International Journal of Recent Research and Review, Vol. VII, Issue 4, December 2014
ISSN 2277 – 8322

Bio Pesticide Formulation for Plant Growth Regulation and Anti Mealy Bug –Borer Activity in Brinjal (*Solanum Melongena* L.) or Egg Plant

Khushal Patel*[1], Kalpesh Ishnava[1] and Hiral Soni[1]
*[1]*Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences, New Vallabh Vidyanagar-3888121, Gujarat, India.*
Email: khushalbiotechstudent@yahoo.co.in

Abstract - In this study main focus was to decrease the load of chemical pesticides and to prepare economically sound biopesticide that can be used commercially with less damage to the brinjal plant. In India mealy bug and borer effect on leaf is highly noticed, chiefly in Brinjal egg plant. Biopesticide Formulation (BPF) was prepared and plant seeds were treated with varying concentration of prepared biopesticide. Certain enzymes and biochemical parameters were estimated to resolve the effect of BPF. BPF was prepared with 9 botanicals; 2 chemicals and mixed with cow urine which is an animal origin. The formulation gave promising results which leads this research to a new arena where modifications can be made and minimum use of botanical results into great activity and affects the use of chemical pesticide on lower side.
Keywords - BPF, Biopesticide, cow urine, pest control, egg plant, botanicals.

I. INTRODUCTION

India is the second largest producer of vegetables after China, about 75 million tons. The existing area under vegetable cultivation in India is around 4.5 million ha. Majority of Indians are vegetarian with a per capita consumption 135 g per day as against the recommended 300 g per day [1]. In near future, there is a need of around 5-6 million tons of food to feed our 1.3 billion Indian population expected by the year 2020. Indian vegetable export is very low because of increased domestic requirement and other limitations in crop production. Vegetables are more prone to insect pests and diseases mainly due to their enderness and softness as compared to other crops and virtual absence of resistance characters because of intensive hybrid cultivation. The insect pests inflict crop losses to the tune of 40 per cent in vegetable production. Synthetic chemicals may be used in plant protection programmes to limit crop damage by pests and pathogens. But because of growing concerns about health and environmental safety, the use of toxic, carcinogenic and/or environmentally damaging chemicals is being discouraged. A survey of monitoring the farm gate samples in different parts of the country recorded pesticide residues above maximum residue limit (MRL) [2-5].

Brinjal or eggplant (*Solanum melongena* L.) is an important solanaceous crop of sub tropics and tropics. India is the second largest brinjal producer in the world (about 84.5 lakh tons) (FAO, 2008). Brinjal occupies about 8.45% of the total area under vegetables in India [6]. Brinjal is attacked by a number of insect pests and nematodes during various stages of crop growth in most of the tropical countries including India. The extent of losses caused by these pests depends on season, variety, soil and other factors [2].

The individual botanicals are not able to control crop pests, when the pest pressure is high or when there is epidemic in the field. Due to this, farmers and sometimes researchers relying on botanicals invariably discard them and switch to persistent and toxic synthetic pesticides. Therefore, a need was felt to have a reliable biopesticide formulation (BPF), which could be applied even at the time of an epidemic, when insect or disease population is high under field conditions. The BPF was prepared and tested for its efficacy in *in vitro* as well as *in vivo* studies. It was prepared by mixing nine natural ingredients of biobotanical origin with one naturally occurring mineral salt along with one animal product, in specific ratios in a liquid (also animal product). These natural products, namely onion,

ginger, *Ocimum*, neem, etc. are reported for their *in vitro* efficacy [7-16].

This communication describes one such product prepared for pest management in brinjal crop. Bioinsecticide and Biochemical analysis of brinjal leaf was done for proteins, chlorophyll, sugar, enzyme, total phenol, amylase, catalase, peroxidise enzyme. The indigenously prepared bio-pesticide is environmentally sound, nature-friendly and economical.

II. MATERIALS AND METHODS

A) Collection of plant material and preparation of BPF

Different plant material was collected from Godhra and surrounding region during May, 2012. The plant seeds and materials were identified by botanists and used for the preparation of biopesticide formulation.

