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MICROBIAL POTENCY, CHARACTERIZATION AND EFFECT OF INDIGENOUSLY ISOLATED UBIQUITOUS BACTERIUM *Bacillus thuringiensis* (BERLINER) IN BIOLOGICAL SUBJUGATION OF LEAF FOLDER *Cnaphalocrocis medinalis* (GUENEE) IN RICE

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ABSTRACT

Ubiquitous bacterium *Bacillus thuringiensis* (*Bt*) (Berliner) has been indigenously isolated from the various crop canopies *viz.,* Rhizosphere, Phyllosphere and Insect cadavers in Puducherry region and the promising isolate was identified based on cultural, molecular, biochemical characteristics and Bioassay studies. Among ten *Bacillus* isolates, the isolate 1, 9 and 12 derived from Rhizosphere and isolate 22 obtained from Insect cadaver had behaved in resemblance with *Bacillus thuringiensis* characteristic features but the isolate 9 had invariably showed its supremacy over other isolates by exhibiting the characteristic features of *Bacillus thuringiensis* like Colonial count, Total cell protein, Bipyramidal crystals, Crystalliferous protein and Bioassay using parasporal inclusions. Characterizing the potency of *Bt* bacterial biopesticide in skirmishing the rice leaf folder *Cnaphalocrocis medinalis* , it was estimated at 22,121 IU/ mg with the LC50 0.6 x 10^8. Thus, *Bacillus* isolate 9 was found to be considered as promising isolate and this was chosen for further studies. The microbial preparation of the promising indigenous *Bt* isolate 9 in various dosages *viz.,* 0.5, 1.0, 1.5, 2.0 and 2.5 g per litre of water were applied over the foliage surface of rice using atomizer. The dosage 2.5 gram per litre of water was effective in causing the highest mean mortality of 61.30 % and the dosage 0.5 g / litre of water was found the least (26.16 %) in 72h. under laboratory conditions. The crystalliferous parasporal inclusions were rampant in *Bt* isolate 9 and they were the rule of thumb for killing the leaf folder larvae in Rice through septicemia.

INTRODUCTION

Rice is the staple food crop grown in almost all the states of India and elsewhere in Asia, Australia and Solomon & Fuji Islands (Kenmore, 1980 ; Narayanasamy, 1985 ; Wilson and Claridge, 1991 ; Udayaprabhakar, 1994 and Van Rie, 2000). In India, rice is being cultivated in an area of 44 Million Hectares with an average yield of 132 Metric Tonnes during the year 2004 which declined to 79 Metric Tonnes during 2009 – 2010 (Anonymous, 2004 and Agarwal, 2011). The economic loss due to the infliction of insect pests in crop plants has been estimated at 41% annually. In rice, it is accounted at 26.3 Metric Tonnes with an estimated value of `9468 crores (Agarwal, 2011). Of rice pests, leaf folder *Cnaphalocrocis medinalis* (Guenee) is the deadliest pest of rice as it assumed a permanent threat to rice cultivation during the past three and half decades in India and it has been there for generations over a cropped area of 21,847 ha. in Puducherry Union Territory. It has attained prominence only with the wide spread introduction of high yielding, broad leaved rice varieties and their associated increases in the use of nitrogenous fertilizers and synthetic chemical pesticides (Yashida *et al.,* 1969 and Yadawa *et al.,* 1972).

The incidence of leaf folder occurs during vegetative as well as panicle initiation stages of the rice crop. The larvae fold the leaf and scrape the green tissues of the leaves from within and cause scorching and leaf drying. Each larvae is capable of destroying several leaves by its feeding (Upadhyay *et al.,* 1975 and Kandibane *et al,* 2010). Sellamal Murugesan and Chelliah (1983) reported that 10% increase in flag leaf damage by the leaf folder significantly reduced the grain yield by 0.13 g per tiller and the number of fully filled grains by 4.5%. Use of Biological Control Agents is one of the effective tools in Integrated Pest Management strategy and among the

