the fungal concentrations, more adult's emergence was observed in 2.4 x 10^5 spores / ml.

5.4. *In vitro* evaluation of *Z. radicans* against rice leaf folder, *C.medinalis*

Among the different treatments like mycelium, conidia, resting spores, combination of mycelium and conidia, conidia and resting spores, mycelium and resting spores, mycelium and conidia and control were tested in this study. Treatment with combination of both conidia & resting spores of *Z. radicans* brought out highest mortality of rice leaf folder larvae (61.83 %) followed by 53.42 % with mycelium & conidia in lab studies (Table 39).

Table 38. Effect of *Z. radicans* on the growth of pupa of *C. medinalis*

Treatments		Pupal growth Parameters			Adults emergence Percentage	
		Pupal Weight (mg)	**Pupal Length (cm)**	**Pupal Duration (Days)**	**Healthy**	**Malformed or dead pupa**
Control	Untreated	205.10	1.53	8.5	80.0 $(62.89)^{a}$	20.0 $(26.55)^{g}$
	Cypermethrin	167.60	1.30	0.0	0.0 $(0.0)^{g}$	100.0 $(90.00)^{a}$
	Neemplus	163.50	1.40	9.0	11.1 $(19.26)^{f}$	88.89 $(70.05)^{b}$
Z.radicans (Spores/ml)	2.4×10^{4}	186.30	1.40	8.7	13.1 $(21.10)^{e}$	86.60 $(68.43)^{bc}$
	2.4×10^{5}	184.10	1.32	8.0	36.1 $(36.86)^{b}$	63.9 $(53.03)^{f}$
	2.4×10^{6}	204.50	1.39	8.5	27.8 $(31.80)^{c}$	72.20 $(58.10)^{de}$
	2.4×10^{7}	189.30	1.35	8.5	25.0 $(30.00)^{d}$	75.0 $(59.31)^{d}$
CD($p_{=0.05}$)		0.58	0.19	0.80	0.65	3.04
SE		0.19	0.03	0.55	0.22	1.06

Each value is a mean of four replications
Figures in parenthesis are arcsin transformed in a column means followed by a common letter are not significantly different (P=0.05) by DMRT

Table 39. *In vitro* Evaluation of *Z. radicans* on rice leaf folder *C. medinalis*

S.No.	Treatments	Percentage infection of leaf folder larvae
1	Mycelium	34.06 (36.40)^{f}
2	Conidia	52.62 (45.12)^{bc}
3	Resting spores	42.38 (39.05)^{d}
4	Mycelium and Conidia	53.42 (45.96)^{b}
5	Conidia and Resting spores	61.83 (47.70)^{a}
6	Mycelium and Resting spores	41.85 (38.76)^{e}
7	Untreated check	5.05 (12.70)^{g}
CD($p_{=0.05}$)		2.66
SEd		1.223

Each value is a mean of four replications

Figures in parenthesis are arcsin transformed
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT
Twenty larvae were used per replications

EXPERIMENT VI

6.1. Effect of powder and oil formulation of *Z. radicans* against biology of rice leaffolder *C. medinalis*

Among the different concentration of powder formulations of *Z.radicans* (Table 40) highest larval mortality (31.25 %), pupal mortality (22.50 %), and adult mortality (20.00 %) were noticed at 8%. Similarly, Larval mortality, pupal mortality, and adult mortality increases with increase in the concentration of formulation. Lowest pupal percentage (68.75 %), adult emergence percentage (46.25 %), and adult percentage (15.00 %) were noticed at 8% concentration. However lowest percentage adult mortality of 6.25, 12.50 and 15.00 recorded at the dose concentration of 2, 4 and 6 per cent respectively.

Pupation per cent, adult emergence per cent and adult per cent decrease with increase in the concentration of formulation (Table 41). Among the different concentration of oil formulations of *Z. radicans*, highest larval mortality (53.75 %), pupal mortality (21.25 %), and adult mortality (17.50 %) were noticed at 8%. Larval mortality, pupal mortality, and adult mortality increases with increase in the concentration of formulation. Lowest pupal percentage (46.25 %), adult emergence percentage (25.00 %), and adult percentage (7.50 %) were noticed at 8% concentration.

Among the oil and powder oil formulation, *Z. radicans* was found better in causing higher mortality of life stages of leaf folder in oil formulation of *Z. radicans.*

6.1.1. Effect of powder and oil formulation of *B. bassiana* against biology of rice brown plant hopper

Among the different concentration of powder formulations of *B. bassiana*, highest nymph mortality (43.75 %), and adult mortality (37.50 %) were noticed at 8%. Nymphal mortality and adult mortality increases with increase in the concentration of formulation. Lowest adult emergence percentage (56.25%), and adult percentage (18.75%) were noticed at 8%. Adult

emergence per cent and adult per cent decrease with increase in the concentration of formulation (Table 42).

However, **a**mong the various concentrations of oil formulations of *B.bassiana*, maximum nymphal mortality (50.00 %), and adult mortality (42.50 %) were noticed at 8%. Nymph mortality, and adult mortality increases with increase in the concentration of formulation. Lowest adult emergence percentage (50.00 %), and adult percentage (7.50 %) were noticed at 8%. Adult emergence per cent and adult per cent decrease with increase in the concentration of formulation (Table 43). Among the oil and powder, oil formulation of *B.bassiana* was found better in causing higher mortality of life stages of leaf folder when compared to powder formulation of *B. bassiana (*Plate 9).

6.2. *In vitro* evaluation of powder and oil formulation of *Z. radicans* against rice leaffolder *C. medinalis.*

Different concentrations of powder and oil formulation of *Z.radicans* were evaluated against leaf folder *in vitro* (Table 44).

Among the different concentrations of powder formulation, mortality of 2.25 % leaf folder larvae was noticed at 2 % and mortality increased to 20.00 % at 8 % after three days of treatment. Mortality of 12.50 % leaffolder larvae was recorded at 2 % and it enhanced to 40.00 % at 8 % after seven days of treatment. However, mortality of 22.50 % leaffolder larvae was noticed at 2 % which increased to 67.50 % at 8 % after ten days of treatment (Plate 10).

Among the different concentrations average larval mortality of 12.50 % was noted at 2 % and 42.50 % at 8 % confirmed that larval mortality increases along with increase in the concentration of formulation.

Among the different concentration of oil formulation, 12.50% mortality at 2 % which increased to 36.50 % at 8 % after three days of treatment. Mortality of 20.00 % was recorded at 2 % and 57.25 % at 8 % after seven days of treatment. However, 37.50 % of mortality was noticed at 2 % & 78.00 % at 8 % after ten days of treatment.

Table 40. Effect of powder formulation of *Z. radicans* against biology of rice leaf folder *C. medinalis*

Sl.No	Dose/ Concentration	Larval mortality (%)	Pupal (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)	Adult (%)
1	2 %	12.50 (20.69)^{d}	87.50 (69.36)^{b}	6.25 (14.47)^{d}	81.25 (64.35)^{b}	6.25 (14.47)^{d}	75.00 (59.97)^{b}
2	4 %	18.75 (25.64)^{c}	81.25 (64.36)^{c}	15.00 (22.78)^{c}	66.25 (54.47)^{c}	12.50 (20.69)^{c}	62.50 (52.52)^{c}
3	6 %	25.00 (30.00)^{b}	75.00 (60.04)^{d}	18.75 (265.64)^{b}	56.25 (48.57)^{d}	15.00 (22.77)^{b}	41.25 (39.94)^{d}
4	8 %	31.25 (33.97)^{a}	68.75 (56.01)^{e}	22.50 (28.30)^{a}	46.25 (42.83)^{e}	20.00 (26.56)^{a}	15.00 (22.77)^{e}
5	Control	0 (0.28)^{e}	100 (90.00)^{a}	0 (0.28)^{e}	100 (90.00)^{a}	0 (0.28)^{e}	100 (90.00)^{a}
SE.d		0.531	1.626	0.436	1.130	0.391	0.883
C.D(p=0.5)		1.243	3.807	1.020	2.647	0.915	2.068

Each value is mean of four replication Figures in parentheses are arc sine transformed values. In a column means followed by a common letter are not significantly different (P =0.05) by DMRT

Table 41. Effect of oil formulation of *Z.radicans* against biology of rice leaffolder *C.medinalis*

S. No.	Dose/ Concentration	Larval mortality (%)	Pupal (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)	Adult (%)
1	2 %	19.25 (26.01)^{d}	80.75 (64.02)^{b}	11.50 (19.81)^{d}	68.75 (56.25)^{b}	12.50 (20.69)^{c}	56.25 (48.57)^{b}
2	4 %	27.50 (31.62)^{c}	72.50 (58.38)^{c}	17.50 (24.71)^{c}	55.00 (47.85)^{c}	18.75 (25.65)^{b}	36.25 (37.02)^{c}
3	6 %	34.50 (35.95)^{b}	65.50 (54.03)^{d}	22.00 (27.98)^{b}	43.75 (41.39)^{d}	22.50 (28.30)^{a}	20.75 (27.08)^{d}
4	8 %	53.75 (47.15)^{a}	46.25 (42.83)^{e}	21.25 (27.43)^{a}	25.00 (29.98)^{e}	17.50 (24.71)^{d}	7.50 (15.89)^{e}
5	Control	0.00 (0.28)^{e}	100.0 (90.00)^{a}	0.00 (0.28)^{e}	100.0 (90.00)^{a}	0.00 (0.28)^{e}	100.0 (90.00)^{a}
SEd		0.582	1.677	0.531	0.845	0.455	0.555
C.D(p=0.5)		1.928	3.927	1.244	1.978	1.067	1.300

Each value is mean of four replication.

