ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Strategic formulation of bio-pesticide using phyto-chemical ingredients for improvised *Abelmoschus esculentus* field efficacy

Govindasamy Vinodhini[1], Ganesan Suganya[1], Selvaraj Selvanaveen[1], Madhava Anil Kumar[1], Jayaraj Iyyappan[2], Muthulingam Seenuvasan[3,*]
[1]Department of Biotechnology, Madha Engineering College, Chennai.
[2]Department of Biotechnology, Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Chennai.
[3]Department of Petrochemical Engineering, SVS College of Engineering, Coimbatore.
***Corresponding Author: E-Mail: msvasan.chem@gmail.com; Mobile: +91 99620 82276**

ABSTRACT

A biopesticidal formulation was prepared using phytochemical extracts of *Wattakakavolubilis, Justiciaadathoda* and *Strychnosnux-vomica*. The phytochemical extracts were prepared by aqueous two phase extraction method and the formulation was optimized using mixture design matrix (MDM), under design of experiments. The ingredients were mixed accordingly in 10 different sets as per the combination given by MSM in earthen pots and buried under ground for fermentation. The fermented solution was collected after 15 days and the filtered extracts were tested for the presence of alkaloids. Simultaneously, the extracts were sprayed on ten *Abelmoschus esculentus* plants and the growth parameters along with the chlorophyll concentration were measured to evaluate the efficacy of the biopesticidal formulation.

KEY WORDS: *Abelmoschus esculentus,* Biopesticide, *Wattakakavolubilis, Justiciaadathoda, Strychnosnux-vomica.*

1. INTRODUCTION

Biopesticides are pesticides which are biologically derived from living organisms and they have gained more importance as they pose less risk to humans and environment (Gasic, 2013; Saxena and Pandey, 2001). They are of broad specific in supressing the pest populations. Biopesticides often are effective in small quantities and are biodegradable (Copping, 2000; Nathan, 2005). The prolonged success of few biopesticidal formulations, there is much more expectations and criteria for new biopesticides (Glare, 2012). The efficacy of a biopesticide is determined by the following criterion; pest control, efficiency of use, consistency of response, host target and non-target susceptibility. The researchers faces the following constraints during the development of a biopesticide are biological, environmental, technological and commercial challenges (Auld and Morin, 1995). The cultivation of *Abelmoschus esculentus* (okra) plants faces many problems mainly by different pests and thus the situation demands the necessity to develop a biopesticide to eradicate this potential threat.

The statistical techniques under design of experiments (DOE) are the most important tool which correlates and establishes a relationship between the independent operational factors and the dependent responses from the experiments (Kumar, 2015; Seenuvasan, 2014). The DOE has different methods for understanding the relationships and modelling the output responses when the independent parameters influence the response to a greater extent (Karthikeyan, 2014; Vidhyadevi, 2014; Kumar, 2012). The biologically derived materials are more advantageous than other conventional available materials with respect to bioremediation (Selvanaveen, 2015; Balaji, 2014; Kumar, 2014), production of bioproducts and bioprocess engineering (Vinodhini, 2015; Seenuvasan, 2014; 2013).

The products from plants as whole or selected potions will be always a good reliable source of commercial importance (Seenuvasan, 2014). *Justiciaadathoda* and *Strychnosnux-vomica* plants has insecticidal activities (Mamun and Ahmed, 2011; Arivoli and Tennyson, 2012). *Wattakakavolubilis* plant is known for its antibacterial and antifungal activity (Yogita, 2013). The present investigation is to screen, extract the phytochemicals from plants bearing insecticidal and antimicrobial activities and to optimize the biopesticide formulation using phytochemicals using MDM and emulate their efficiency in eradicating the pests and disease found in *Abelmoschus esculentus* plants.

2. MATERIALS AND METHODS

2.1. Chemicals: The chemicals used in this study are of highest purity and of analytical grade and were purchased from a standard commercial chemical supplier. The reagents were prepared using sterilized double distilled water. All the chemicals, media and the glass wares used in the investigation were steam sterilized prior use.