B) Preparation of formulation

The Biopesticide Formulation comprised of a number of total 12 ingredients; nine of them were of bio-botanical in origin; two were natural mineral salts and one was an animal product (cow dung) respectively, all mixed in a liquid (cow urine) which is also an animal product. The indigenous cow breed used for BPF ingredients was normal, healthy and milking and having no infection. Fresh cow dung was taken in the early morning and sieved through a muslin cloth to get its extract. The ratio and proportion of ingredients was maintained as availability of sources. Ratio of ingredients along with plant part used for preparing 1000 ml of BPF was standardized and is given in (Fig. 1, Table I).

The raw material used for this formulation was mashed and mixed thoroughly in cow urine of indigenously breed cow in an earthen pot. The pot was then buried in soil for 25 days for fermentation. Then the contents of the pot were thoroughly mixed and the solution was considered as 100% stock solution.

Table I
Composition of Biopesticide Formulation

Ingredients	Weight of dry formed all ingredients(gram)
Phyllanthus emblica (amla) fruit	200
Curcuma zedooria (turmeric)	300
Lycopersicon esculentum (tomato) Leaf powder	300
Potassium aluminum sulphate dodecahydrate (naturally occurring mineral salt)	250
Azadirachta indica leaves	275
Calotropis procera	250
Allium sativum Linn (garlic) powder	200
Fresh cow-dung extract (cow dung taken in morning hours)	150
Allium cepa (onion) powder	175
Ferula narthexboiss	100
Ocimum canum (tulsi leaves)	200
Cow urine	2600
Total weight	5000