Figures in parentheses are arc sine transformed values. In a column means followed by a common letter are not significantly different (P =0.05) by DMRT

Table 42. Effect of powder formulation of *B. bassiana* against biology of BPH

Sl.No	Dose/Concentration	Nymph mortality	Adult Emergence	Adult mortality	Adult (%)
1	2 %	18.75 (25.00)^{d}	81.25 (64.36)^{b}	17.50 (24.72)^{d}	63.75 (52.54)^{b}
2	4 %	25.00 (29.98)^{c}	75.00 (59.99)^{c}	27.50 (31.62)^{c}	47.50 (43.55)^{c}
3	6 %	31.25 (33.96)^{b}	68.75 (56.02)^{d}	31.25 (33.97)^{b}	37.50 (37.74)^{d}
4	8 %	43.75 (41.39)^{a}	56.25 (48.57)^{e}	37.50 (37.75)^{a}	18.75 (25.65)^{e}
5	Control	0.00 (0.28)^{e}	100 (90.00)^{a}	0 (0.28)^{e}	100.00 (90.00)^{a}
SEd		0.649	1.249	0.620	0.743
C.D(p=0.05)		1.52	3.159	1.452	1.739

Each value is mean of four replication Figures in parentheses are arc sine transformed values. In a column means followed by a common letter are not significantly different (P =0.05) by DMRT

Table 43. Effect of oil formulation of *B. bassiana* against biology of rice BPH

S. No.	Dose/ Concentration	Nymph mortality	Adult emergence	Adult mortality	Adult (%)
1	**2 %**	25.00 (30.00)^{d}	75.00 (60.00)^{b}	18.75 (25.65)^{d}	56.25 (48.75)^{b}
2	**4 %**	37.50 (37.75)^{c}	62.50 (52.52)^{c}	25.00 (30.00)^{c}	43.75 (41.25)^{c}
3	**6 %**	44.75 (42.15)^{b}	55.25 (47.88)^{d}	32.60 (34.09)^{b}	22.75 (27.52)^{d}
4	**8 %**	54.20 (48.75)^{a}	46.80 (44.73)^{e}	42.46 (40.38)^{a}	3.34 (7.27)^{e}
5	**Control**	0.00 (0.28)^{e}	100.00 (90.00)^{a}	0 (0.28)^{e}	100.00 (90.00)^{a}
6	**SEd**	0.803	1.075	0.665	0.584
7	**C.D (p=0.05)**	1.880	2.517	1.558	1.368

Each value is mean of four replication Figures in parentheses are arc sine transformed values.
In a column means followed by a common letter are not significantly different (P =0.05) by DMRT

Table 44. *Invitro* evaluation of powder and oil formulation of *Z. radicans* against rice leaffolder

Sl.No	Dose Concentration	Powder formulation				Dose	Oil formulation			
		3DAT	7DAT	10DAT	Average		3DAT	7DAT	10DAT	Average
1	**2 %**	2.25 (8.62)^{d}	12.50 (20.65)^{d}	22.50 (28.28)^{d}	12.50	**2 %**	12.50 (13.89)^{d}	20.00 (18.43)^{d}	37.50 (28.22)^{d}	23.33
2	**4 %**	10.00 (18.43)^{c}	22.50 (28.28)^{c}	35.00 (36.23)^{c}	22.50	**4 %**	22.50 (20.46)^{c}	30.00 (24.53)^{c}	52.50 (29.58)^{c}	35.00
3	**6 %**	12.50 (20.46)^{b}	30.00 (33.17)^{b}	52.50 (46.42)^{b}	31.70	**6 %**	31.25 (20.46)^{b}	43.75 (29.63)^{b}	62.50 (34.15)^{b}	45.83
4	**8 %**	20.00 (26.56)^{a}	40.00 (39.20)^{a}	67.50 (55.25)^{a}	42.50	**8 %**	36.50 (24.12)^{a}	57.25 (28.22)^{a}	78.00 (38.98)^{a}	56.25
5	**Control**	0 (0.28)^{e}	0 (0.28)^{e}	0 (0.28)^{e}	0	**Control**	0 (0.28)^{e}	0 (0.28)^{e}	0 (0.28)^{e}	0.0
C.D (p=0.05)		5.41	3.877	4.133	-	**C.D (p=0.05)**	8.96	6.55	5.23	-
SEd		2.310	1.657	1.765	-	**SE.d**	4.11	3.00	2.40	-

Each value is mean of four replication Figures in parentheses are arc sine transformed values. In a column means followed by a common letter are not significantly different (P =0.05) by DMRT

DAT=Days After Treatment

Whereas, average larval mortality of 23.33 % at 2 % was recorded which increased to 56.25 % at 8 % concentration of formulations. Among the oil and powder, oil formulation of *B.bassiana* was superior in causing higher mortality of leaffolder when compared to powder formulation.

6.3. *In vitro* evaluation of powder and oil formulation of *B.bassiana* against rice brown plant hopper.

Different concentrations of powder and oil formulation of *B. bassiana* were evaluated against brown plant hopper *in vitro* (Table 45).

Among the different concentrations of powder formulation, mortality of 2.25 % brown plant hopper was noticed at 2 % and mortality increased to 17.50 % at 8 % after three days of treatment. Mortality of 15.00 % was noticed at 2 % which enhanced to 42.50 % at 8 % after seven days of treatment. However, after ten days of treatment of 20.00 % was noticed at 2 % and mortality increased to 65.00 at 8 %. However, average larval mortality of 11.70 % was noticed at 2 % and 41.70 % at 8 % larval mortality which increased along with increase in the concentration of formulation.

Whereas, in oil formulation among the different concentrations, mortality of 5.00 % brown plant hopper was noticed at 2 % and mortality increased to 22.50 % at 8 % after three days of treatment. Mortality of 17.50 % brown plant hopper was noticed at 2 % and mortality increased to 45.00 % at 8 % after seven days of treatment. Mortality of 27.50 % brown plant hopper was noticed at 4 % and 46.70 % at 8 % after ten days of treatment (Plate 11).

Among the different concentrations average larval mortality of 16.70 % was noticed at 2 % and increased to 46.70 % at 8 % larval mortality increases along with increases in the concentration of formulation. Among the oil and powder, oil formulation of *Z. radicans* were found better in causing higher mortality of brown plant hopper when compared to powder formulation of *Z. radicans.*

Table 45. *In vitro* evaluation of powder and oil formulation of *B. bassiana* against rice brown plant hopper

Sl.No	Dose Concentration	Powder formulation				Dose	Oil formulation			
		3DAT	7DAT	10DAT	Average		3DAT	7DAT	10DAT	Average
1	2 %	2.25 (9.26)^{d}	15.00 (22.50)^{d}	20.00 (18.43)^{d}	11.70	2 %	5.00 (12.74)^{d}	17.50 (24.68)^{d}	27.50 (31.58)^{d}	16.70
2	4 %	7.50 (13.89)^{c}	17.50 (18.43)^{c}	27.50 (20.46)^{c}	17.50	4 %	18.50 (25.45)^{c}	27.50 (31.58)^{c}	42.50 (40.67)^{c}	27.50
3	6 %	15.00 (22.50)^{b}	32.50 (24.53)^{b}	50.00 (28.22)^{b}	32.50	6 %	15.00 (22.56)^{b}	37.50 (37.74)^{b}	60.00 (50.79)^{b}	37.50
4	8 %	17.50 (26.56)^{a}	42.50 (29.88)^{a}	65.00 (31.55)^{a}	41.70	8 %	22.50 (28.28)^{a}	45.00 (42.11)^{a}	75.00 (60.01)^{a}	46.70
5	Control	0 (0.091)^{e}	0 (0.286)^{e}	0 (0.286)^{e}	0	Control	0 (0.28)^{e}	0 (0.28)^{e}	0 (0.28)^{e}	0.0
C.D (p=0.5)		11.18	7.21	5.66	-	C.D (p=0.5)	4.483	3.334	3.225	-
SEd		5.13	3.31	2.13	-	SEd	1.914	1.425	1.377	-

