

**DEVELOPMENT OF PLANT ESSENTIAL OIL BASED
FORMULATION TO CONTROL *CALLOSOBRUCHUS MACULATUS*
IN STORED LEGUME GRAINS**

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INDIAN INSTITUTE OF TECHNOLOGY DELHI
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IN STORED LEGUME GRAINS**

by

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CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY**

Submitted

*In fulfillment of the requirements of the degree of
DOCTOR OF PHILOSOPHY*

to the



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MAY 2009**

*TO MY FAMILY
WITH LOVE*

CERTIFICATE

This is to certify that the thesis entitled "**Development of plant essential oil based formulation to control *Callosobruchus maculatus* in stored legume grains**" being submitted by Ms. Poonam Bakhshi (nee Kaushik) to the Indian Institute of Technology, Delhi for the award of Doctor of Philosophy is a record of bonafide research work carried out by her under the guidance and supervision in conformity with the rules and regulations of Indian Institute of Technology, Delhi.

The research report and results presented in this thesis have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.

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(Prof. Santosh Satya)

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ABSTRACT

*Stored grain insect-pests alone pose a serious problem to humanity by damaging and contaminating about 15-20 % of stored grains. In this context, essential oils have gained a wide recognition for being safer insecticide in comparison to chemical pesticides. Essential oils exhibit insecticidal activity, antifeedancy, and repellency against arthropods, fungi, mites etc. They show excellent fumigation activity due to their high volatility against various insect-pests at immature and adult stages. These multifarious biocidal activities of essential oils render them as a right candidate for developing formulations against stored grain insect-pests. Surprisingly, none of the botanical formulation (including based on essential oils) for controlling storage pests is available in agrochemicals market, thus demanding intensive research in this area. In view of above scenario, the present research therefore envisages developing desired formulations from promising bioactive essential oils and testing their bioefficacy under lab conditions and finally exploring the feasibility of their application under actual field conditions. An important aspect of the study includes targeting the control of most serious insect-pest (i.e. *Callosobruchus maculatus*) in decentralized storage systems, prevalent in urban and rural areas.*

*Based on the literature survey, 31 plant essential oils belonging to 14 different families were selected for bioassay studies and were subjected to chemical constituent identification by GC-analysis. GC results revealed that the major constituents present in essential oils were in agreement with the previously reported literature. Different bioassay (i.e. fumigation, contact toxicity, repellency screening, grain damage assessment), were conducted to evaluate the bioactivity of essential oils against *C. maculatus*. The modeling of these research findings (LC_{50}/LC_{95}) revealed that *C. longa* (0.32/0.70), *C. citratus* (0.40/3.23) and *C. paradisi* (0.32/2.12) oils stand excellent on the basis of their cumulative performance in various bioassay. Therefore, *C. longa* essential oil (turmeric oil) was selected for the detailed investigation for formulation development. GC-MS spectrum of turmeric oil showed the presence of ar-turmerone and turmerone as its major chemical constituents.*

*Two formulations i.e. Controlled release formulation (CRF, Na-Alg beads) and Microemulsion (ME), were prepared taking turmeric oil as active ingredient against *C. maculatus*. Since no study on dose optimization of essential oil for a given storage duration was available in literature, intensive discussion based on holistic perspective was required for designing the experiments. An innovative methodology for studying release kinetics of active ingredient of these formulations using GC method was evolved. Infact understanding the active ingredient release pattern in formulation matrix is a crucial factor for deciding the dose of CRF to be used in grain storage system. Surprisingly, literature seems to be silent on this crucial aspect of botanical*

formulations. In case of CRF, very interesting results were obtained. The release kinetics experiments were performed under airtight and partially open conditions for a period of four months. CRF started releasing mortal dosage (from 4th day of preparation) of turmeric oil, and maintained upto 85 days in airtight conditions. However, in partially open conditions, CRF released, all active ingredient within 30 days. FTIR spectral data revealed that there was no chemical interaction between inert ingredients and active ingredient in CRF. Similarly, release kinetics experiments of ME formulation in impregnated jute bags were designed and setup for a period of one month. It was found that no substantial release of active ingredient takes place from the surface of jute bag even after 30 days. The turmeric oil 5ME formulation was found to be stable at 10, 30 and 40^o C but was unstable at 54^o C as there was complete separation of oil.

*In lab level bioefficacy evaluation (i.e. artificial insect inoculation) of CRF and ME, both showed excellent bioefficacy by producing 100% insect mortality @ 0.5g CRF/500g/1L and @ 0.2 mg/cm²/100g/250ml respectively and no infestation of *C. maculatus* was observed even after 90 days of treatment in stored *Vigna mungo*. Under simulated field conditions as well, bioefficacy evaluation of CRF and ME revealed that 3.0 g of bead/1.5 Kg of *V. mungo*/2.5 L and @ 0.5 mg/cm² turmeric oil/ 1Kg *V. mungo* gave 100 % mortality of *C. maculatus* in plastic pearlpet jars/mud pots and jute/cotton bags respectively.*

*Overall, the present study brings to the fore the insecticidal potential of turmeric oil against *C. maculatus* and provides guidelines for preparation and application of CRF and ME formulations against the most serious storage insect-pest (*C. maculatus*). Since natural pest infestation in grain storage system would be far less as compared to artificial inoculation conditions, lower concentration of these formulations will be sufficient for 100 % *C. maculatus* mortality. An important experimental observation w.r.t. the special biological tendency of *C. maculatus* to remain on the upper layers of grain mass, was identified in the present study. This observation would prove of immense significance in dose optimization in different geometrical storage structures thereby, reducing the amount of insecticide drastically. This study would give an innovative facet of advancement of green botanical formulation for stored grains and thus opening a new avenue for safer pesticides for stored legumes especially at urban household and rural level.*

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