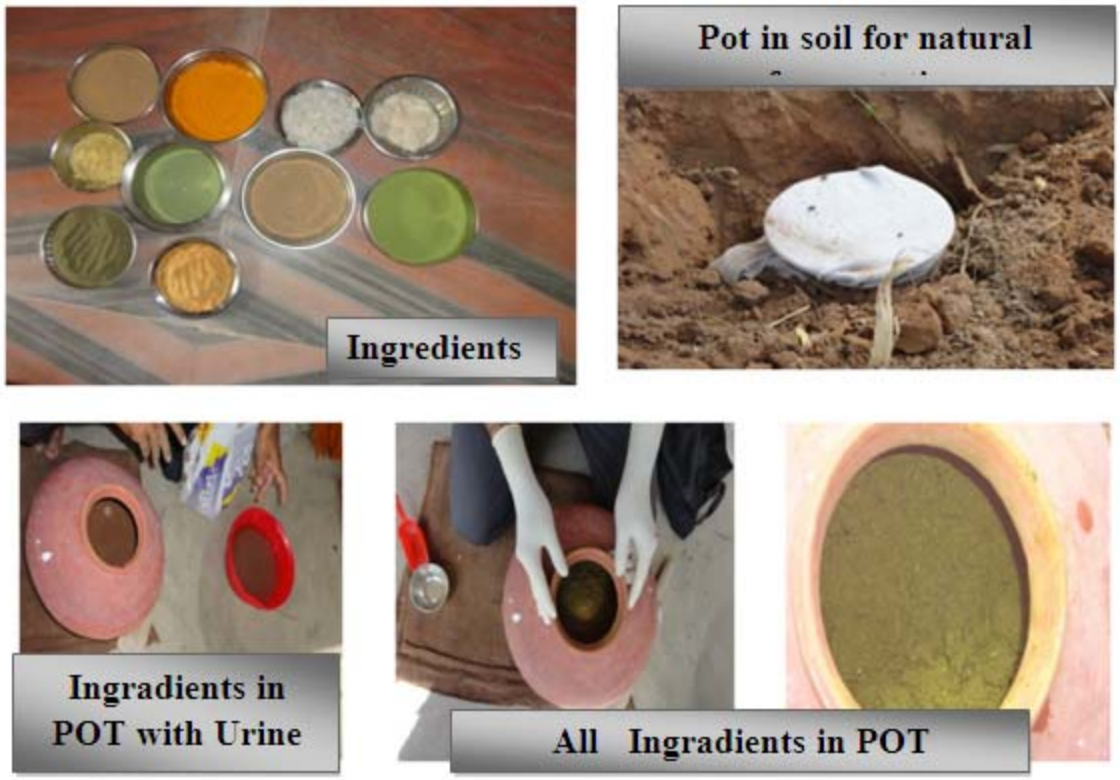

Fig. 1 Preparation of BPF

For *in vitro* bio-insecticide studies on *mealy bug* and borer, larvae of the insect were collected from fields. 5% of this formulation was prepared by serial dilution of the crude formulation with distilled water. The bioassay studies were carried out using leaf dip method [11]. Ten 6-days old, third in star larvae were released on each disc in an individual petri plate. For control sample, the leaf disks were dipped in distilled water for the same time. Four replications were used for each concentration, including control. Observations were recorded on larval mortality in each treatment at 1,2,3,4 h intervals up to 8 h after treatment.

Four sprays of BPF at 5% each at the flowering, fruiting and two sprays in between (at an interval of 20 days) the maturing stage.

C) Brinjal Cultivation

2*2 land was wetted and seeds INDU 10, F1 hybrid, Brinjal were sowed at 1 cm gap. Two sets were separated – one with Spray of 5% formulated biopesticide on that surface and one with without spray indicates it as control. Spray same liquid on every 15 days on experiment. Allow them to grow till 22 – 25 days. After 8cm – 10cm growth, transferred them in field and sowed the ropes in 1*1 ft area. Spraying was done in every 15 days. Mean while checked out few parameters within these days with the gap of 15 days observation.Estimation of Protein was done by Lowry's Method; sugar was determined by Anthrone method [17]. Total Phenol was examined and calculated according to method explained [18]. Amylase, Peroxidase and Catalase enzyme activity was determined by the method described [19].

D) Leaf disc Method

For *in vitro* bio-insecticide studies Brinjal pest, the insect were collected from fields and were reared on brinjal leaves. Five concentrations (0%, 1%, 3%, 5% and 10%) of this formulation were prepared by serial dilution of the crude formulation with distilled water. The bioassay studies were carried out using leaf dip method.Insects were released on each disc dipped in formulated liquid in an individual petri-plate. For control sample, the leaf discs were dipped in distilled water for the same time. Four replications were used for each concentration, including control. Observations

were recorded on insects' mortality in each treatment [11].

III. RESULT AND DISCUSSION

A) Estimation of Protein & chlorophyll:

Protein concentration was estimated by Lowry's method. It was observed that as days are increasing, protein level is also increasing in the experiment plants and compared to control. After the 70 days protein production was next to stable (Fig. 2).

Chlorophyll is extracted in 80% acetone and the absorbance at 663nm and 645nm are taken in spectrophotometer. Using the absorption coefficients, the amount of chlorophyll is calculated. The estimation of chlorophyll a, chlorophyll b and total chlorophyll at different time intervals (15, 30, 45, 60, 70 Days) is estimated in both control and experiment after spraying at different time interval of plant growth regulator formulation and biopesticide formulation. Chlorophyll-a at different time intervals (15, 30, 45, 60, 70 Days) is compared with control and experiment. Chlorophyll-a is not as much changed in the control and experiments. It is of equal amount. Chlorophyll-b was changed in the control and experiments. It was three times more amount of Ch – b produced (21.03 mg/g) after 70 days in experiment compared to control (Fig. 3). It has good effect of plant growth regulator formulation. Total chlorophyll has three times more amount of total chlorophyll production (20.14 mg/g) after 70 days compared to control.