2.2. Collection and extraction of phytochemicals: Fresh leaves of *Wattakakavolubilis, Justiciaadathoda* and *Strychnosnux-vomica* were washed with tap water and followed by thorough washing in double distilled water and were dried at 70°C overnight. The dried material was subject to aqueous two phase extraction and the resulting phytochemical extract was used as the ingredients in the formulating the biopesticide.

2.3. Formulation of biopesticide: The MDM provided by Minitab software (Ver. 14.0, U.S. Federal Government Commonwealth of Pennsylvania, USA), was used to optimize the formulation of the biopesticide. The extracts of *Wattakakavolubilis, Justiciaadathoda* and *Strychnosnux-vomica* were used as the ingredients, in varying proportions ranging from 0 to 1.0, as shown in Table 1. The ingredients were added according to the ratio given by the MDM in an earthen pot and buried under ground for fermentation for 15 days. The fermented solution was filtered using a muslin cloth and the concentrated solution was diluted with water, then sprayed on to the leaves of *Abelmoschusesculentus* on daily basis for 10 days and results were recorded. The plant growth, total number of plant and damaged leaves, presence or absence of pest and total chlorophyll concentration were recorded. These are the data were also considered as parameters by the evaluation of before and after sprayed effect on *Abelmoschus esculentus* plants.

2.4. Modeling of biopesticide production: The efficiency of the formulated biopesticide was tested by means of the total chlorophyll concentration of *Abelmoschus esculentus* plant leaves. The regression analysis is used to establish the relationship between performance and biopesticide formulation given by the MDM and the observed responses were fitted to the polynomial model

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{23} X_2 X_3 + \beta_{13} X_1 X_3 \quad (1)$$

Where Y is the dependent variable (mg total chlorophyll/g tissue); X_1, X_2 and X_3 are the phytochemical extracts of *Wattakakavolubilis*, *Justiciaadathoda* and*Strychnosnux-vomica* used in the formulation;β_1, β_2 and β_3 are the linear coefficients and $\beta_{12}, \beta_{13}$and $\beta_{23}$are the interaction coefficients respectively. The adequacy of the regression model was evaluated using regression coefficients, analysis of variance (ANOVA), probability (the P-value), Fisher variance ratio (the F-value) and Student's t-test.

2.5. Determination of total chlorophyll concentration: The biopesticide exposed plant leaf of *Abelmoschus esculentus*were analyzed for the chlorophyll content (Hiscox and Israelstam, 1979). 4 mL of dimethysulphoxide (DMSO) was added to one-third of the leaf tissue and incubated at 65˚C for 2.5 h with occasional vortexing. The chlorophyll concentration was measured spectrophotometrically at 645 and 663 nm using DMSO as blank solution and the chlorophyll concentration was determined using the following equations (Arnon, 1949).

$$\text{mg chlorophyll a/g tissue} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W} \quad (2)$$

$$\text{mg chlorophyll b/g tissue} = 22.9(A_{645}) - 4.68(A_{663}) \times \frac{V}{1000 \times W} \quad (3)$$

$$\text{mg total chlorophyll/g tissue} = 20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W} \quad (4)$$

Where A is the absorbance at specific wavelengths; V is the final volume of chlorophyll extract in DMSO (mL) and W is the fresh weight of leaf tissue extracted (g).

3. RESULTS AND DISCUSSION

3.1. Optimized biopesticide formulation: The efficacy of the bio pesticide formulation was evaluated in terms of various plant growth parameters and the quantifiable assessed parameter was the total chlorophyll concentration (mg total chlorophyll/g tissue) of the leaves of *Abelmoschus esculentus* (Table 1). The chlorophyll concentration was 0.90 (mg total chlorophyll/g tissue) when the ratio of the ingredients were 0.16, 0.16 and 0.66 (run #10). The developed polynomial model for the observed response was used to determine the optimal formulation for improved field efficacy and validation of the model is described as follows:

$$\text{mg total chlorophyll/g tissue} = 0.01X_1 + 0.26X_2 + 0.58X_3 + 1.64X_1X_2 + 1.62X_2X_3 + 1.55X_1X_3$$

Table.1.MDM for the biopesticide formulation and response as mg total chlorophyll/g tissue

