UGC MAJOR RESEARCH PROJECT
ON

FORMULATING BOTANICAL FUMIGANT TABLETS AS AN ALTERNATIVE TO SYNTHETIC FUMIGANTS FOR SUSTAINABLE MANAGEMENT OF CERTAIN STORED GRAIN INSECT PESTS

FINAL REPORT
(From 01-04-2013 TO 31-03-2017)

(UGC NO. F. 42-746/2013(SR) DATED 25-03-2013)

Submitted by

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Summary of the work done on the UGC - Major Research Project

1	**UGC Reference No. & Date**	**F.No. 42-746/2013(SR) dated 25-03-2013**
2	**Name of the Principal Investigator**	**Dr. C.KATHIRVELU** Associate Professor
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4	**Department and university where the project has undertaken**	Department of Entomology, Faculty of Agriculture, Annamalai University.
5	**Title of the project**	**Formulating botanical fumigant tablets as an alternative to synthetic fumigants for sustainable management of certain stored grain insect pests**
6	**Date of Implementation**	**01.04.2013**
7	**Tenure of the Project**	**4 years from 01.04.2013 to 31.03.2017**
8	**Total grant allocated**	**Rs. 10,87,000**
9	**Total grant received**	**Rs. 10,01,000**
10	**Final expenditure**	**Rs. 8,11,054**

EXECUTIVE SUMMARY

Food grain losses due to insect infestation during storage are a serious problem, particularly in the developing countries. Losses caused by insects include not only the direct consumption of kernels, but also accumulation of exuviae, webbing and cadavers. High levels of the insect detritus may result in grain that is unfit for human consumption and loss of the food commodities, both, in terms of quality and quantity. It is estimated that more than 20,000 species of field and storage pests destroy approximately one-third of the world's food production, valued annually at more than $100 billion among which the highest losses (43%) occurring in the developing world (Dubey *et al.,* 2008). The quantitative and qualitative damage to stored grains and grain product from the insect pests may amount to 20-30% in the tropical zone and 5-10% in the temperate zone (Rajendran and Sriranjini, 2008). Food grain production in India has reached 250 million tonnes in the year 2010-2011, in which nearly 20-25% food grains are damaged by stored grain insect pests (Rajashekar *et al.,* 2010).

Since the 1950's, synthetic insecticides have been used extensively in grain facilities to control stored product insect pests. Fumigants such as methyl bromide, phosphine, cyanogens, ethyl formate, or sulfuryl fluoride rapidly kill all life stages of stored product insects in a

commodity or in a storage structure. Fumigation is still one of the most effective methods for the prevention of stored product losses from insect pests but they develop resistance against chemical fumigants slowly. Resistance to phosphine is so high in Australia and India, it may cause control failures (Donahaye, 2000).

Although chemical insecticides are effective, their repeated use has led to residual toxicity, environmental pollution and an adverse effect on food besides side effect on humans (Kumar *et al.,* 2007). Their uninterrupted and indiscriminate use not only has led to the development of resistant strains but also accumulation of toxic residues on food grains used for human consumption that has led to the health hazards. In view of all these problems, several insecticides have either been banned or restricted in their use. The increasing serious problems of resistance and residue to pesticides and contamination of the biosphere associated with large-scale use of broad spectrum synthetic pesticides have led to the need for effective biodegradable pesticides with greater selectivity. This awareness has created a worldwide interest in the development of alternative strategies, including the discovery of newer insecticides. However, newer insecticides will have to meet entirely different standards. They must be pest specific, non phytotoxic, nontoxic to mammals, ecofriendly, less prone to pesticide resistance, relatively less expensive, and locally available. This has led to re-examination of the century-old practices of protecting stored products using plant-derivatives, which have been known to resist insect attack (Talukder, 2006).