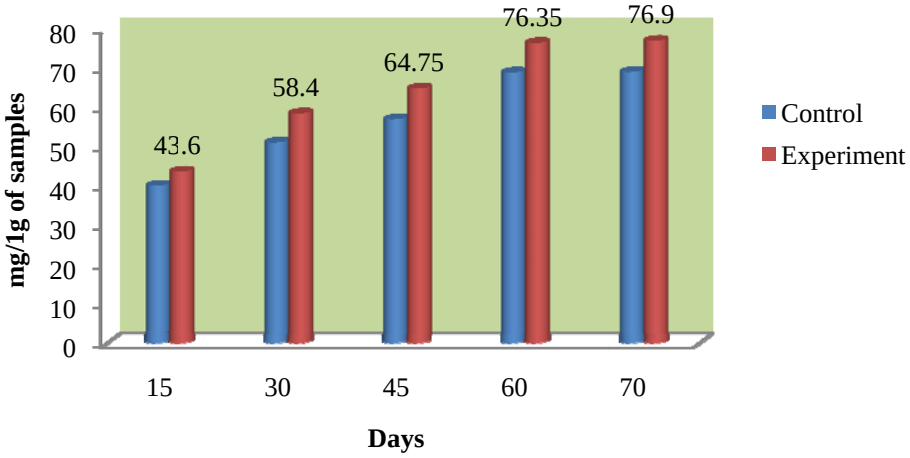

Fig.2: Estimation of protein

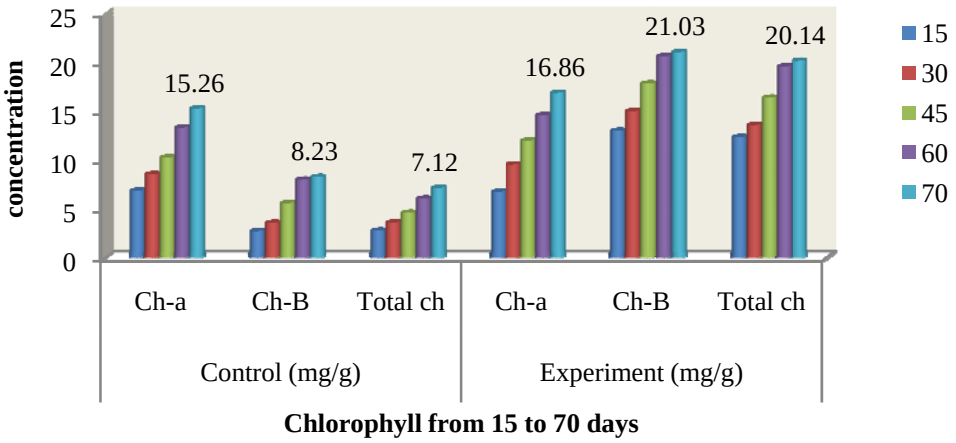

Fig.3: Estimation of Chlorophyll

B) Estimation of Total Sugar & Phenol

Total sugar was increasing in the control and experiments (Fig.4). Experiment has double times more amount of total sugar produced (78.23 mg %) after 70 days compared to control.

Total phenol at different time intervals (15, 30, 45, 60, 70 Days) is compared with control and experiment. After 15 days and 30 days, 20 % of increase in value compared to control and then after 70 days decrement in production of total phenol by 10%. After the 45th day total phenol level becomes steady. It has 10% times more amount of total phenol produced (61.11 mg/g) (Fig. 5) after 70 days compared to control. Spraying the BPF in experiment amount of up to 45 days, 10% production is increasing after the production of total phenol getting declined.