Each value is mean of four replication. Figures in parentheses are arc sine transformed values. In a column means followed by a common letter are not significantly different (P =0.05) by DMRT

DAT=Days After Treatment

EXPERIMENT VII

Safety studies on natural enemies

7.1. Safety level of fungal formulation on non target organism-Compatibility studies on parasitoids

7.1.1. Effect of entomopathogenic fungi *B. bassiana* and *Z. radicans* against Braconids and Trichogrammatids

1. B. bassiana:

Data (Table 46) showed that highest mortality of Braconids and Trichogramma parasitoids were recorded at 1×10^9 spores/ml and lowest mortality of 12.50 % and 6.25 % were at 1×10^6 spores/ml. Mortality of Braconids and Trichgramma increased with increase in the concentration of *B. bassiana*. Mortality percentage of Braconids (37.50 %) was high at 1×10^9 spores/ml of *B. bassiana* compared to Trichogramma (31.25 %).

2 ***Z. radicans:***

Lowest mortality of Braconids (33.25 %) and Trichogramma (25.00 %) parasitoids were recorded at 1×10^9 spores/ml and lowest mortality of 15.00 % and 0 % were at 1×10^6 spores/ml. Mortality of Braconids and Trichgrammatids increase with increase in the concentration of *Z. radicans*. Mortality percentage of Braconids (33.25 %) were high at 1×10^9 spores/ml of *Z. radicans* when compared to (25.00 %) in Trichogramma.

Among the *B. bassiana* and *Z. radicans*, *Z. radicans* caused lesser mortality of parasitoids when compared to *B. bassiana*

Table 46. Effect of entomopathogenic fungai *B. bassiana* and *Z. radicans* against Braconids and Trichogrammatids

S.NO	Treatment (No. of spores/ml)	*B. bassiana*		*Z. radicans*	
		Braconids	**Trichogrammatids**	**Braconids**	**Trichogrammatids**
1	1×10^{6}	12.50 $(15.14)^{d}$	6.25 $(7.71)^{d}$	15.00 $(22.50)^{d}$	0 $(0.286)^{d}$
2	1×10^{7}	22.50 $(20.46)^{c}$	17.50 $(26.56)^{c}$	18.75 $(22.57)^{c}$	6.75 $(7.71)^{c}$
3	1×10^{8}	31.21 $(33.75)^{b}$	25.00 $(30.00)^{b}$	27.50 $(20.46)^{b}$	17.50 $(26.56)^{b}$
4	1×10^{9}	37.50 $(31.50)^{a}$	31.25 $(33.75)^{a}$	33.25 $(34.25)^{a}$	25.00 $(30.00)^{a}$
5	Control	0 $(0.28)^{e}$	0 $(0.28)^{e}$	0 $(0.28)^{e}$	0 $(0.28)^{e}$
6	CD (0.05)	19.19	16.11	18.96	15.29
7	SEd	9.00	7.55	8.89	7.17

Each value is mean of four replication

Figures in parentheses are arc sine transformed values. In a column means followed by a common letter are not significantly different (P =0.05) by DMRT

7.2. Effect of entomopathogenic fungi *Z.radicans* against parasitoids (Ichneumonids and Braconids)

The highest mortality of Ichneumonids at 1×10^9spores/ml was recorded in Sethiathoppu (27.35%) followed by B.Mutlur (19.10%) and Annamalainagar isolates (18.30%). Whereas, the lowest mortality of Ichneumonids was also recorded in Annamalainagar (3.65%) followed by B. Mutlur (3.80 %) and Sethiathoppu (4.25%) and at 1×10^6 spores/ml (Table 47).

The highest mortality of Braconids was recorded in Sethiathoppu isolates (32.25%), followed by B.Mutlur (26.30%) and Annamalainagar (20.10%) at 1×10^9spores/ml. B. Mutlur (Table 48).

Hence, the mortality of Ichneumonids and Braconids increased with increases in the concentration and depends upon the virulent against parasitoid. However, the Annamalainagar isolate was least virulent to parasitoids against *Z. radicans* in laboratory conditions. These experiments proved *Z. radicans* was safe against parasitoids

7.3. Effect of *Z. radicans* against honey bees, *Apis cerana indica*

The highest mortality of honey bees was recorded in Sethiathoppu (19.25%) followed by B.Mutlur (17.25%) and Annamalainagar (16.15%) at 1×10^9 spores/ml and the lowest mortality of honeybees was also recorded in Annamalainagar (2.20%) followed by B.Mutlur (2.95%) and (3.30 %) in Sethiathoppu at 1×10^6 spores/ml. The mortality of honey bee increases with increase in the concentration of *Z. radicans*. Mortality of the bees was found on these treated with corn flour only, control ranged between 5-6% respectively (Table 49).

Table 47. Effect of entomopathogenic fungi *Z. radicans* against Ichneumonids

Sl. No	Treatment (No. of spores/ml)	Mortality percentage of Ichneumonids		
		Annamalainagar isolate	B.Mutlur isolate	Sethiathoppu isolate
1	1×10^6	3.65 (11.01)^d	3.80 (11.24)^d	4.25 (11.89)^d
2	1×10^7	5.40 (13.43)^c	6.50 (14.76)^c	9.50 (17.94)^c
3	1×10^8	12.00 (20.25)^b	11.20 (19.54)^b	15.00 (22.77)^b
4	1×10^9	18.30 (25.31)^a	19.10 (25.90)^a	27.35 (31.51)^a
5	Control	0.00 (0.28)^e	0.00 (0.28)^e	0.00 (0.28)^e
C. D (p= 0.05)		0.86	0.80	0.97
SE(d)		0.37	0.35	0.43

Each value is mean of three replications.
Figures in parentheses are arc sine transformed values.
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

Table 48. Effect of entomopathogenic fungi *Z. radicans* against Braconids

Sl. No	Treatment (No. of spores/ml)	Mortality percentage of Braconids		
		Sethiathoppu Isolate	B.Mutlur isolate	Annamalainagar isolate
1	1×10^6	8.20 (16.63)^d	9.45 (17.89)^d	6.20 (14.41)^d
2	1×10^7	17.35 (24.60)^c	14.40 (22.29)^c	11.00 (19.36)^c
3	1×10^8	21.60 (27.68)^b	20.15 (26.65)^b	15.60 (23.25)^b
4	1×10^9	32.25 (34.59)^a	26.30 (30.84)^a	20.10 (26.62)^a
5	Control	0.00 (0.28)^e	0.00 (0.28)^e	0.00 (0.28)^e
C. D (p= 0.05)		1.18	1.08	0.92
SE(d)		0.52	0.48	0.41

Each value is mean of three replications.
Figures in parentheses are arc sine transformed values.
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

Table 49.Effect of *Z. radicans* against Indian honey bee *Aphis cerana indica*

Sl. No	Treatment (No. of spores/ml)	Mortality percentage of honey bees		
		Annamalainagar isolate	Sethiathoppu isolate	B.Mutlur isolate
1	1×10^{6}	2.20 (8.52)^{e}	3.30 (10.46)^{e}	2.95 (9.89)^{e}
2	1×10^{7}	5.45 (13.49)^{c}	7.50 (15.89)^{c}	7.60 (15.99)^{c}
3	1×10^{8}	11.10 (19.45)^{b}	14.60 (22.45)^{b}	12.30 (20.52)^{b}
4	1×10^{9}	16.15 (23.68)^{a}	19.25 (26.01)^{a}	17.25 (24.53)^{a}
5	Control	5.80 (13.93)^{d}	5.00 (12.91)^{d}	5.30 (13.30)^{d}
C. D (p= 0.05)		0.85	0.90	0.81
SE(d)		0.38	0.40	0.36

Each value is mean of three replications.
Figures in parentheses are arc sine transformed values.
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

EXPERIMENT VIII

8.1. Effect of synthetic media on the cultural characteristics of *Z. radicans*

Biomass and radial growth recorded on Czapek's Dox Agar (101.5 mg and 57.63 mm) were very less and that were slightly supporting the fungal growth (Table 50). However SMAY (654.31mg and 73.33mm), SDAY (486.20mg and 62.00mm), PDA (426.28 mg and 63.61mm) and RBA (354.22 mg and 76.67 mm) were highly supporting for biomass production and radial growth of entomopathogenic fungi *Z. radicans* but SMAY was higher than biomass and radial growth recorded in control medium (364.6mg and 69.30mm). The lowest spore germination and infectivity were recorded in Czapek's Dox Agar (53.20 % and 37.80 %). The highest spore germination and infectivity were recorded in SMAY (79.41% and 65.60%), SDAY (64.19% and 55.55%), PDA (62.13 % and 50.30 %), RBA (66.71% and 46.23%) but SMAY were higher than control medium (68.04% and 52.03%). It is clear that the synthetic media like Czapek's Dox Agar was slightly supporting the growth and infectivity of entomopathogenic fungi, *Z. radicans*. The above studies showed that SMAY was highly supporting for the growth of *Z. radicans* and it can be well suited for the mass production of entomopathogenic fungi.