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biological control agents, microbes especially bacterial pathogens are increasingly found potential against crop pests (Li *et al*., 1987; Carozzi *et al* ., 1991; Jayachandran, 1994 and Johnson and McGaughey, 1996). In India Hofte and Whitley (1989) was the first to report feasibility of *Bacillus thuringiensis* var. *kurstaki* effective against lepidopterous pests which include *Cnaphalocrocis medinalis* and *Scirphophaga incertulus* (Srivastava and Nayak, 1978; Philip and Jacob,1982; Khan 1995; Singh *et al*., 2000 and Yadugiri, 2010). Subsequently, Halder and Ellar (1989) reported that the high dosage of *Bacillus thuringiensis* was found effective against a number of lepidopterous pests. While enormous researches have been conducted in testing the biocontrol potential of numerous isolates of native bacterium available in Indian soil, plant and water in the laboratory and field level, tapping this bacterial entity to looking beyond has been very few and scanty (Travers *et al*., 1987 ; Bora *et al*., 1994 and Ichimatsu *et al*., 2000). However, Ramamourti *et al*. (2007) have made in-roads into the study and collected five strains of *Bacillus thuringiensis* from rhizosphere and phyllosphere of various crop ecosystems in Puducherry Union Territory. This maiden initiative is the first of its kind in Puducherry. Among Five indigenously isolated *Bacillus thuringiensis* strains, the strain christened PKKVK 1 was found to be effective against Leaf folder *Cnaphalocrocis medinalis* showing 73.30% mortality in rice under Laboratory conditions.

The indigenously isolated strains are found potential in rapid killing of insect pests by virtue of their virulence, effectiveness and adaptability to native crop settings. In Puducherry, Agricultural as well as Horticultural crops are being grown mainly by farming community and cultivable lands in this region have been exclusively endowed with various types of soils such as wet land, dry land and garden land soils and the crop canopy is highly conducive for the augmentative appearance of microbial strains especially *Bacillus* bacterial strains coinciding with coastal microclimate known to be pristine microhabitat. This indicated that there is vast scope for tackling Leaf folder and other Lepidopterous foliage feeding pests through bacterial pathogens which are already adopted to the crop ecosystem. The present study was hence undertaken to isolate a well-adapted promising bacterial entity *Bacillus thuringiensis* and to develop a bacterial biopesticide using indigenously isolated effective strains for Leaf folder problem in rice in Puducherry.

MATERIALS AND METHODS

Collection and isolation of Indigenous *Bacillus thuringiensis* from Biosphere

Forty six soil samples from the rhizosphere, three samples from the phyllosphere and five samples of insect cadavers were collected aseptically from the major crops including Rice, Groundnut, Blackgram, Bhendi, Chilli, Clusterbean, Sugarcane, Marigold, Tomato, Tapioca and Banana where there was no history of application of *Bacillus thuringiensis* in and around Puducherry region. Collection was undertaken when crop environments were always humid owing to its coincidence with winter season of the year aided by the North East Monsoon (October-December) best suited for the growth and proliferation of the bacterium.

One gram of soil adhering the lateral roots of respective crops measuring 5-15 cm along the length of roots were collected aseptically after removing the 2-4 inches top litter layer and placed in sterilized polythene bags (25 x 15 cm) and sealed airtight. Microbial samples associated with three long leaf surfaces of various crop plants due to bacterium were collected in sterile glass petriplates (9cm diameter) and the plates were sealed with parafilm. Similarily, the moribund larvae of Rice leaf folder, Bhendi fruit borer, Tomato fruit borer and Naiad of Rice small grasshopper suspected to be seen with bacterium by their external symptoms were also collected along with a piece of adhering substrate in sterile glass vials (15 x 10 cm) using hand gloves from the scores of crops. Later, the gathered microbial samples were examined for bacterial infections and insects showing "Epizootics or Enzootics Toxemia" were subjected to isolation and pathogenicity studies.

Isolation of *Bacillus thuringiensis* was done using the collected microbial samples from various crop canopies following serial dilution plate technique in Katnelson's Tryptose Agar and Nutrient Agar media. The colonies thus isolated from the microbial samples were identified for the presence of *Bacillus thuringiensis* employing the parameters like colonial growth, gram nature, colonial count, virulence, spore, crystal and vegetative cell ratio & their structures parasporal inclusions, toxin protein, protein positioning, biochemical characteristics and pathogenicity to Leaf folder larval with variation in the magnitude of infectivity.