Run	X_1	X_2	X_3	mg total chlorophyll/g tissue
1	1.00	0.00	0.00	0.02
2	0.00	1.00	0.00	0.27
3	0.00	0.00	1.00	0.56
4	0.50	0.50	0.00	0.55
5	0.50	0.00	0.50	0.66
6	0.00	0.50	0.50	0.80
7	0.33	0.33	0.33	0.81
8	0.66	0.16	0.16	0.53
9	0.16	0.66	0.16	0.68
10	0.16	0.16	0.66	0.90

3.2. Statistical significance of the biopesticide formulation: The greater F-value explains the variation in the data and also describes whether the estimated component effects are real or significant. The associated P-value was 0.001 (i.e. α=0.05 or 95% confidence) indicating that the developed regression models is statistically significant (Table 2). The regression (R^2), adjusted regression and predicted regression coefficients were 98.89, 97.51 and 80.37% respectively, which indicates that the model have a good fit with the target and highly significant (Zhou, 2007).

Table.2.Analysis of variance (ANOVA) for mg total chlorophyll/g tissue for the linear and interactions regression model

Source	Degrees of freedom	Sum of square	Sum of adjusted squares	Adjusted average squares	F-ratio	P-value
Regression	5	0.626540	0.626540	0.125308	71.40	0.001
Linear	2	0.241233	0.178746	0.089373	50.93	0.001
Quadratic	3	0.385307	0.385307	0.128436	73.18	0.001
Residual error	4	0.007020	0.007020	0.001755		
Total	9	0.633560				
R^2= 98.89%		**$R^2_{predicted}$= 80.37%**		**$R^2_{adjusted}$ = 97.51%**		

3.3. Graphical representation of optimized biopesticide formulation: The two and three dimensional (2D and 3D) surface plots estimate the optimal formulation of the biopesticde. The 2D contour plot (Fig.1) indicates that there is a considerable interaction between the ingredients on (mg total chlorophyll/g tissue). The 3D mixture surface plot (Fig.2) represents interaction of the ingredients and their subsequent effect on the response (Liu, 2009). Furthermore, tailoring of the process parameters has to be done in order evaluate the efficacy of the formulated pesticide for other commercially important plant crops (Kumar, 2015). The main effect plots for mg total chlorophyll/g tissue is the graphical illustrations to select the optimal level of the ingredients for formulation of the biopesticide as shown in Fig.3.