Inference

It is inferred that among the various supplemented media tested, SEMA was found efficient in all the growth characteristics and infectivity of the fungus.

Table 50. Effect of synthetic media on the cultural characteristics of *Z. radicans*

Sl. No	Synthetic media	Biomass (mg)*	Radial growth (mm)*	Spore germination (%)**	Infectivity (%)**
1	SMAY	654.31 (25.59)^{a}	73.33 (8.62)^{ab}	79.41 (63.02)^{a}	65.60 (54.08)^{a}
2	SDAY	486.20 (22.07)^{b}	62.00 (7.93)^{de}	64.19 (53.23)^{bc}	55.55 (48.17)^{b}
3	RBA	354.22 (18.84)^{d}	76.67 (8.81)^{a}	66.71 (54.76)^{bc}	46.23 (42.82)^{d}
4	PDA	426.28 (20.66)^{c}	63.61 (8.04)^{cd}	62.13 (52.01)^{c}	50.30 (45.15)^{cd}
5	Czapek's Dox Agar	101.5 (10.12)^{e}	57.63 (7.66)^{e}	53.20 (46.82)^{d}	37.80 (37.92)^{e}
6	Control (Carrot)	364.6 (19.11)^{d}	69.30 (8.38)^{bc}	68.04 (55.58)^{b}	52.03 (46.15)^{bc}
C. D (p= 0.05)		0.86	0.37	3.58	2.58
SE(d)		0.39	0.17	1.63	1.17

Each value is mean of three replications.
Figures in parentheses are square root/arc sine transformed values. In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

8.2. Effect of oil based bio-formulation and granular formulation of *Z. radicans* against biology of leaf folder, *C. medinalis*

Among the different treatments of oil based formulation (Table 51) of *Z. radicans*, the highest larval mortality (38.75%), pupal mortality (26.25 %), adult mortality (22.50%) and also the lowest pupal formation (61.25%) and adult emergence (35.00 %) was noticed in *Z. radicans* + Sunflower oil + Glycerol treatment. The lowest larval mortality (11.25 %), pupal mortality (8.25 %) and adult mortality (8.25 %) were noticed in *Z. radicans* alone.

Whereas among the different concentrations of granular formulation (Table 52) of *Z. radicans*, the maximum larval mortality (27.50 %), pupal mortality (19.25 %), adult mortality (16.50 %) and also the lowest pupal percentage (72.50 %), adult emergence percentage (53.25 %) and adult mortality (16.50 %) were recorded on 81.3 spores/mm^{-2} concentration. However, the lowest larval mortality (14.25 %), pupal mortality (8.25 %) and adult mortality (5.75 %) observed on 12.2 spores/mm^{-2} concentration. Pupation percentage and adult emergence percentage decreased with increase in the concentration of spores. However, in the preliminary studies it was found that among different food grains tested Broom corn millet *Panicum miliaceum* was most promising, however further studies should be carried out to recommend it for commercial production.

Among the oil and granular formulation, oil formulation of *Z. radicans* was found to be better in causing higher mortality of life stages of leaf folder when compared to granular formulation of *Z. radicans*.

Table 51. Effect of oil based bio-formulations of *Z. radicans* against biology of rice leaf folder *C. medinalis*

Sl. No	Treatment	Larval mortality (%)	Pupal formation (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)
1	*Z. radicans* alone	11.25 (19.59)^{f}	88.75 (70.38)^{b}	8.25 (16.68)^{f}	80.50 (63.77)^{b}	8.25 (16.67)^{e}
2	*Z. radicans* + Glycerol	17.50 (24.72)^{e}	82.50 (65.25)^{c}	13.78 (21.78)^{e}	68.65 (55.93)^{c}	9.25 (17.69)^{d}
3	*Z. radicans* + Sunflower oil	31.25 (33.48)^{b}	68.75 (55.99)^{f}	24.25 (29.48)^{b}	44.50 (41.82)^{f}	22.25 (28.30)^{a}
4	*Z. radicans* + Sunflower oil+ Glycerol	38.75 (38.48)^{a}	61.25 (51.48)^{g}	26.25 (30.81)^{a}	35.00 (36.25)^{g}	22.50 (28.30)^{a}
5	*Z. radicans* + Mustard oil	21.25 (27.43)^{d}	78.75 (62.52)^{d}	17.25 (24.53)^{d}	61.50 (51.63)^{d}	12.75 (20.91)^{c}
6	*Z. radicans* + Mustard oil + Glycerol	28.50 (32.25)^{c}	71.50 (57.71)^{e}	19.75 (26.37)^{c}	51.75 (45.98)^{e}	15.25 (22.97)^{b}
7	Control	0.00 (0.28)^{g}	100 (89.71)^{a}	0.00 (0.28)^{g}	100 (89.71)^{a}	0.00 (0.28)^{f}
C. D (p= 0.05)		0.76	0.63	0.59	0.72	0.85
SE(d)		0.35	0.29	0.27	0.33	0.39

Each value is mean of three replications.

Figures in parentheses are arc sine transformed values.

In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

Table 52. Effect of granular formulations of *Z. radicans* against biology of rice leaf folder, *C. medinalis*

Sl. NO	Spore concentration (Spores/mm^{-2})	Larval mortality (%)	Pupal formation (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)
1	12.2	14.25 (22.16)^{d}	85.75 (67.79)^{b}	8.25 (16.68)^{d}	77.50 (61.66)^{b}	5.75 (13.85)^{d}
2	34.6	16.50 (23.95)^{c}	83.50 (66.02)^{c}	9.25 (17.69)^{c}	74.25 (59.48)^{c}	7.75 (16.16)^{c}
3	54.3	23.25 (28.81)^{b}	76.75 (60.59)^{d}	13.25 (21.40)^{b}	63.50 (52.81)^{d}	13.75 (21.75)^{b}
4	81.3	27.50 (31.50)^{a}	72.50 (58.35)^{e}	19.25 (26.03)^{a}	53.25 (46.84)^{e}	16.50 (23.95)^{a}
5	Control	0.00 (0.28)^{e}	100 (89.71)^{a}	0.00 (0.28)^{e}	100 (89.71)^{a}	0.00 (0.28)^{e}
C. D (p= 0.05)		1.38	1.17	0.72	0.73	0.85
SE(d)		0.61	0.52	0.32	0.33	0.37

Each value is mean of three replications.
Figures in parentheses are arc sine transformed values.
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

8.3. Semi field evaluation of oil based bio-formulation and granular formulation of *Z. radicans* against leaf folder *C. medinalis*

Different treatments of oil and granular formulations of *Z. radicans* were evaluated against leaf folder larvae in pot culture condition (Table 53). Among the different treatments of oil based formulations, *Z. radicans* caused the lowest larval mortality of 7.25%, 16.50%, 22.75% and 28.25% after 3, 5, 7 and 10 DAT respectively. Whereas, *Z. radicans* + Sunflower oil + Glycerol treatment caused the highest larval mortality of 26.25%, 42.15%, 58.25% and 72.25% after 3, 5,7 and 10 DAT respectively.

Among the various treatments, the lowest mean mortality of 18.68% was noticed on *Z. radicans* alone treatment. However, the highest mean mortality of 49.72% was recorded on *Z. radicans* + Sunflower oil + Glycerol treatment. Because, the combination of sunflower oil and adjuvant (Glycerol) increased the efficiency of the fungus causing significant mortality of leaffolder.