Colonial growth and colonial count of *Bacillus* bacterium were measured following the standard microbiological techniques (Booth, 1971) using colony counter. *Bt* isolates indexing was done by dividing the population of crystalliferous *Bt* isolates by the total population of *Bacillus* for each sample collected from different sources. Population was expressed as Number of colony forming units per gram of soil; per square cm of leaf area and per larvae for insect cadaver.

Virulence Indexing of *Bacillus* isolates was done following the procedure of the number of small Vegetative cells was enumerated using haemocytometer after 20 h. of incubation. The number of spores and crystals were enumerated by staining the heat fixed culture smear with Amidoblack and Safranin stain. Finally, the cells, spores and crystals at Five Microscopic Fields were observed under Phase Constrast Microscope (40 X) and the percent vegetative cells and the Spore crystal ratio were calculated using the formula

$$\text{Percent Vegetative Cells} = \frac{\text{Total Vegetative Cells} \times 100}{\text{Total Vegetative Cells + Spores}}$$

$$\text{Spore Crystal Ratio} = \frac{\text{Total Spores}}{\text{Total Crystals}}$$

Parasporal Crystalline bodies morphology were observed using their shape under Phase Contrast Microscope (100 X) and imaged. Total cell protein was estimated using ten millilitres of 72 h incubated *Bacillus thuringiensis* culture added with five millilitres of cell lysis buffer to lyse and release the proteins into the buffer. The culture with cell lysis buffer was centrifuged at 10,000 rpm for 15 min. using high speed cooling centrifuge. The supernatant in centrifuged tubes was discarded and the pellet settled at bottom was washed with 10 ml 1M Sodium Chloride to remove the cells of *Bacillus thuringiensis* associated serine and metalloproteases. Then, the pellet was washed twice with sterile distilled water. The suspension was centrifuged at 10,000 rpm for 15 min. The pH of the supernatant was adjusted to 8 using Tris Hydrochloride. Cell protein present in the supernatant was quantified by Lowry's method (Lowry *et al*., 1951) using Biospectrophotometer at 600 nm and expressed as mg/g.

Crystallaceous protein position present in *Bacillus thuringiensis* cultures were qualitatively located in protein lanes using Medium protein molecular weight markers by the Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis Technique (Laemmli, 1970).

Biochemical tests on *Bacillus thuringiensis* cultures like Methyl Red Reaction, Voges Proskauer Test, Citrate, Urease Tests, Starch Hydrolysis, Gelatin Hydrolysis besides Indole, Catalase, Mannitol and Glucose utilization were carried out employing standard protocols.

Rearing of Target Insect Leaf folder *Cnaphalocrocis medinalis*

Larvae of leaf folder *Cnaphalocrocis medinalis* were collected from the leaf folder infested rice leaves (Variety : CR 1009) during maximum tillering and flowering phases in the rice fields situated at Eastern Block Farm of PKKVK (Study place) and adjoining villages as well.

The collected larvae were reared in sterile petriplates (15 cm diameter) by providing fresh rice leaves under Laboratory conditions. After a week time, the pupae obtained were transferred into another sterile petriplate (15 cm diameter) kept on wet cotton for developing into adults. Thirty adults were at once transferred into a fresh fecudity cage for egg laying and honey swab was provided as food.

Bioassay of ubiquitous bacterium *Bacillus thuringiensis* against rice leaf folder

Bioassay test of ubiquitous bacterium *Bacillus thuringiensis* isolated from various crop canopies which were found pathogenic, was carried out with rice leaf folder. Four different concentrations of bacterial parasporal suspensions such as 10^7, 10^8, 10^9 and 10^{10} were prepared with sterile distilled water containing 0.02% Tween 80 and sprayed directly on fresh rice foliage bits infesting 25 leaf folder larvae in each sterile petriplate (15 cm diameter) toweling filter paper beneath @ 4 ml / petriplate. Mortality counts were made three days after spraying. Three replications were maintained for each treatment. Control received only 0.02% Tween 80. Among four concentrations, the concentration which gave a least LC 50 was selected and the data collected from dosage mortality responses were subjected to Probit Analysis and appropriate LC 50 values were worked out. Sporulated Broth of *Bacillus thuringiensis* was stabilized by lowered the p_H into 4 and added Potassium Sorbate @ 2 g/litre with Sulphuric acid. Then,

