Essential oil entrapment in Alginate gel to control *Callosobruchus maculatus*- Preliminary Investigation

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Introduction:

Biopolymers are playing an important role in all aspects of human life (Miyazaki et al, 1994). Their application in the areas such as food, pharmaceutical industries is remarkable (Stanford and Baird, 1983). Alginate is a naturally occurring biopolymer used in food and pharmaceutical industry for its gelling, film-forming, stabilizing, thickening, and emulsifying properties (Wang http://www .glue.umd.edu/~nsw/ench485/lab7b.htm). It also has several unique properties enabling its application as a matrix for entrapment and /or delivery of variety of active ingredients i.e. pesticides, drugs, proteins and cells (Bahadir, 1987; Mamdouh et al, 2004, Smith, 1994; Basic et al, 1996). These properties includes inert aqueous environment within matrix, mild room temperature encapsulation process, free of non-polar solvents, high gel porosity for high diffusion rates of molecules, ability to control porosity with simple coating procedures, dissolution and biodegradation of the system under normal physiological conditions. These extraordinary properties of alginate make it suitable for its application in the field of agrochemicals as well for the controlled release of the active ingredient at a desired rate (Bahadir, 1987; Bahadir and Pfister, 1985).

Infestation of soil fungi has been cured by using the carbofuran based alginate formulations successfully (Kulkarni et al, 2000). The application of pesticide to the stored grains is necessary to protect it from the attack of the pests even if part of the life cycle it completes in the grain. However, the residual toxicity of the chemical pesticides in food is a matter of great concern due to human health hazards. This could be minimized by using biologically compatible and naturally available less toxic pesticides such as neem seed oil (*Azadirachta indica* A. Juss.) (Singh and Singh, 1996). Application of alginate in biopesticide formulation has also been reported by Knudsen et al, 1990. Spices and their extractives are known to have various effects on insect pests, including stored grain insects (Shaaya 1991), possessing characteristic flavors and odors due to the essential oils (which are allelopathic agents against predation by insects and infestation). In this context essential oils emerges as a big hope since they are often active against a limited number of species including specific target insects, are often biodegradable to non-toxic products, highly volatile and are potentially suitable for use in Integrated pest management, they could lead to the development of new classes of safer insect control agents (Shaaya et al., 1997). Essential oil of turmeric is known to have insecticidal

properties against many insects (Agular et al, 1994). Further *Callosobruchus maculatus* is a widespread oligophagous tropical seed beetle attacking commercially important legumes, causing huge economic losses to mankind.

Keeping all the above facts in mind, controlled release formulation based on turmeric as active ingredient against *Callosobruchus maculatus* was prepared. Therefore the aim of the present work was to formulate controlled release, calcium alginate beads of turmeric essential oil, and to determine the efficacy of the formulation against *Callosobruchus maculatus.*

Materials and Methods

Insects: *Callosobruchus maculatus* originally procured from Entomology Division of IARI, were obtained from laboratory cultures maintained for the last two years without any insecticide application on *Vigna mungo* at 28 (±1) ^{o} C, 70 (±5) % RH and a photoperiod of 12:12 h light : dark. The insects were isolated with technique of Bandara and Saxena (1995). Newly emerged adults were used for the tests.

Essential oils and chemicals: The essential oil of *Curcuma longa* was purchased from Kaanta Chemicals, Tilak Bazar, New Delhi. Triton-X-100 was purchased from Lobachemie, Sodium-alginate from Sigma and Calcium chloride from Merck. All chemicals were of highest grade commercially available. Double distilled water was used for the preparation of controlled release, calcium alginate-turmeric formulation.

Formulation Preparation: Preparation of calcium alginate formulations was carried out from aqueous solutions of sodium alginate (10 %, 5 %) to which 10 ml of 5 % emulsion of *Curcuma longa* was added gradually while stirring the thick gel of sodium alginate. Surfactant used for emulsion preparation was Triton-X-100. The Triton-X-100 concentration in the final formulation was 4 %. The beads (approx 6mm dia)were prepared at room temperature by dripping the alginate and turmeric solution from a height of approx 20 cm into 100ml of stirred 5 % $CaCl_2$ solution from a burette at the rate of 4 drops/min. Beads were left undisturbed in the calcium solution for 6 hrs for curing and then dried in paper folds. Beads without active ingredient were also prepared as control formulation.

Bioassay: 0.2 g of prepared beads were added to Petri-plate, to which were added newly emerged 20 unsexed adults. Each concentration and control was replicated 5 times. The mortality was recorded after 1 DAT, 2 DAT and 3 DAT (DAT-Day After Treatment).

Statistical Analysis: The statistical analysis for calculations was performed using online software (www.ehabsoft.com/ldpline/onlinecontrol.htm) based on Abbott's formula, (1925).

Results and Discussion

Data on effect of different concentration of calcium alginate on the mortality of adult *Callosobruchus maculatus* are shown in Table 1. Turmeric is known to have

insecticidal properties towards many insects (Agular et al, 1994). Further, it has been reported as toxicidal against *Callosobruchus maculatus* as well. It was observed that at lower concentration of alginate more than 85 % mortality was observed in comparison to higher concentrations where the observed mortality was 50 %. The difference in the *Callosobruchus maculatus* mortality was observed at different concentrations of alginate, though the active ingredient i.e. turmeric essential oil, concentration was same. This difference in mortality can be attributed to the difference in alginate concentration in both the formulations and on the basic structure of alginates. Further the porosity of an alginate gel can be significantly reduced by partially drying the beads (Smidsrod, 1973). Drying of beads is necessary to ensure in stored grains applications as insect infestation is directly related the moisture content in the stored grains. Completely dried beads which purely consist of turmeric essential oils can diffuse out the essential oil very efficiently, though we observed difference in mortality of *Callosobruchus maculatus* due to different alginate concentrations. It could be observed that alginate beads with low alginate concentration could kill *Callosobruchus maculatus* adults within 1DAT but this is related to very fast diffusion of turmeric essential oil from the pores of alginate beads. Low mortality in high alginate concentration beads could be attributed to the low diffusion rate through the pores of alginate beads. High alginate concentration beads can be successfully exploited to furnish prolonged security from insect infestation. Therefore this formulation is an appropriate tool to protect grains from *Callosobruchus maculatus* infestations as it can produce quick mortality and can be employed to safeguard grins over a period of time by tailoring the alginate concentration in beads.

Conclusion

It is evident from the data and above discussions that alginate entrapment is an effective, cheap and safer method for entrapment of active ingredients. This active ingredient can be pesticide (in the form of chemicals, essential oils, plant extracts etc), biocontrol agents, proteins (for drug delivery), cells etc. In designing a formulation for stored grain pests it is necessary to ensure that the active ingredient should not be toxic to humans as the exposed grains are generally consumed by humans directly without any further processing. Sodium alginate is another safer constituent of the formulation which has already known applications in food and pharmaceutical industries. Developed formulation is based on food grade constituents which are totally acceptable in food commodities without any toxic effects. Hence the developed formulation is advantageous in the respect that it is totally acceptable to be used in stored food grains as it is non-toxic to the humans. Moreover it provides an effective kill against *Callosobruchus maculatus*, to safeguard the economically important legumes during storage.

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Table1: % mortality of *Callosobruchus maculatus* at different alginate concentrations.