In the studies on granular formulation of different (Table 54) concentrations, 10g *Z. radicnas*/pot caused the lowest larval mortality of 4.50, 13.25, 16.50 and 21.50 % after 3, 5, 7 and 10 DAT respectively. Whereas, 40g *Z. radicnas*/pot recorded the highest larval mortality of 22.50, 41.00, 56.25 and 68.50 % after 3, 5, 7 and 10 DAT respectively. However, among the different concentrations average larval mortality of 13.94 % was noticed at 10g *Z. radicans*/pot treatment and increased to 47.06 % at 40g *Z. radicans*/pot treatment. Larval mortality increases along with increase in the concentration of formulation. Among the oil and granule, oil formulations of *Z. radicans* was found better and caused significant leaf folder larval mortality compared to granular formulation of *Z. radicans*.

Table 53. Semi – field evaluation of oil based bio-formulation of *Z. radicans* against rice leaf folder *C. medinalis*

Sl. No	Treatment	Per cent larval mortality				Mean
		3 DAT	5 DAT	7 DAT	10 DAT	
1	*Z. radicans* alone	7.25 (15.61)^{f}	16.50 (23.95)^{f}	22.75 (28.48)^{e}	28.25 (32.09)^{e}	18.68 (25.03)^{e}
2	*Z. radicans* + Glycerol	12.50 (20.69)^{e}	21.50 (27.61)^{e}	29.75 (33.04)^{d}	36.50 (37.15)^{d}	25.06 (29.62)^{d}
3	*Z. radicans* + Sunflower oil	21.25 (27.44)^{c}	35.50 (36.56)^{c}	44.00 (41.53)^{bc}	57.50 (49.29)^{b}	39.56 (38.71)^{b}
4	*Z. radicans* + Sunflower oil+ Glycerol	26.25 (30.81)^{a}	42.15 (40.47)^{a}	58.25 (49.73)^{a}	72.25 (58.19)^{a}	49.72 (44.80)^{a}
5	*Z. radicans* + Mustard oil	18.50 (25.46)^{d}	33.00 (35.05)^{d}	42.50 (40.67)^{c}	50.00 (44.98)^{c}	36.00 (36.54)^{c}
6	*Z. radicans* + Mustard oil + Glycerol	23.25 (28.81)^{b}	38.25 (38.19)^{b}	44.75 (41.97)^{b}	58.25 (49.73)^{b}	41.12 (39.68)^{b}
7	Control	0.00 (0.28)^{g}	0.00 (0.28)^{g}	0.00 (0.28)^{f}	0.00 (0.28)^{f}	0.00 (0.28)^{f}
C. D (p= 0.05)		0.33	0.55	1.09	0.88	5.56
SE(d)		0.15	0.25	0.49	0.40	1.86

Each value is mean of three replications. Figures in parentheses are arc sine transformed values. In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

DAT = Days after treatment.

Table 54. Semi – field evaluation of granular formulation of *Z. radicans* against rice leaf folder *C. medinalis*

Sl. No	Treatment (gram/pot)	Per cent larval mortality				Mean
		3 DAT	5 DAT	7 DAT	10 DAT	
1	10	4.50 (12.23)^{d}	13.25 (21.34)^{d}	16.50 (23.95)^{d}	21.50 (27.61)^{d}	13.94 (21.29)^{d}
2	20	9.25 (17.70)^{c}	18.75 (25.65)^{c}	24.25 (29.49)^{c}	32.25 (34.59)^{c}	21.12 (26.86)^{c}
3	30	15.50 (23.17)^{b}	29.50 (32.88)^{b}	36.50 (37.15)^{b}	43.25 (41.10)^{b}	31.18 (33.58)^{b}
4	40	22.50 (28.30)^{a}	41.00 (39.79)^{a}	56.25 (48.57)^{a}	68.50 (55.84)^{a}	47.06 (43.13)^{a}
5	Control	0.00 (0.28)^{e}	0.00 (0.28)^{e}	0.00 (0.28)^{e}	0.00 (0.28)^{e}	0.00 (0.28)^{e}
C. D (p= 0.05)		0.78	0.86	0.64	0.62	6.70
SE(d)		0.34	0.37	0.27	0.26	3.04

Each value is mean of three replications. DAT = Days after treatment
Figures in parentheses are arc sine transformed values.
In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

8.4. Field Efficacy of granular formulation of *Z. radicans* against rice leaf folder *C. medinalis*

In the experiments on the different doses of *Z. radicans* granules, 400g *Z. radicans* granules/plot was found effective by recording 25.29 per cent reduction over control. This was followed by 20.70 % 300g/plot treatment (Table 53).

During the second spray (Table 54) also, similar trend was noticed where the maximum reduction of leaf folder population was noticed in the plot treated with the dose of 400g/plot treatment (57.23 per cent) over control. Whereas, lowest reduction of *C. medinalis* population was recorded on dose of 100g /plot (46.02 per cent reduction over control). Among the different treatment of oil and granular formulations, oil formulation of *Z. radicans* was caused the highest per cent reduction over control when compared to granular formulation of *Z. radicans*.

8.5. Field efficacy of oil based bio-formulation of *Z. radicans* against rice leaf folder *C. medinalis*

The studies on effect of various treatments against rice leaf folder (Table 55) indicated that *Z. radicans* + Sunflower oil + Glycerol resulted significant and higher reduction of infestation to the tune of 35.25 per cent reduction over control after the first spray. This was followed by treatments with *Z. radicans* + Mustard oil + Glycerol, *Z. radicans* + Sunflower oil and *Z. radicans* + Mustard oil combinations which recorded 26.33, 26.13 and 24.24 per cent respectively.

During the second spray (Table 56), *Z. radicans* + Sunflower oil + Glycerol recorded the maximum of 69.96 per cent reduction over control. This was followed by *Z. radicans* + Mustard oil + Glycerol and *Z. radicans* + Sunflower oil combination treatments resulted 59.37 and 57.87 per cent respectively.

Table 53. Field efficacy of granular formulations of *Z. radicans* against rice leaf folder *C. medinalis* – I spray

Sl. No	Dose (gm/plot)	I spray - % larval mortality				Mean	% reduction over control
		Pre count	3	7	10		
1	100	17.02 (4.25)	17.05 (4.25)	16.03 (4.13)	15.35 (4.04)	16.14 (4.14)	17.70
2	200	17.65 (4.32)	17.00 (4.24)	16.22 (4.15)	15.13 (4.02)	16.12 (4.14)	17.80
3	300	18.33 (4.39)	16.00 (4.12)	15.36 (4.04)	14.28 (3.91)	15.21 (4.07)	20.70
4	400	16.27 (4.16)	15.12 (4.02)	14.32 (3.91)	13.50 (3.81)	14.31 (4.03)	25.29
5	Control	18.72 (4.44)	18.12 (4.37)	19.90 (4.57)	20.80 (4.67)	19.60 (3.90)	
C. D (p= 0.05)		0.15	0.18	0.19	0.18	0.15	
SE(d)		0.06	0.08	0.08	0.08	0.05	

Each value is mean of three replications.
Figures in parentheses are transformed square root values.

Table 54. Field efficacy of granular formulations of *Z. radicans* against rice leaf folder *C. medinalis* – II spray

Sl. No	Dose (gm/plot)	II spray - % larval mortality				Mean	% reduction over control
		Pre count	3	7	10		
1	100	15.35 (4.04)	13.12 (3.76)	11.28 (3.50)	10.42 (3.38)	11.61 (3.55)	46.02
2	200	15.13 (4.02)	14.02 (3.86)	12.00 (3.61)	9.00 (3.16)	11.67 (3.56)	45.74
3	300	14.28 (3.91)	12.00 (3.61)	10.24 (3.35)	8.00 (3.00)	10.08 (3.33)	53.14
4	400	13.50 (3.81)	11.15 (3.49)	9.31 (3.21)	7.15 (2.85)	9.20 (3.19)	57.23
5	Control	20.80 (4.67)	20.25 (4.61)	21.12 (4.70)	23.16 (4.91)	21.51 (4.74)	-
C. D (p= 0.05)		0.18	0.17	0.17	0.16	0.17	-
SE(d)		0.08	0.07	0.07	0.06	0.07	-

Each value is mean of three replications.

Figures in parentheses are transformed square root values.