Potency of *Bacillus thuringiensis* Broth was determined by observing suspended solids using the formulae

$$\text{Potency of } \textit{Bacillus thuringiensis} = \text{Potency of the standard X } \frac{\text{LC50 of the standard (mg/ml)}}{\text{LC50 of the sample (mg/ml)}}$$

Biopesticide Formulation

Promising culture of *Bacillus thuringiensis* grown on Luria Bertani medium was centrifuged at 10000 rpm for one hour. Supernatant was discarded and the pellet was weighed and transferred into a sterile plate. Primary powder was prepared by mixing 97% of the cell concentrate with 2% of Tween-80 and 1% of Carboxy Methyl Cellulose. Then, the Wettable powder was formulated using 20% Primary Powder, 75% of Bentonite, 0.75% of Wessalon S, 2% Tween-80 and 2% Gelatin. After through mixing, the biopesticide was shade dried and the contents were sieved through bacterial sieve to get fine powder with the particle size of 44 microns. The fine powdered bacterial biopesticide was packaged in a sterile polythene bag and sealed air tight and stored at room temperature or at Refrigerator at 4^oC till use.

Effect of Indigenously isolated ubiquitous bacterium *Bacillus thuringiensis* against rice Leaf folder

To check the effectiveness of promising *Bacillus thuringiensis* bacterial biopesticide against rice leaf folder *Cnaphalocrocis medinalis* under laboratory conditions, different dosages of bacterial biopesticide like 0.5, 1.0, 2.0 and 2.5 g / litre of water were taken in two litres conical flask containing a litre of mono sterile distilled water each. The bacterial biopesticide in various dosages were sprayed using a pneumatic mini sprayer over rice leaves (Variety : CR 1009) kept in the plastic vials (3.5 x 3.5 cm) toweling filter paper at the bottom. Then, six hours pre-starved twenty five larvae were taken and one larvae per vial was allowed. Rice leaves treated only with water served as control. Three replications were maintained in each treatment. Symptoms and mortality of the larvae due to molecular mechanisms of *Bacillus thuringiensis* were recorded for every 24 h until the larvae in the control grew to pupae, adult and egg stages. The larvae pronounced dead when they seized all body movements and no longer responded to touch up by the dissecting needle.

The data obtained in the laboratory experiments were analysed following the various methods described by Panse and Sukhatme (1978) and Gomez and Gomez (1983) using Randomized Block Design and the critical difference values were used to compare the treatment means by Duncan's Multiple Range Test (Duncan, 1956). The LC 50 of the bacterial suspension *Bacillus*

thuringiensis was calculated using Probit Analysis (Finney, 1964).

RESULTS AND DISCUSSION

The Crystalliferous *Bt* isolation index and type of crystals produced characterization of indigenously isolated bacterial samples for authentication of *Bacillus thuringiensis* isolates using certain cultural characteristics like shape, motility, gram reaction, presence of vegetative cells, spores and crystals, crystal morphology, colonial count and virulence ; molecular characteristics like cell protein and protein positioning and biochemical characteristics following standardised methods as outlined in Bergey's Manual of Systematic Bacteriology were done and the findings are presented (Table- 1). of the fifty four samples collected from different sources of crop canopies like Rhizosphere, Phyllosphere and Insect cadavers, ten samples were found to possess the characteristic features of *Bacillus* bacterium which were positive for gram stain, spore and crystal staining with the bacterial population ranged between 2.01 x 10^1 to 4.56 x 10^7.

Puttasamy, 2007) confronting with the development of insect resistance to chemical insecticides sprayed for the control of multitudinous agricultural crop pests. Further, the colonial count of *Bt* in the present study was most similar with colonial count ranged between 4.23 x 10^5 – 6.52 x 10^5obtained by isolation from soil (Chatterjee *et al*, 2007).