Bio-pesticides obtained from plant sources are safer, devoid of residue problems and almost negligible application risks as compared to synthetic chemical pesticides. It has been demonstrated by many workers that numerous plant species showed insecticidal, antifeedant, repellent, antigrowth and oviposition inhibiting properties. In recent years interest has been shown in plant products and their components for fumigant action may have advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability. Although the plant products do not possess quick knock down effect unlike synthetic insecticides, they constitute no health hazards, surface persistence and lasts for long time with no adverse effects on seed germiability, cooking quality and milling. Further, they are less expensive and easily available. Most of the food grains produced are being stored at farmers level under the most primitive conditions of storage and hence they are easily accessible to the attack by a variety of insect pests and other agents in storage.

As on now, use of powder formulation of plants is practiced for the management of stored grain pests though the direct application of grain protecting plant powders on food grains are harmful as they may stick to seed coat of grain, create cleanliness problem, leave high pungency, bitterness, oiliness and may toxic for human consumption. Although the tablet formulation is usually considered safer than direct mixing of active materials with food grains and no attempt is made to formulate a tablet with botanicals and their fumigant action against stored grain pests is also less studied. To fulfill this lacuna in the arena of stored grain insect pest management, a new formulation of bio fumigant tablets as an alternative to the existing synthetic fumigants is therefore required. Hence, a maiden attempt of research work was carried out.

Objectives

1. To screen certain identified plant species suitable for fumigant activity on insects and selection of important promising plant species
2. Synthesis of a few biofumigants as tablet formulation
3. Evaluation of selected biofumigant tablets against key insect pests of stored grains in

laboratory

4. To find out their mechanism of action against key insect pests
5. To assess safety of the biofumigants on selected grains and non target organisms
6. To determine shelf life of the selected biofumigant tablet formulation
7. To assess efficacy of the selected biofumigant tablet formulation against stored grain insects in godown conditions.

Methodology

Mass culturing of test insects

The test insects namely pulse beetle, *Callosobruchus chinensis* L., Rice weevil, *Sitophilus oryzae* L., Lesser grain borer, *Rhyzopertha dominica* F., Red flour beetle, *Tribolium castaneum* Herbst adults and *Corcyra cephalonica* larvae were obtained and mass cultured.

Collection and preparation of botanicals

Twenty five plant species were collected from in and around Annamalainagar area and also from other places and shade dried for 45 days. Shade drying is to prevent the loss of active principle from the plants. The dried plant materials were powdered using electric blender and sieved through strainer and the fine powder was used for extraction using Soxhelt extraction apparatus. The powders were extracted with the solvent acetone. These extracts were evaluated against key pests of stored produce for their fumigant, repellent and contact action.

Soxhlet extraction

The ordinary method of extraction was not efficient to yield good amount of active principle of the plant material. To extract more active principle from all the plant materials, Soxhlet extraction was used.

Effect of selected plant extracts against test insects in laboratory

i) Fumigant action

The fumigant activity of the plant extracts were tested according to a protocol suggested by Singh *et al.* (1989).

ii) Repellent action

To study the repellent activity of plant extracts, filter paper strips (6x4 cm) was dipped in solvent extracts and air-dried for few minutes. Then the filter paper was placed inside the plastic container and attached into the arm of olfactometer. Newly emerged 10 adult target insects were introduced into the olfactometer set up and the repellancy was observed daily for four days.

iii) Contact toxicity

In the impregnated filter paper assays, filter paper discs (What man No.2 Dia 8 cm) were impregnated with 1 ml of extract. The discs were air dried and placed on the bottoms of pertidishes or plastic containers (Dia 8 cm). Ten number of freshly emerged adults of target insects were placed in the treated paper with a source of food. In case of *C. cephalonica*, the second instar larvae were tested. The container was closed and maintained under constant condition.

Selection of promising plant species for formulation of biofumigant tablets

Based on the results of above laboratory studies, the promising plant species were selected for synthesis and evaluation of biofumigant tablets.

Collection and preparation of carrier materials

The carrier materials *viz.* chalk powder (CP), kitchen ash (KA) and fly ash (FA) were also obtained for preparation of tablet formulation. These carrier materials were further sized through fine dust (35 micron) and made as fine powder so as to enable to mix properly with the formulation.