Table 55. Field efficacy of oil based bio-formulations of *Z. radicans* against rice leaf folder *C. medinalis* – I spray

Sl. No	Treatment	I spray - % larval mortality				Mean	% reduction over control
		Pre count	3	7	10		
1	*Z. radicans* alone	17.50 (4.30)	16.10 (4.14)	15.50 (4.06)	15.00 (4.00)	15.60 (4.07)	22.26
2	*Z. radicans* + Glycerol	16.55 (4.19)	16.00 (4.12)	15.70 (4.09)	15.00 (4.00)	15.56 (4.07)	22.85
3	*Z. radicans* + Sunflower oil	18.83 (4.45)	16.40 (4.17)	15.20 (4.02)	13.10 (3.75)	14.90 (3.99)	26.13
4	*Z. radicans* + Sunflower oil+ Glycerol	18.30 (4.39)	16.00 (4.12)	13.20 (3.77)	10.00 (3.32)	13.06 (3.75)	35.25
5	*Z. radicans* + Mustard oil	17.00 (4.24)	16.00 (4.12)	15.60 (4.07)	14.25 (3.91)	15.28 (4.03)	24.24
6	*Z. radicans* + Mustard oil + Glycerol	19.45 (4.52)	16.10 (4.13)	14.70 (3.96)	13.80 (3.85)	14.86 (3.98)	26.33
7	Control	17.55 (4.31)	20.50 (4.64)	19.00 (4.47)	21.00 (4.69)	20.17 (4.60)	
C. D (p= 0.05)		0.20	0.19	0.18	0.17	0.18	
SE(d)		0.09	0.09	0.08	0.08	0.08	

Each value is mean of three replications.

Figures in parentheses are transformed square root values.

Table 56. Field efficacy of oil based bio-formulations of *Z. radicans* against rice leaf folder *C. medinalis* – II spray

Sl. No	Treatment	II spray - % larval mortality				Mean	% reduction over control
		Precount	3	7	10		
1	*Z. radicans* alone	15.00 (4.00)	13.30 (3.78)	12.20 (3.63)	10.00 (3.32)	11.80 (3.58)	48.11
2	*Z. radicans* + Glycerol	15.00 (4.00)	13.00 (3.74)	11.60 (3.55)	9.40 (3.23)	11.33 (3.51)	50.18
3	*Z. radicans* + Sunflower oil	13.10 (3.75)	11.15 (3.48)	9.35 (3.22)	8.23 (3.04)	9.58 (3.25)	57.87
4	*Z. radicans* + Sunflower oil+ Glycerol	10.00 (3.32)	8.15 (3.02)	7.25 (2.87)	5.10 (2.47)	6.83 (2.80)	69.96
5	*Z. radicans* + Mustard oil	14.25 (3.91)	13.25 (3.77)	12.00 (3.61)	9.10 (3.18)	11.45 (3.53)	49.65
6	*Z. radicans* + Mustard oil + Glycerol	13.80 (3.85)	11.28 (3.50)	9.09 (3.18)	7.35 (2.90)	9.24 (3.20)	59.37
7	Control	21.00 (4.69)	23.23 (4.92)	22.62 (4.86)	22.37 (4.83)	22.74 (4.87)	
C. D (p= 0.05)		0.17	0.16	0.15	0.14	0.15	
SE(d)		0.08	0.07	0.70	0.06	0.07	

Each value is mean of three replications.
Figures in parentheses are transformed square root values.

The millet based technology for production granular cultures of *Z. radicans* was easy, inexpensive and highly efficient compared to *B.bassiana* for the management of major rice pests and the procedure for mass production is superior than other methods for mass production. This new technique is simple to follow and there is no need of additives, drying freezing and milling. This technology is amenable for mass production of *Z. radicans* .*Z. radicans* produce specialized resting spores which could have potential as an alternative commercial inoculums as they are robust and long lived.

From this study it was clear that the test fungus *B. bassiana* and *Z. radicans* are able to grow on a variety of cheap, easily available grains and on a wide variety of agriculture products and by products of both solid and liquid state and this can be useful to farmers to culture these fungi easily. These rearing media, grains can be used for the mass multiplication of the fungus and it may increase its efficiency as biocontrol agent which is also economic and easily available.

However, further intensive research studies are required on mechanism of resting spore production and germination, to withstand abiotic factors like temperature and humidity for wider scale of production in large quantity to make it commercialize with novel formulation and application technology to develop it as effective myco- insecticide.

According to crop Statistics of Ministry of Agriculture, India is the second largest producer of agricultural crops. Agrowastes of about 1500 lakh tons is produced every year (The Times of India, 19,10,2015) which costs about 96,000 crores. Though it is proved that agrowastes possess significant potential in the production of entomopathogens, For mass culturing these materials were not popular and preferred for use in many places due to its quality, purity, shelf life and effectiveness. As entomopathogens are sensitive, chances for contaminations, practical utility in field application are limited due to so many factors. However, focusing further studies may provide promising scope; good chances for production of better refined material from potential agro wastes are possible in futures.

SUMMARY

The following are the excerpts of important findings of the present investigations.

1. In the preliminary survey on Entomofungal pathogens, rice leaf folder larvae recorded maximum fungal infection percentage followed by brown plant hopper in selected major rice growing districts when compared to other districts. In the confirmative survey also, among the different places surveyed in three districts of Tamil Nadu, places under Cuddalore district recorded highest infection percentage.

2. Among the evaluated isolates, *Z. radicans* of Annamalainagar isolate and *B. bassiana* of Annamalainagar isolate recorded maximum pathogenicity of fungal infected cadavers of *Cnaphalocrocis medinalis* and *Nilaparva lugens* than stemborer, due to minimum pesticides application among all the surveyed field plots.

3. Among different months of study, incidence of fungal infection was highest during second fortnight of December and January.

4. Very high relative humidity was recorded at Annamalainagar in Cuddalore district when compared to remaining surveyed areas as it is near to the seacoast recorded maximum cadavers.

5. Among the weather parameters, relative humidity and minimum temperature exerted positive correlation with incidence of entomopathogenic fungi, where as negative influence was found with rainfall.

6. High relative humidity influenced the incidence of entomopathogenic fungi on brown plant hopper, rice leaf folder in December and January month during samba season.

7. In the first season, the combined effect of fungal concentration and gamma irradiation doses recorded better results in increasing the mortality of brown plant hopper and leaffolder than each

treatment applied separately. There was positive correlation between mortality, fungal concentration and gamma irradiation doses.

8. Among two entomopathogens, there was remarkable enhancement of the growth and infectivity of Z. *radicans* with the addition of 100% fish scale powder.

9. Similarly, there was appreciable increase of the growth and infectivity of *Z. radicans* with the addition of the prawn waste @ 60 per cent.

10. In the initial studies, Potato dextrose agar and among the millets broomcorn millet grain medium followed by sorghum medium was efficient and ideal for culturing *Z. radicans*

11. Temperature range of 25-30°C and Relative Humidity range of 90-95% were optimum for *Z. radicans* multiplication *in vitro*

12. Biomass production and spore germination of *Z. radicans* were uniform up to second culturing. Radial growth and infectivity diminished from fourth subculturing.

13. *Z. radicans* could be stored for five months in soil of rice field without much loss of its virulence.

14. Application of entomopathogenic fungi on brown plant hopper (*B.bassiana*) , rice leaf folder(*Z. radicans)* in recommended concentrations are safe, most feasible and effective.

15. Highest average mortality of the life stages of brown planthopper and leaffolder were recorded at 8% oil formulations of *B.bassiana* and *Z. radicans* respectively.

16. Among the powder and oil formulations of *B.bassiana* and *Z. radicans* higher concentration of oil formulations recorded highest mortality of the life stages of brown planthopper and leaffolder.

17. Among the selected insecticides, chlorpyriphos was rated as moderately toxic on 7^{th} and 14 days after inoculation (80-90%). Imidacloprid, triazophos and cypermethrin were moderately toxic on 7^{th} day after inoculation and toxicity reduced on 14 days afiter inoculation and they were become slightly harmful. Other insecticides like monocrotophos, spinosad, Econeem and quinalphos were rated as slightly harmful on 7^{th} and 14 days after inoculation.

18. For culturing of *Z. radicans,* Sabouraud-Egg-Milk-Agar medium was found suitable.

19. A novel sporulation medium with yeast extract and sunflower oil (4:8) mixture was formulated for the production of resting spores of *Z. radicians.*

20. The temperature of 20°C was optimum for germination of azygospores of *Z. radicans*.

21. Among the different concentrations of *Z. radicans*, least larval length and larval weight was noticed in 2×10^5 spores/ml and least larval duration was observed at 2×10^6 spores/ml. least pupation percentage was observed at 2×10^4 and 2×10^7 spores/ml.

22. Among the different concentrations of *Z. radicans*, least pupal length, pupal weight and pupal duration was recorded at 2×10^5 spores/ml concentration. Lowest healthy adult's emergence and highest malformed pupa was observed in the concentration 2×10^4 spores/ml.