The bacterial cells present in the ten samples were found in rod shaped which produced polymorphic crystals. As per as the types of crystals produced by ten isolates, three crystal type parasporal inclusions *viz.*, Bipyramidal, Cuboidal and Rhomboidal were evident. Among ten *Bacillus* isolates, five isolates produced Bipyramidal crystals which were found predominant followed by cuboidal in four isolates and Rhomboidal in only one isolate. The study gains support from Asokan and Puttasamy, 2007 who observed Bipyramidal, Cuboidal and Rhomboidal crystals in indigenously isolated *Bt* samples. The shape of the crystal depends on the protoxin composition (*i.e*) Bipyramidal Crystals contain *Cry* 1, Cuboidal contains *Cry 2* and Rhomboidal

Table 1 Characterization of Indigenously isolated bacterial samples of *Bacillus thurtngtensts* obtained in and around Pondicherry

Sl. No	Isolate Index	Crops	Place	Crop canopy	Cultural Characters								Molecular Characters			Biochemical Characters **								
					Shape	Motility	Gram Reaction	% vegetative cell	S:C ratio	Crystal Morphology	Colony count KTA	Colony count NA	Virulence #	Cell protein (µg/ml)	Protein positioning	Methyl red	Vogues proskaur	Citrate	Urease	Starch hydrolysis	Gelatin hydrolysis	Indole	Catalase	Mannitol	Glucose
1.	Isolate 1	Rice	Sellipattu	Rhizosphere	Rod	Motile	G+	8.5	0.94	Bp	6.36x 10^6	5 x 10^4	+++	118.9	135KDa	+	-	+	-	+	+	-	+	+	-
2.	Isolate 2	Rice	P.S Palayam	Rhizosphere	Rod	Motile	G+	10.08	1.00	Cb	2.23 x 10^4	3 x 10^3	++	73.56	65 KDa	+	-	+	-	+	+	-	+	+	-
3.	Isolate 3	Rice	Ariyankuppam	Rhizosphere	Rod	Motile	G+	9.8	1.06	Rb	6.12x 10^3	9 x 10^3	++	67.10	73 KDa	+	-	+	-	+	+	-	+	+	-
4.	Isolate 4	Brinjal	Ariyankuppam	Phyllosphere	Rod	Motile	G+	9.7	1.05	Cb	6.26x 10^5	12 x 10^2	++	88.74	65 KDa	+	-	+	-	+	+	-	+	+	-
5.	Isolate 5	Groundnut	Sellipattu	Phyllosphere	Rod	Motile	G+	9.7	1.06	Cb	2.01x 10^4	3 x 10^3	++	95.93	65 KDa	+	-	+	-	+	+	-	+	+	-
6.	Isolate 9	Brinjal	Andiarpalayam	Rhizosphere	Rod	Motile	G+	8.55	0.96	Bp	4.56x 10^7	4 x 10^6	+++	135.9	135 KDa	+	-	+	-	+	+	-	+	+	-
7.	Isolate 12	Brinjal	Pillaiyarkuppam	Rhizosphere	Rod	Motile	G+	9.12	0.99	Bp	2.25x 10^6	1 x 10^5	+++	127.8	135 KDa	+	-	+	-	+	+	-	+	+	-
8.	Isolate 15	Groundnut	Kalapet	Rhizosphere	Rod	Motile	G+	8.23	1.06	Bp	3.56x 10^4	1 x 10^2	+++	72.73	135 KDa	+	-	+	-	+	+	-	+	+	-
9.	Isolate 16	Cluster bean	P.S Palayam	Rhizosphere	Rod	Motile	G+	10.48	1.04	Cb	2.23x 10^5	2 x 10^4	++	75.74	65 KDa	+	-	+	-	+	+	-	+	+	-
10.	Isolate 22	Rice	PKKVK Farm	Insect cadaver	Rod	Motile	G+	9.2	0.96	Bp	1.56x 10^5	2 x 10^6	+++	105.8	135 KDa	+	-	+	-	+	+	-	+	+	-

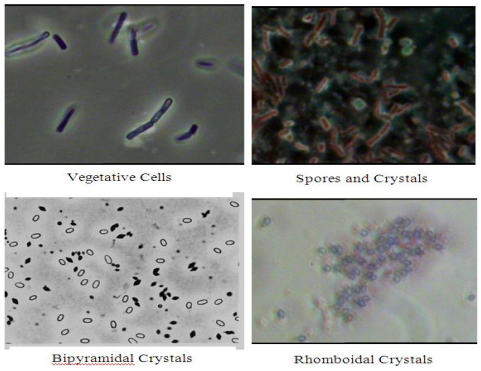
G+ Positive
G- Negative
Bp Bipyramidal crystals
Cb Cuboidal Crystals
Rb Rhomboidal Crystals
KTA Katznelson's Tryptose Agar
NA Nutrient Agar