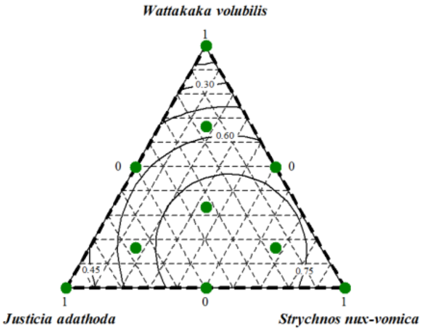

Fig.1. 2D-mixture contour plots for mg total chlorophyll/g tissue

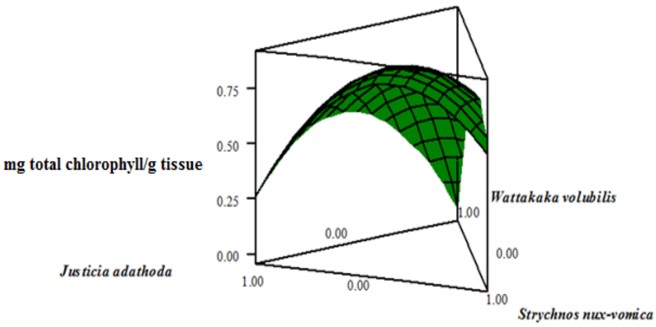

Fig.2. 3D-mixture response surface plot for mg total chlorophyll/g tissue

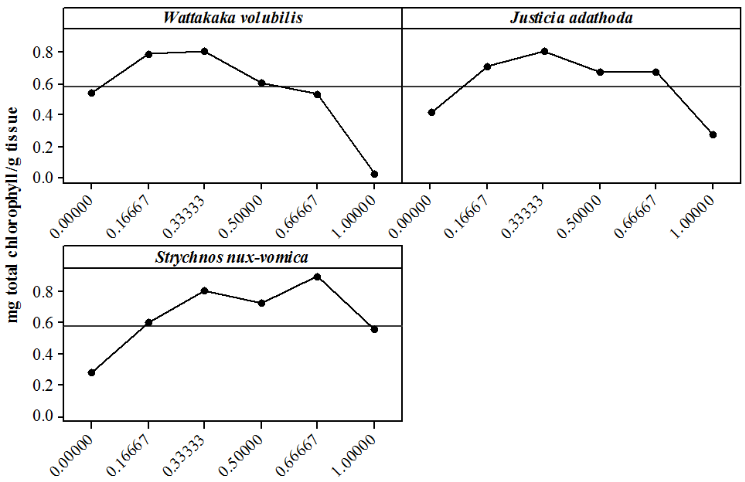

Fig.3.Main effect plots of the individual ingredients for mg total chlorophyll/g tissue

4. CONCLUSION

The MDM was successfully used to formulate the biopesticide containing phytochemical extracts as ingredients. The efficacy of the formulated biopesticide was evaluated in terms of the higher chlorophyll content in the leaves of *Abelmoschus esculentus* plants. The pests were also eradicated and the plants were found to be healthier.

REFERENCES

Arivoli S, Tennyson S, Larvicidal efficacy of *Strychnosnuxvomica* Linn. (Loganiaceae) leaf extracts against the filarial vector *Culexquinquefasciatus* Say (Diptera: Culicidae), World J. Zool., 7, 2012, 6–11.

Arnon D.I, Copper enzymes in isolated chloroplasts, Polyphenoloxidase in *Beta vulgaris,* Plant Physiol., 24, 1949, 1–15.

Auld B.A, Morin L, Constraints in the development of bio herbicides, Weed Technol., 9, 1995, 638–652.

Balaji N, Kumar K.S, Seenuvasan M, Kumar M.A, Efficacy of laccase to mineralize the aromatic amines released during the oxido-reductive cleavage of sulphonated reactive azo dye, Int. J. Appl. Eng. Res., 9, 2014, 9116–9118.

Copping L.G, Menn J.J, Biopesticides: a review of their action, applications and efficacy, Pest Manag. Sci., 56, 2000, 651–676.

Gasic S, Tanovic B, Biopesticide formulations, possibility of application and future trends, Pestic. Phytomed., 28, 2013, 97–102.

Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Köhl J, Marrone P, Morin L, Stewart A, Have biopesticides come of age?, Trends Biotechnol., 30, 2012, 250–258.

Hiscox J.D, Israelstam G.F, A method for the extraction of chlorophyll from leaf tissue without maceration, Can. J. Bot., 57, 1979, 1332–1334.

Karthikeyan S, Kumar M.A, Maharaja P, Rao B.P, Sekaran G, Process optimization for the treatment of pharmaceutical wastewater catalyzed by poly sulpha sponge, J. Taiwan Inst. Chem. E., 45, 2014, 1739–1747.

Kumar M.A, Kumar V.V, Ponnusamy R, Daniel F.P, Seenuvasan M, Anuradha C.D, Sivanesan S, Concomitant mineralization and detoxification of acid red 88 by an indigenous acclimated mixed culture, Environ. Prog. Sustain. Energy, 34, 2015, 1455–1466.

Kumar M.A, Kumar V.V, Premkumar M.P, Baskaralingam P, Thiruvengadaravi K.V, Anuradha C.D, Sivanesan S, Chemometric formulation of bacterial consortium-AVS for improved decolorization of resonance-stabilized and hetero-polyaromatic dyes, Bioresour.Technol., 123, 2012, 344–351.

Kumar M.A, Vijayalakshmi A, Lincy E.A.R, Palin M.D, Seenuvasan M, Kumar V.V, Sivanesan S, Biotransformation of reactive black HEBL into 3-nitroso-3-azabicyclo (3.2.2) nonane by an acclimated mixed culture, Int. J. ChemTech Res., 6, 2014, 4172–4179.