Synthesizing bio fumigant tablets

The method of preparing tablet formulation was developed in the Department of Entomology. The shade dried plant leaves were thoroughly ground to a fine powder and the raw materials were then homogenized separately by using electric blender. The powdered plant materials were used to prepare tablet formulation @ 55:40:5 ratio of plant materials, carriers and rice gruel. Distilled water was added to prepare a consistent thick paste to formulate the tablet (35mm×12mm) with an average weight of 7.63g each manually with a wooden plank prepared separately for making appropriate sized tablets. A wooden roller was used for spreading the dough and then tablets were shade dried at room temperature. Blank tablet was also prepared from carrier agent and rice gruel and used as untreated check. The dried biofumigant tablets were packed using airtight polythene covers. Various combinations of plant materials and carriers were used for preparation of tablets.

Efficacy of biofumigant tablets against target insect pests in laboratory condition

Fumigant toxicity

The bio-apparatus for the direct fumigation toxicity test consisted of a plastic cup (height 8cm) inserted into a plastic container (height 12cm, diameter 8cm). The biofumigant tablets were placed on the bottom of this cup. The top of the plastic cup was covered with a muslin cloth so that fumes emitting from tablets would reach the insects. After the plastic cup was inserted into the container, adult insects were introduced into it and the top was covered with a polythene film. Observations on the adult mortality were recorded at an interval of 12 hours for six days after the introduction of insects.

Effect of biofumigant tablets on respiration (O_2 consumption and CO_2 emission) of target insects

The effect of biofumigants on the respiration of target insects was monitored by using Lutron AQ- 9901 SD gas analyser. The gas analyser comprises a separate probe for measuring CO_2, O_2, temperature and relative humidity. The probes were inserted in to a closed container placed with biofumigant tablet, nutritional source and target insects. The Co_2 and O_2 level in the air tight condition of each grains and target insects were measured separately prior to experiment. This instrument was used to determine oxygen consumption and carbon dioxide release by an insect under treatment at an interval of 24 hrs. From this experiment, the respiration distruption in insects due to biofumigants was determined.

Evaluation of biofumigant tablets against target insects in storage godown

In this experiment, the target insects and their respective food source was taken in gunny bags and treated with the biofumigants. The mortality percent of the insect pests was recorded and compared with check.

Effect of biofumigants on seed germination and growth

The impact of biofumigant tablet treated seeds/ grains were tested for germination, seedling growth and development after storing six to eight months.

Shelf life of biofumigant tablets

Storage of the biofumigant tablet formulation for various periods and temperatures was studied in respect of their efficacy against the insect pests in grains.

Impact of biofumigant tablets on the rheology of food commodities

The biofumigant treated seeds or grains was used in this experiment, to determine the adverse effects on the rheological properties of the food commodities like quality of flour, on its dough-development characteristics and on the taste and flavour.

Safety of biofumigant tablet formulation to non target organisms

Safety of biofumigant tablet formulation to non target organism, a braconid parasitoid,

Bracon hebetor Say was observed using fumigant toxicity test.

Economics of production of the biofumigant tablets

The cost of production of biofumigant tablets was worked out in comparison with a commercial synthetic fumigant tablet formulation.

Statistical analysis

The data on the effect of the various plant extracts and tablet formulation of plant species against all the test insects were analysed using Completely Randomized Block Design (CRD) as per Goulden (1952). Analysis of variance was worked out and the mean values were compared using least significant difference (LSD). All the percentage data were subjected to arc sine transformation.

Experimental Results

The following are the results of the bioassay tests using extracts of pesticidal plant species against various stored insect pests under laboratory condition.

- In fumigant toxicity test, the plant species namely, *Euclyptus globulus, Mentha piperita, Artemesia vulgaris, Allium sativum* were found promising and *Vitex negundo, Ocimum basilicum, Acorus calamus* and *Brassica juncea* were on the same level in causing mortality of the test insects.
- The repellant activity of the pesticidal plant species against test insects were found maximum in *M. piperita, O. basilicum, A. vulgaris, V. negundo, E. globulus* and *A. indica*.
- The plant species, *A. sativum, Azadirachta indica, V. negundo, B. juncea* and *O. canum* performed better in causing mortality of the target insects in contact toxicity studies.