Virulence
\+ Less than Ten spores very poor sporulation
++ Only very few but more than ten spores
+++ Abundant sporulation
* No sporulation

** Biochemical Characters
\+ Positive
\- Negative

The characteristic features of *Bt* in the present study corroborates with a battery of *Bt* strains from a wide array of samples ranging from soil, stored products, litters, leaves, seed dust and insect cadavers have successfully been isolated with proven desirable characteristics such as increased virulence and broad spectrum activity against crop pests by many scientists (Travers *et al.*, 1987, Martin and Travers, 1989, Chilcott and Wigley, 1993, Kim *et al*, 1998, Prabagaran *et al*, 2003 and Asokan and

contain *Cry 3* type of protoxin that are specific to target insects (Zelanzny *et al.*, 1990). All the isolates did not grow anaerobically and the colonies produced by the isolates were circular, white with undulated margin. From Table – 1, it is obvious that the isolate 16 had produced maximum percent vegetative cells of 10.48 % and the minimum was found noticed in isolate 15 (8.23%) after 20h. incubation in Minimum Sporulation Medium.

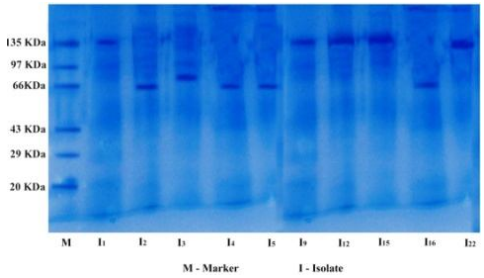


This indicated the variance of both spores and crystals which could be seen in almost all the isolates of *Bacillus*. Isolates 1, 2, 9, 14 and 22 produced spore crystal ratio of 1 and less than 1 while the isolates 3, 4, 5, 15 and 16 had the spore crystal ratio with more than one after 72h. of incubation. Similar findings observed by Normand Dubois (1968) who had the *Bacillus thuringiensis* cell concentration at 2 x 10^5 cells / ml with the spore crystal ratio of 1.

Maximum amount of cell protein was found recorded (135.9µg/ml) in isolate 9 followed by isolate 12, isolate 1, isolate 22 and isolate 3 had the protein level as low as 70µg/ml. Prabagaran (2000) had clearly described the productivity range of cell protein 36.50 – 65.00 mg / ml in indigenously isolated *Bt* strain PBT 372 using various natural sources of biomass.

On the basis of molecular weight, the protein profiles of *Bacillus* isolates were figured by SDS PAGE. The electrophoragram (Scans of protein profiles) revealed 135 KDa was rampant in the protein profiles of isolates 1, 9, 12, 15 and 22, 73 KDa in isolate 3 and 65 KDa in isolates 2,4, 5 & 16. The size of the crystal protein plays a crucial role in killing of insects that too protein level with 135 KDa is considered to be effective against on slaught of lepidopterous insects.

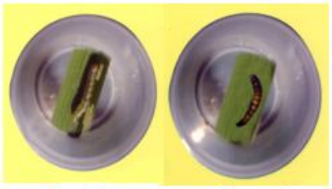
Protein profile of indigenously isolated Bacterial samples confirming *Bacillus thuringiensis*



No clear biochemical differences among *Bacillus* isolates were detected. All the *Bacillus* isolates showed a positive reaction to Methyl red, Citrate, Starch hydrolysis, Gelatin hydrolysis, Catalase and Mannitol tests and in contrast, the negative reaction was witnessed in Vogues proskauer, Urease, Indole and Glucose tests. The study corroborates with the findings of Allywyn *et al.*(2007) who narrated the characterization of various *Bt* isolates based on Biochemical characters.