23. Among the synthetic media tested, SMAY, SDAY and PDA were found to be suitable for culturing *Z. radicans* which yielded the highest (79.41%, 64.19% and 66.71%) spore density, colony growth and the maximum infectivity against *C. medinalis*.

24. Among the natural amended media, potato media was found to be the most suitable to *Z. radicans* which obtained the maximum (81.26%) spore density, (78.04mm) colony growth and (847.5mg) biomass production. Spore harvested from potato achieved the highest mortality against *C. medinalis.*

25. Among various agricultural products rice was superior cultural media for both *B.bassiana* and *Z. radicans.* Among various agricultural waste products sugarcane baggase for*B.bassiana* and FYM for *Z. radicans* are promising submedia for culturing. Application of entomopathogenic fungi (*Z. radicans*) at the recommended concentration are safe enough to be used along with the parasitoids in an IPM programme.

26. All isolates of *Z. radicans* were caused less mortality of honey bees. Applications of *Z.radicans* at the recommended doses are safe enough to be used with the pollinators in an IPM programme.

27. Among the oil based bio-formulation and granular formulation of *Z. radicans*, *Z. radicans* + Glycerol + Sunflower oil was recorded the highest (38.75% larval, 26.25% pupal and 22.50% adult) mortality of life stages of rice leaf folder.

28. Among the oil and granular formulation of *Z. radicans* tested in semi-field and field conditions, *Z. radicans* + Glycerol + Sunflower oil was recorded the highest (49.72% in semi-field and 69.96% field conditions) larval mortality of rice leaf folder, *C. medinalis*.

29. The highest mortality of leaf folder was recorded in oil formulation of *Z. radicans* when compared to granular formulation.

30. Higher efficiency of oil formulation compared to other formulation might be due to oil prevent the desiccation of the conidia and helps in longer survival period and better penetration of peg into integuments.

31. The best IPM strategy to contain the leaf folder menace in low land rice was the combined application of conidia in the form of wettable powder and resting spore formulation of *Z. radicans.*

32. Broom corn millet {Proso millet} (*Panicum miliceum*) is most promising solid substrate to prepare granular culture of *Z. radicans.* This new technique requires no need of drying, freezing , additives and milling, in addition it is easy to handle and cost effective and effective.

33. *Z. radicans* produce specialized resting spores which could have potential as an alternative commercial inoculums as they are robust and long lived.

The present research studies showed that *B. bassiana* is definitely a popular entomopathogen and can be found on so many host plants, however *Z. radicans* which was found to be more in this coastal part of Tamil Nadu during rainy and winter season due to high humidity and lesser temperature in rice fields was more promising . It can be also easily mass cultured on rice, however Broom corn millet has more potential due to its cost, easy for multiplication. As these studies were conducted in the limited area, the scope for mass culturing in large scale requires further standardization of all the important parameters which needs more facilities, conditions and resources for successful mass culturing to produce effective myco insecticide against major pest like leaffolder in this part of Tamil Nadu.

However further intensive research in the above aspects will enhance the quality at reasonable cost and best performance for production of effective mycoinsectcide.

Plate1. Millet market: major producers, by volume in % global, 2018
Distribution of small millets in India

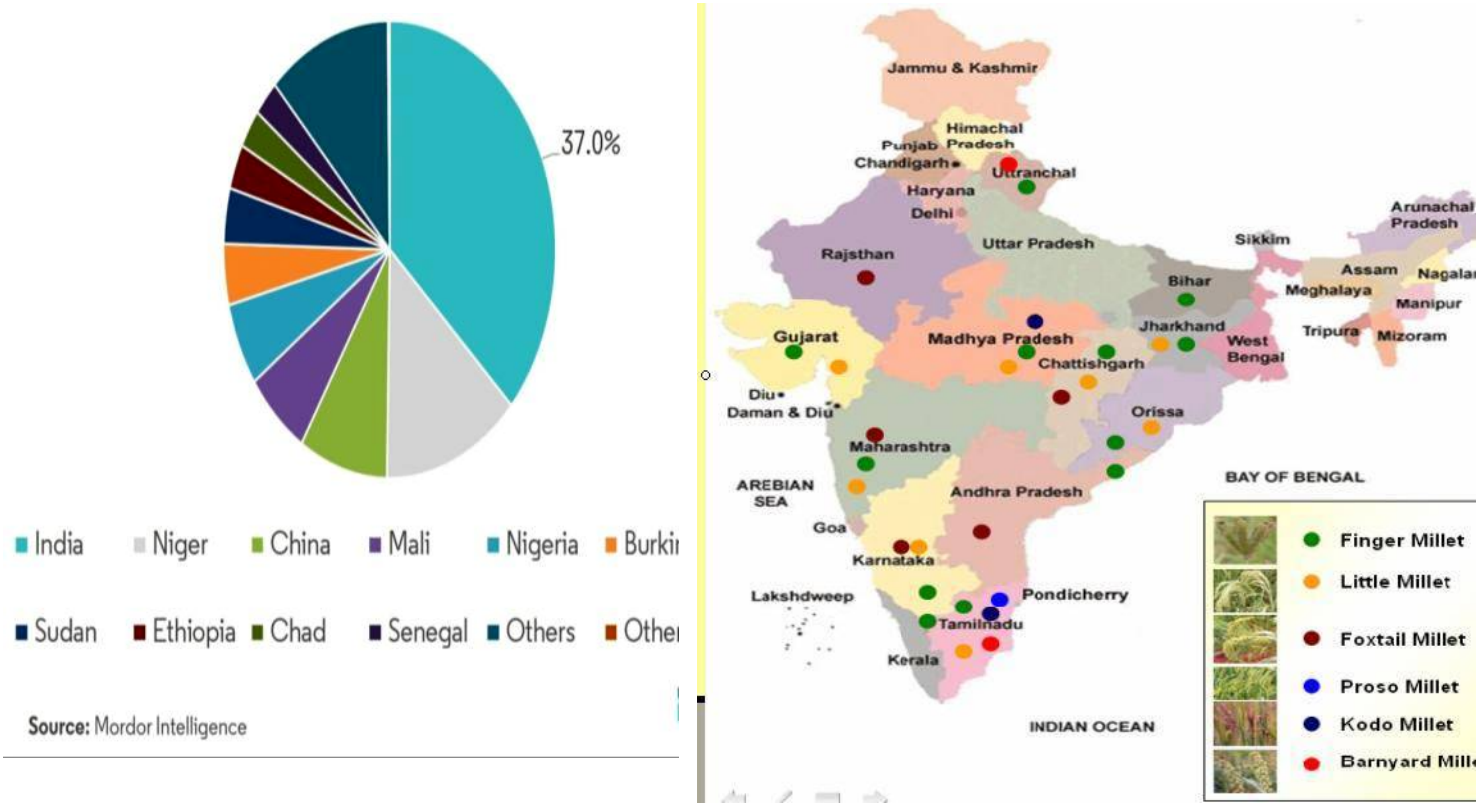


Plate 2. The nutrition details of different food grains

Seed	Protein(g)	Fat (g)	Ash (g)	Crude Fibre(g)	Carbs (g)	Energy Kcal	Ca Mg	Fe Mg	Thiamin Mg	Riboflavin Mg	Niacin Mg
Rice (brown)	7.9	2.7	1.3	1.0	76.0	362	33	1.8	0.41	0.04	4.3
Wheat	11.6	2.0	1.6	2.0	71.0	348	30	3.5	0.41	0.10	5.1
Maize	9.2	4.6	1.2	2.8	73.0	358	26	2.7	0.38	0.20	3.6
Sorghum	10.4	3.1	1.6	2.0	70.7	329	25	5.4	0.38	0.15	4.3
Pearl Millet	11.8	4.8	2.2	2.3	67.0	363	42	11.0	0.38	0.21	2.8
Finger Millet	7.7	1.5	2.6	3.6	72.6	336	350	3.9	0.42	0.19	1.1
Foxtail Millet	11.2	4.0	3.3	6.7	63.2	351	31	2.8	0.59	0.11	3.2
Proso Millet	12.5	3.5	3.1	5.2	63.8	364	8	2.9	0.41	0.28	4.5
Little millet	9.7	5.2	5.4	7.6	60.9	329	17	9.3	0.30	0.09	3.2
Barnyard millet	11.0	3.9	4.5	13.6	55.0	300	22	18.6	0.33	0.10	4.2
Kodo millet	9.8	3.6	3.3	5.2	66.6	353	35	1.7	0.15	0.09	2.0

*For 100g of proximate analysis carried out on rice, wheat, major and minor millets of India