All the above results of the pesticidal plants against stored product pests in laboratory conditions were thoroughly compared with each other and the results were referred and in comparison with the available literatures. Finally, there were five plant species selected for making tablet formulation for further evaluation against target insect pests in both laboratory and godown condition using carrier materials like chalk powder, flyash and kitchen ash.

- The plant species namely, *M. piperita, V. negundo, O. basilicum, A. vulgaris* and *E. globulus* were chosen and the biofumigant tablets were formulated using them.
- Various combinations of the above plant species and carrier materials were used for formulating biofumigant tablets and they were evaluated against key pests of stored produce in laboratory and godown conditions.
- Among the combinations of biofumigant tablets, *V. negundo* and *M. piperita* in combination with carrier materials were found promising against *C. chinensis* and *R. dominica* respectively in laboratory condition.
- The plant species namely, *O. basilicum* and *M. piperita* with carrier combinations of biofumigant tablets were found effective in controlling the insects, *S. oryzae* and *C. cephalonica* respectively in laboratory condition using fumigant bio assay method. *A. vulgaris* and *E. globulus* and their carrier combinations were found better against *T. castaneum.*
- In godown condition, the biofumigant tablets formulated with *V. negundo* in combination of various carrier materials proved their supremacy over other plant species against *C. chinensis*. The *S. oryzae* was effectively checked by *M. piperita* combinations.
- The *R. dominica* and *T. castaneum* were controlled effctively by *A. calamus* and *A. vulgaris* combinations respectively in godown condition.
- *O. basilicum* was found better in managing *C. cephalonica* larvae when compared to

other plant species and their combination treatments in godown condition.

- Regarding the loss of weight in grains, it was studied that a minimum damage of grains due to insects was caused by usage of biofumigant tablets against them.
- The shelf life of the biofumigant tablets were found against *C. chinensis* in godown condition. It was observed that the tablets were effective upto 45 DAT after that no significant differences were observed between treatments and control.
- Regarding the germination of seeds, all the combinations of biofumigant tablets tested with grains/ seeds were more or less similar in their effect and did not affect germination of the paddy and pulse seeds considerably.
- The biofumigant tablet formulation did not have any impact on the natural enemies and on the rheological properties of the food commodities like quality of flour, its dough-development, taste and flavour.
- Among the carrier materials used for formulating biofumigant tablets, it is found that chalk powder was better in their consistent and stable for long period of storage and handling.
- Almost all the plant species used in the formulations of biofumigant tablets are available in and around the farmers field or they can grow in their own field, therefore, no / less cost is involved when it is used in pest management of stored grains.

Conclusion

The plant species namely, *M. piperita*, *V. negundo, O. basilicum, A. vulgaris* and *E. globulus* were chosen from twenty five plant species after the results obtained from series of assays conducted in the laboratory and the biofumigant tablets were formulated using them. The formulated tablets were tested in the laboraroty and godown conditions against target pests and identified as promising to the safe storage of grains and effective against various target insect pests like Pulse beetle, *C. chinensis*, Rice weevil, *S. oryzae*, Lesser grain borer, *R. dominica*, Red flour beetle, *T. castaneum* and Rice moth, *C. cephalonica*. The biofumigants do not have toxic effect on human beings and there will be no fear of poisoning to non target organisms and also easy to handle. Regarding the carrier used in the formulation of biofumigant tablets, it was observed that the formulated tablets were found consistent till the end of experiment and the order of preference was as follows: kitchen ash < fly ash < chalk powder. The formulated tablets did not affect the germination of pulses and paddy as well as no changes observed from the flour of the grains and seeds.

Further research on the same line of study may enhance the method of synthesizing the tablets with machines or equipments for large quantity and its effectiveness and usage in the farmer's level. After standardizing the ingredients of tablet formulation with the suitable plant species and their extracts or alkaloids will pave a way for the commercialization and patenting the product with easily available form and low cost.