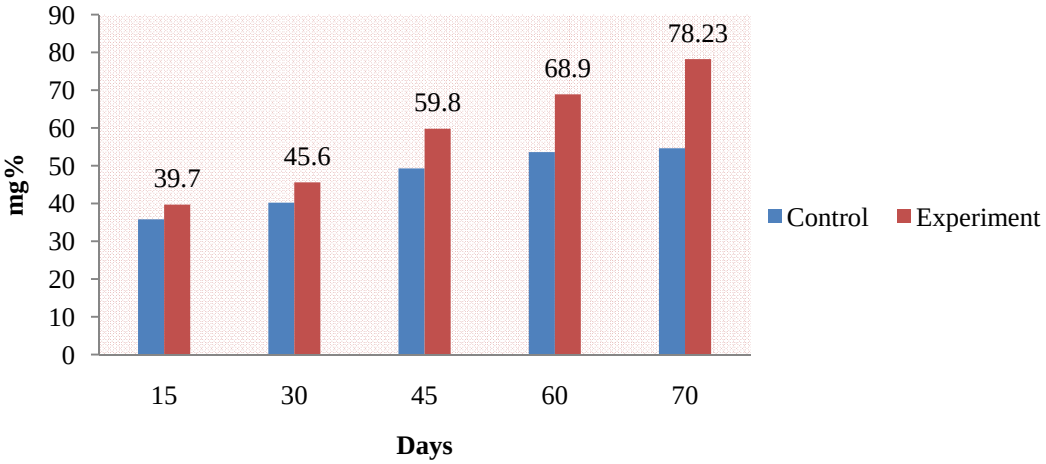

Fig.4: Estimation of total Sugar

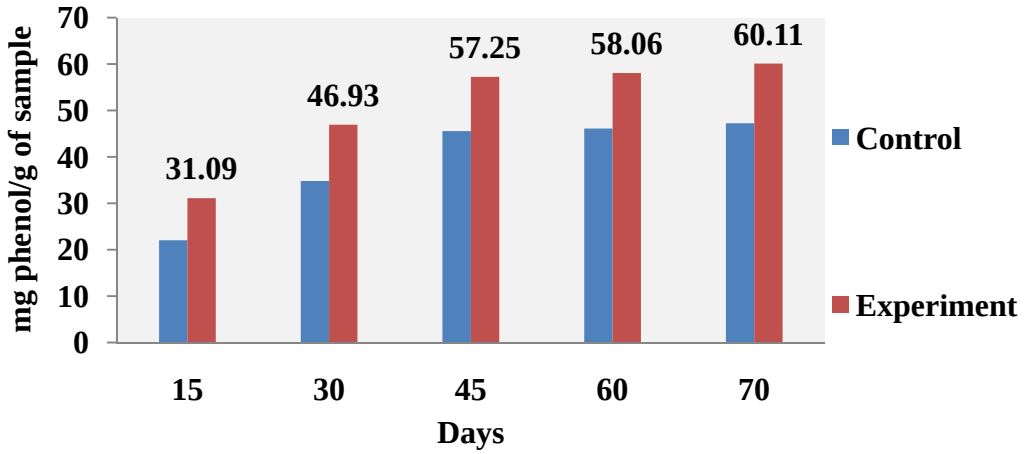

Fig. 5: Estimation of total phenol

C) Estimation of Amylase & Catalase

Amylase was changed at steady rate in the control and experiments (Fig. 6). After 15 days and 30 days, 2 % increment was observed compared to control, then after 70 days; steady and slow production of amylase up to steady level of production. After 45 days production of amylase gives steady results.

Catalase activity study with control and experiments as shown in (Fig. 7). After 15 days and 30 days, 20 % increasing compared to control than after 70 days steady increasing and slow production of catalase. As time increases the productions of catalase compared to experiment increasing. Spraying of the BPF in experiment has role for growth and development for the plants

.