Liu L, Lin Z, Zheng T, Lin L, Zheng C, Lin Z, Wang S, Wang Z, Fermentation optimization and characterization of the laccase from *Pleurotusostreatus* strain 10969, Enzyme Microb.Tech., 44, 2009, 426–433.

Mamun M.S.A, Ahmed M, Prospect of indigenous plant extracts in tea pest management, Int. J. Agril. Res. Innov. & Tech., 1, 2011, 16–23.

Nathan S.S, Kalaivani K, Murugan K, Chung P.G, The toxicity and physiological effect of neem limonoids on *Cnaphalocrocismedinalis* (Guenee) the rice leaf folder, Pestic. Biochem. Phys., 81, 2005, 113–122.

Saxena S, Pandey A.K, Microbial metabolites as eco-friendly agrochemicals for the next millennium, Appl. Microbiol. Biot., 55, 2001, 395–403.

Seenuvasan M, Kumar K.S, Abinandan S, Anugraha C, Umamageshwari K, Kumar M.A, Balaji N, Statistical analysis on stress induced lipid accumulation along with the major cell components of *Chlorella* sp., Int. J. ChemTech Res., 6, 2014, 4186–4193.

Seenuvasan M, Kumar K.S, Kumar M.A, Iyyappan J, Suganthi J.R.G, Response surface estimation and canonical quantification for the pectin degrading Fe_3O_4-SiO_2 nano biocatalyst fabrication, Int. J. ChemTech Res., 6, 2014, 3618–3627.

Seenuvasan M, Kumar K.S, Malar G.C.G, Preethi S, Kumar M.A, Balaji N, Characterization, analysis, and application of fabricated Fe_3O_4-chitosan-pectinase nanobiocatalyst, Appl. Biochem. Biotech., 172, 2014, 2706–2719.

Seenuvasan M, Malar G.C.G, Preethi S, Balaji N, Iyyappan J, Kumar M.A, Kumar K.S, Fabrication, characterization and application of pectin degrading Fe_3O_4-SiO_2 nanobiocatalyst, Mater. Sci. Eng. C, 33, 2013, 2273–2279.

Seenuvasan M, Malar G.C.G, Preethi S, Balaji N, Iyyappan J, Kumar M.A, Kumar K.S, Immobilization of pectinase on to co-precipitated magnetic nanoparticles for its enhanced stability and activity, Res. J. BioTech., 8, 2013, 24–30.

Seenuvasan M, Selvi P.K, Kumar M.A, Iyyappan J, Kumar K.S, Standardization of non-edible *Pongamiapinnata* oil methyl ester conversion using hydroxyl content and GC-MS analysis, J. Taiwan Inst. Chem. E., 45, 2014, 1485–1489.

Selvanaveen S, Yuvarani K, Padmini B, Kumar M.A, Abraham S.S.O, Vinodhini G, Seenuvasan M, An indigenous nosocomial opportunistic pathogen in the degradation of tannery effluent, Res. J. Pharm. and Tech., 8, 2015, 316–321.

Vidhyadevi T, Murugesan A, Kalaivani S.S, Kumar M.A, Ravi K.V.T, Ravikumar L, Anuradha C.D, Sivanesan S, Optimization of the process parameters for the removal of reactive yellow dye by the low cost *Setaria verticillata* carbon using response surface methodology: Thermodynamic, kinetic and equilibrium studies, Environ.Prog. Sustain. Energy, 33, 2014, 855–865.

Vinodhini G, Kumar M.A, Balamanikandan S, Seenuvasan M, Assessment of antimicrobial property of a secondary metabolite produced by an enriched bacterial culture isolated from soil, Res. J. Pharm. and Tech., 8, 2015, 51–53.

Yogita S, Prachi A, Arun J, Maya B, Antibacterial and antifungal activity of roots of *Wattakaka volubilis,* Global J.Pharmacol., 7, 2013, 283–287.

Zhou J, Liu X, Huang K, Dong M, Jiang H, Application of the mixture design to design the formulation of pure cultures in Tibetan kefir, Agr. Sci. China, 6, 2007, 1383–1389.