Bioassay studies of Bacterial isolates with Leaf folder *Cnaphalocrocis medinalis*

Results pertaining to determination of Lethal Concentration (LC50) of ten indigenously isolated *Bacillus* pathogen towards rice leaf folder through bioassay studies are presented (Table – 2). Among the isolates, *Bacillus* isolate 9 registered the lowest LC50 value of 0.6 x 10^8 parasporal inclusions ml^{-1} to bring about 50% mortality. This was closely followed by isolate 12 and isolate 1 with LC50 of 4.3 x 10^8 and 4.9 x 10^8 parasporal inclusions ml^{-1} respectively. Conversely, *Bacillus* isolate 15 was least infective with the highest LC50 of 22.5 x 10^8 parasporal inclusions ml^{-1}. Conclusively, the lethal concentration of *Bacillus* isolate 9 with fiducial limit values ranging from 0.20 x 10^8 to 1.6 x 10^8 parasporal inclusions ml^{-1} at 95% Fiducial Limit and with LC50 of 0.6 x 10^8 parasporal inclusions ml^{-1} was found to be the best of all. Followed by this was isolate 12 with fiducial limit values ranging from 1.5 – 11.8 and LC50 value of 4.3 x 10^8 .The present findings corroborates with the findings of Hapal Singh *et al.* (2008) where the higher concentration level of LC50 was significantly more effective than lower level LC50 in all the isolates of *Bt* in reducing larval survival of *Cnaphalocrocis medinalis* in rice. Besides this, higher concentration of *Bacillus thuringiensis* had reduced food consumption by rice leaf folder and enhanced significant larval mortality (Singh *et al.*, 2000).



Epizootics of indigenous *Bt* Isolate 9 on rice leaf folder larvae

The potency of indigenously isolated promising *Bt* isolate 9 was estimated to be 22,121 IU/mg and the present microbial potency is in tune with the standard potency values prescribed in the *Bacillus thuringiensis* Production Handbook (Lisansky *et al.*, 1993) and the LC50 value of BTK HD 1 was arrived at 44.76 as standard for Rice Leaf Folder (Harpal Singh *et al.*, 2008)

Thus, the present investigation obviously revealed the characteristics behaviour of *Bacillus thuringiensis* of ten indigenously isolated bacterial isolates. Among ten *Bacillus* isolates, the isolate 1, 9 and 12 derived from Rhizosphere and isolate 22 obtained from Insect cadaver had behaved in resemblance with *Bacillus thuringiensis* characteristic features but the isolate 9 had invariably showed its supremacy over other isolates by exhibiting the characteristic features of *Bacillus thuringiensis* like Colonial count, Total cell protein, Bipyramidal crystals, Crystalliferous protein and Bioassay using parasporal inclusions. Thus, *Bacillus* isolate 9 was found to be

considered as promising isolate and this was chosen for further studies.

Optimum dosage of Biopesticide *Bacillus thuringiensis* using indigenously promising bacterial isolate 9 against Rice Leaf Folder

Table 3 illustrates the various dosages of Bacterial Biopesticide *Bacillus thuringiensis* using promising isolate 9 *viz.,* 0.5, 1.0, 1.5, 2.0 and 2.5 g / lit of water differed significantly among themselves in causing percent mortality of rice leaf folder under *in vitro* conditions. The data reveals that higher dosages of Bacterial Biopesticide *Bacillus thuringiensis viz.,* 2.0 g and 2.5g per litre of water had caused more than 50% mean mortality of Rice Leaf Folder larvae in 72h. Among the dosages tested, the dosage 2.5g per litre of water had caused the maximum mean mortality of Rice Leaf Folder (61.30%) while the least mean mortality (26.16%) was recorded with 0.5 g per litre of water (Fig. 1). The present findings is in tune with the findings of Halder and Ellar (1999) who reported that the high dose of *Bacillus thuringiensis* is effective against lepidopteran insects particularly rice leaf folder *Cnaphalocrocis medinalis.* This was reaffirmed in testing the efficacy of indigenously isolated biopesticide using promising *Bt* isolate PKKVK 9 against leaf folder *Cnaphalocrocis medinalis* in Rice (Ramamourti *et al.,* 2010). Further, the high dosage (2.5 kg/ha) of *Bt* registered the lowest leaf folder damage of 8.21% when compared to untreated control in Rice field (Kandibane *et al.,* 2010).

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