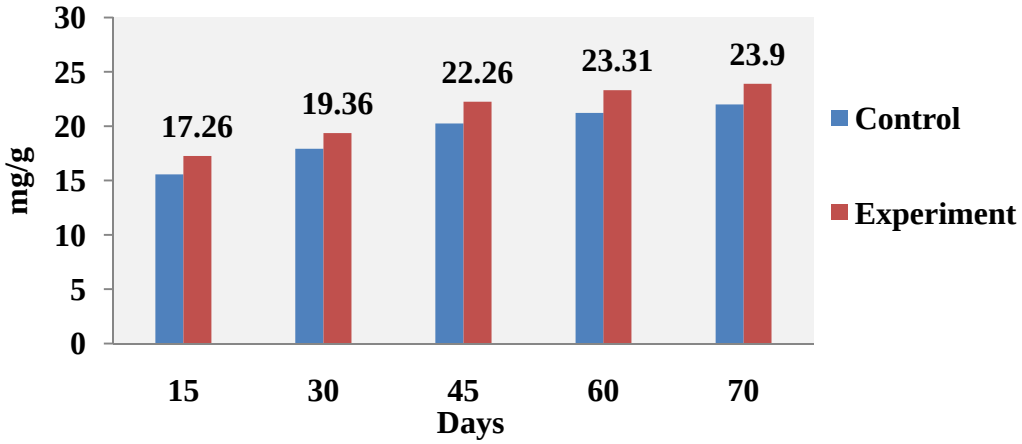

Fig.6: Estimation of Amylase

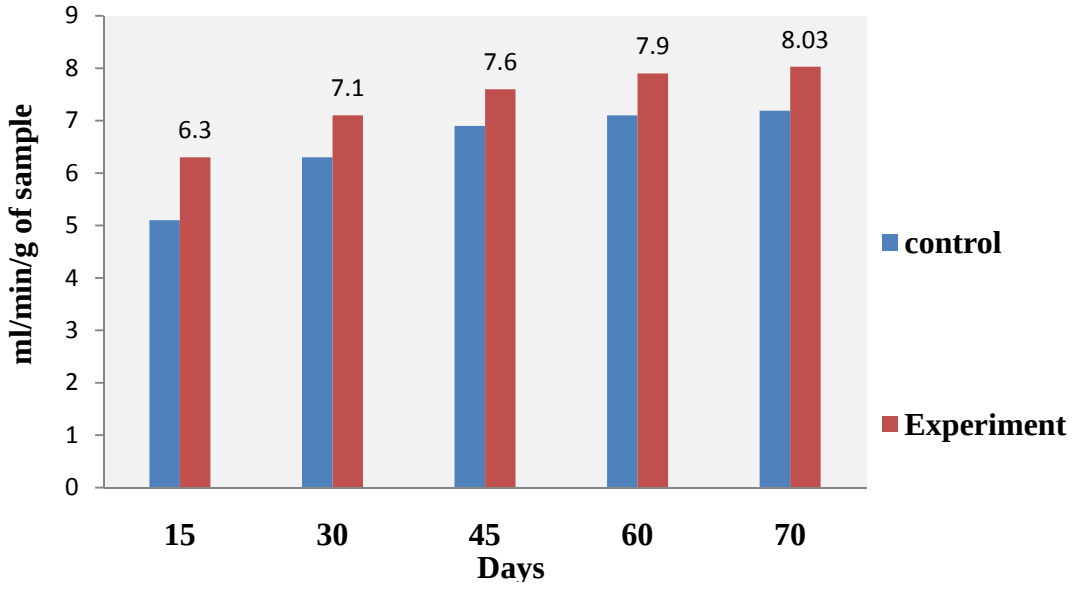

Fig.7: Estimation of Catalase

D) Estimation of peroxides

Peroxides changes were observed in the control and experiments (Fig. 8). After 15 days, 20 % increase in OD compared to control, then after 70 days steady increase and slow production of peroxides. As times are increasing, the productions of peroxides in experiment compared to control increasing.