Plate 3. Different types of food grains used in media preparation

Sorghum

Pearl millet

Finger millet

Soya bean

Borken Wheat

Broken Rice

Proso millet (Broomcorn millet) *Panicum miliaceum*

Plate 4. Field view of rice crop

Plate 5. Mass culturing of rice Leaf folder *C. medinalis*

Plate 6. Collection of Cadavers from the Rice fields at Annamalai University

Leaf Folder **BPH Cadaver**

Rice leaf folder, *C. medinalis* infested with *Zoophthora radicans*

Brown planthopper, *N. lugens* infected with *B. bassiana*

Plate 7. Preparation of oil formulation of *Z. radicans*

Oil formulation

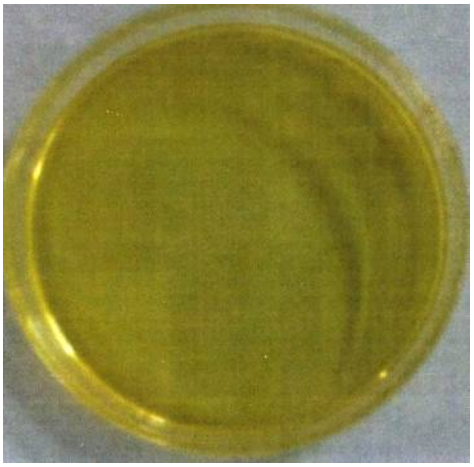
Corn

Plate 8. Preparation of powder formulation of *Z. radicans* and *B. bassiana*

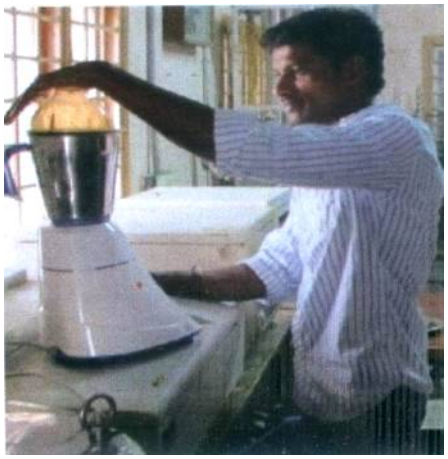
Fungal mat

Talcum powder

Gum

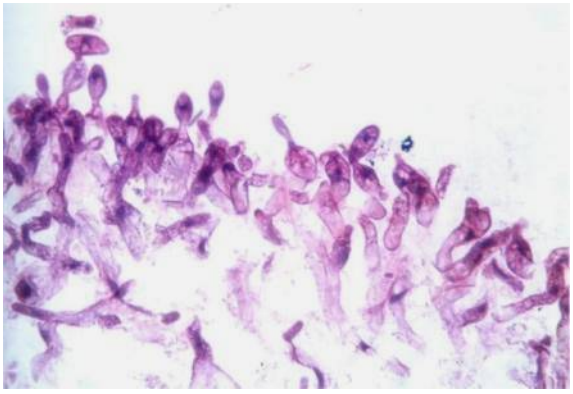


Grinding

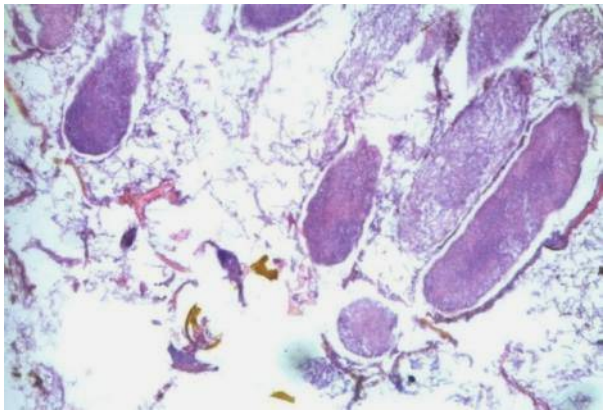


Powder formulation

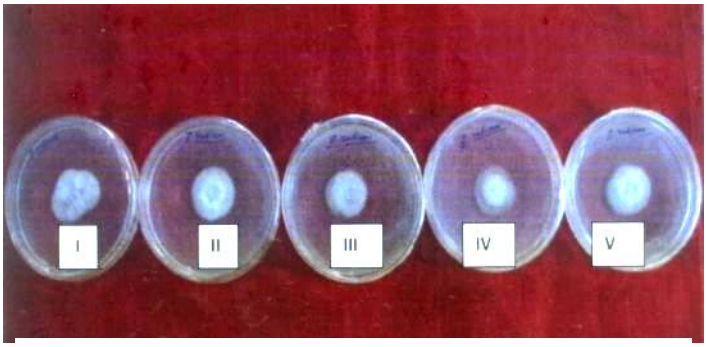
Plate 9. Sectional view (Microtomy) of leaf folder *C. medinalis*



Rows of conidioshores arising from the body wall of leaf folder, *C. medinalis*



Different isolates of *Zoophthora radicans*



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Different isolates of *B.bassiana*

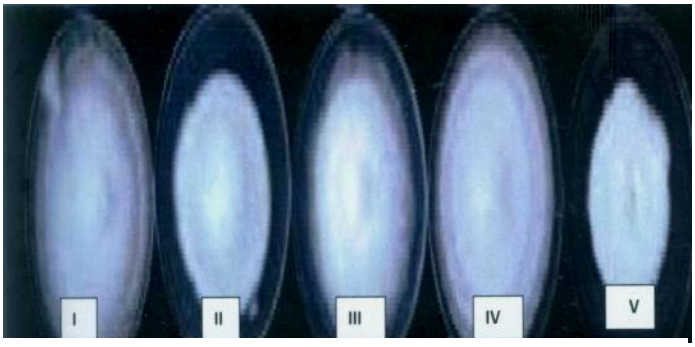

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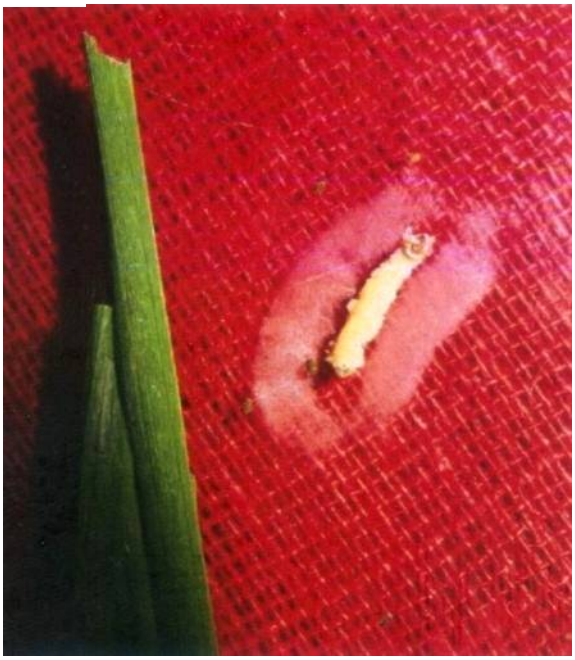
Plate 10. Evaluation of different powder formulation of *Z. radicans* against biology of leaf folder

Powder formulation

Different concentration of oil Formulation of *Z. radicans*

Infected 3rd instar larva @

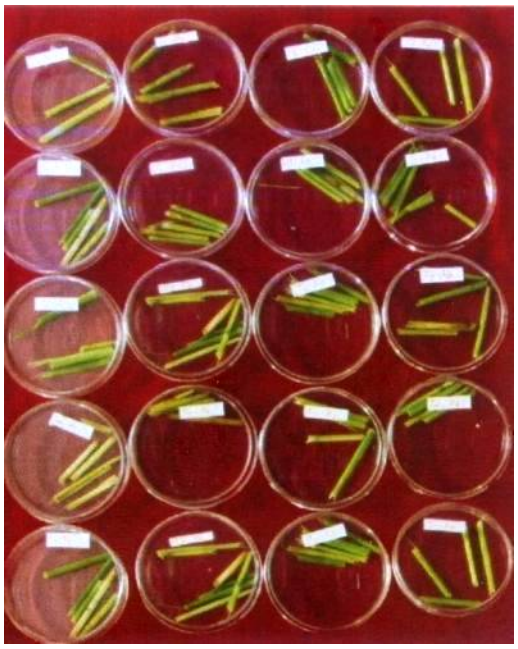


Infected 5rd instar larva @

Pupal malformation @ 8%

Plate 11. Evaluation of oil formulation of *B. bassiana* against biology of Brown plant hopper

Different concentration of oil formulation of *B. bassiana*



Infected BPH

Infected BPH Nymph @ 8%

Infected BPH adult @ 8%