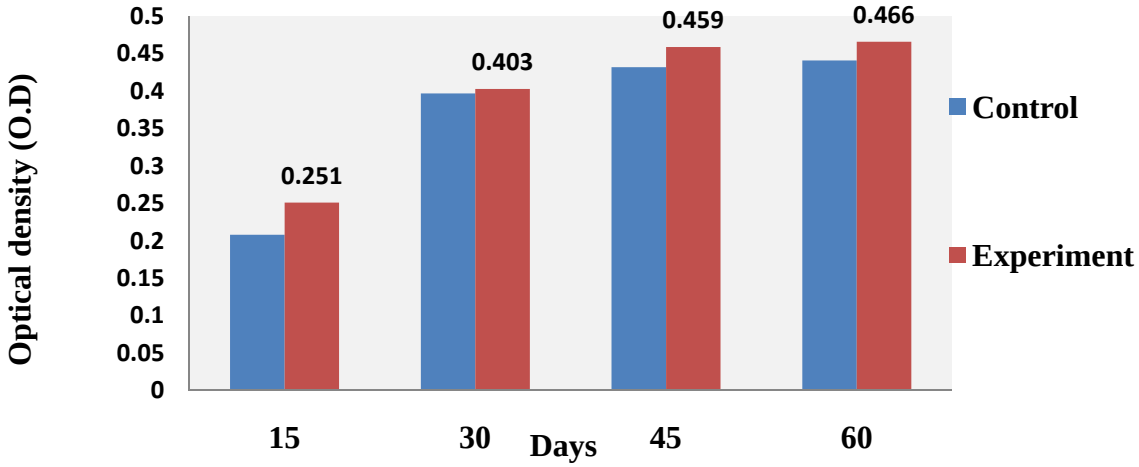

Fig.8: Estimation of peroxides

E) Leaf disc Method

Results revealed that all the treatments with biopesticides in the present study significantly reduced the population of mealy bug over untreated check. After first spraying, among the biopesticides, mealy bug population after 3 hr states to 60% of mortality rate. In three leaves corresponding to time, is increasing the death of mealy bug and few remains slowly active. 5 hr after the entire mealy bug are dead. 5 hr after entire population was observation no viable mealy bug. Five

concentrations (0%, 1%, 3%, 5% and 10%) of this formulation were prepared by serial dilution of the crude formulation with distilled water out of this 5% and 10% are more effective. It has shown that 5% gives sufficient effect on it. (Fig. 9).

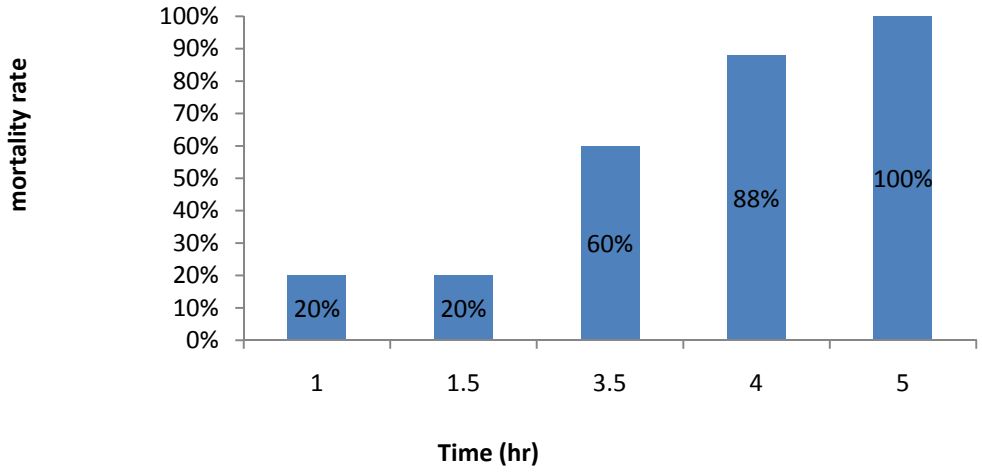

Fig.9 : Mortality Rate of borer against BPF on brinjal (*Solanum melongena* L.) leaf

IV. CONCLUSION

The technique may be validated against other crops, as it is economical, socially acceptable, leaves no toxic residues in the environment, uses easily accessible inputs and therefore can strengthen the national IPM programmes. The field dosage 5% was decided according to laboratory studies for brinjal crop. It can be increased or decreased according to the target pest and field crop studied. The BPF may not give rise to phytotoxicity, because it has proved to be a nutrient supplier for plant growth with enzyme activity studies. We did not test the BPF beyond 5% as limited time, but it may not be harmful for use beyond that value. Research of such new arena can be further moved on with environmental effects and physiological changes in plant to get better outcomes, but till now this BPF for Brinjal to control Mealy bug was done successfully.

V. REFERENCES

[1] A new molecule for the management of thrips of chillies.: In: *International vegetable conference*, Dhandapani, N., Dhivahar, P. and Murugan, M., Thiacloprid –Bangalore, India. 259 ; Nov.11-14; 2002.

[2] Monitoring of pesticide residues in farmgate vegetable samples in West Bengal.; Kole, R. K., Banerjee, H. and Bhattacharyya, A.; *Pesticide Research Journal*,vol.14; pp 77–82;2002,

[3] Magnitude and frequency of pesticide residues in farmgate samples of cauliflower in Punjab, India; Mandal, K. and Singh, B., *Bulletin of Environmental Contamination and Toxicology;* vol.85; pp 423–426; 2010.

[4] Monitoring of pesticides from farmgate samples of vegetables in Haryana; Madan, V. K., Kumari, B., Singh, R. V., Kumar, R. and Kathpal, T. S., *Pesticide Research Journal;* vol.8; pp 56–60; 1996.

[5] Insecticide residues in farmgate vegetable samples in Punjab*;* Chahal, K. K., Singh, B., Kang, B. K., Battu, R. S. and Joia, B. S., *Pesticide Research Journal*; vol.9; pp 216-226;1997.

[6] Effectiveness of thiamethoxam 25WG against the insect pests of brinjal under field conditions.; Patnaik, H. P., Mohapatra, L. N. and Maity, B. K.; *Journal of Plant Protection and Environment*, vol. 1; pp 39-46; 2004.

[7] Control of plant diseases by natural products: allicin from garlic as a case study.; Slusarenko, A. J., Patel, A. and Portz, D; *European Journal of Plant Pathology*, vol.121;313-322; 2008.

[8] *In vitro* assay of some plant extracts against *Fusarium oxysporum* f. sp. *lycopersici* causal agent of tomato wilt.; Ogechi, A. N. and Marley, P. S.; *Journal of plant Protection Research;* vol. 46;pp 3-10; 2006.

[9] Isolation, characterization and insect growth inhibitory activity of major turmeric constituents and their derivatives against *Schistocerca gregaria* (Forsk) and *Dysdercus koenigii* (Walk).; Chowdhury, H., Walia, S. and Vinod, S. S., *Pest Management Science;* vol. 56;pp 1086-1092; 2000.

[10] Insecticidal and fungicidal potential of *Allium* substances as biofumigants; Jacques, A., Ingrid, A., Sabine, D.-A., Michaël, R., Frédéric, M. and Magali, P.; *Agroindustria* vol.3; pp 5-8; 2004.

[11] Potential of fipronil and *Bacillus thuringiensis* to control *Plutella xylostella* on cabbage crop; Arora, S. and Gopal, M., *International Pest Control*; vol.47; pp 251-256; 2005.

[12] A case study: major insect pest associated with different vegetable crops in cold arid region, Ladakh of Jammu and Kashmir; Pandey, A. K., Namgyal, D., Mehdi, M., Mir, M. S. and Shikh, B. A.; *Journal of Entomological Res*earch,vol.30;99 169-174; 2006.

[13] Coordinate gene activity in response to agents that induce systemic acquired resistance; Ward ER, Uknes SJ, Williams SC, Dincher SS, Wiederhold DL, Alexander DC, Ahl-Goy P, M´etraux JP and Ryals JA ; *The Plant Cell* vol. *3*; pp 1085–1094; 1991.

[14] Acquired resistance in *Arabidopsis*; Uknes S,Mauch-Mani B,Moyer M, Potter S,Williams S, Dincher S, Chandler D, Slusarenko A, Ward E and Ryals J; *The Plant Cell* ; vol.4; pp 645–656; 1992.

[15] A mutation in *Arabidopsis* that leads to constitutive expression of systemic acquired resistance; Bowling SA, Guo A, Cao H, Gordon AS, Klessig DF and DongX; *The Plant Cell* vol. 6; pp 1845–1857; 1994.

[16] Plant defensins: novel antimicrobial peptides as components of the host defense system; Broekaert WF, Terras FRG, Cammue BPA and Osborn RW; *Plant Physiology* ; vol.108;1353–1358; 1995.

[17] Variation of soluble sugar content and specific leaf weight during the genetic improvement of soybean cultivars.; Wang XH, Xu KZ, Li DY, Zhang ZA, Wu ZH, Chen ZY, Zhang XR; *Soybean Science*; vol. 26; pp 879-884; 2007.

[18] Phenolic compounds andantioxidant properties in the snow alga *Chlamydomonas ni*V*alis* after exposure to UV light.; Duval, B.; Shetty, K.; Thomas, W. H.; *Journal of Applied Phycology;* vol.11; pp. 559-566; 1999.

[19] A note on the effect of rust infection on peroxidase isozymes in flax.; Andreev, l. An d m. Shaw; *Canadian Journal of Botany* ; vol. 43; pp 1479-1483; 1965.