# Entrapment of Essential oil in Bio-polymeric Coatings for Controlling *Callosobruchus maculatus*

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## Introduction

Currently serious environmental and health implications of chemical pesticides being used to control insect pests, has created an urgency for searching other alternative stratagem for this purpose. Essential oils are getting special status in agriculture and grain storage systems, Bioactivity of essential oils against stored grain insect pests has been reported by several authors (Shaaya et al, 1997; Papachristos et al, 2003, 2004). Inspite of plethora of literature, suitable formulation, based on essential oils for controlling the stored grain pests is not commercially avilable. Therefore, preparation of such formulation of essential oil is a challenging task. For designing a formualtion for stored grain pests, it is important to consider: a) Controlled release of active ingredient for sustained control of insect pest. b) Toxicity of all the ingredients used, as the grains are going to be consumed directly after this stage.

In agrochemical sector, particularly in stored grains, controlled release of bioactive constituents is highly instigated. Entrapment of active ingredient in polymeric or bio-polymeric matrices leads to its controlled release thus indicating the practical utility of the formulations. Infact, use of biodegradable films of natural polysaccharides blends entrapped with active ingredient for the preparation of films and coatings, for various purposes has been increasing in the last few years (Immirzi et al, 2003). These entrapped coatings releases the active ingredient i.e essential oil in a controlled manner for its long term effect. Bio-polymers like sodium alginate, agar, gelatin, guar-gum, chitosan etc are gaining importance as entrapment marices. These bio-polymers have applications in food, pharmaceuticals, pesticides, and cells encapsulation (Stanford and Baird, 1983; Bahadir, 1987; Mamdouh et al, 2004; Smith, 1994; Basic et al, 1996; Courts 1973, James 1977; Fox et al, 1997; Prodhomme et al, 1989; Brode et al,1991). These are low cost and have excellent viscosifying properties. Hence, specific characteristics their potential can be harnessed for preparing formulation for stored grains protection against insect pests.

*Callososbruchus maculatus* is a serious pest of stored grains causing enormous qualitative and quantitative loss of commercial leguminous crops and Turmeric essential oil is known to have excellent bio-efficacy against this insect pest (Agular et al, 1994). The present paper aims at preparing the essential oil (Turmeric essential oil) entrapped bio-polymeric coatings (sodium alginate, gelatin and guar gum) and testing their bio-efficacy against *Callosobruchus maculatus*.

## Materials and Methods:

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

**Essential oils and chemicals**: The essential oil of *Curcuma longa* was purchased from Kaanta Chemicals, Tilak Bazar, Delhi. Tween-80 was purchased from Lobachemie. Sodium-alginate from CDH and food grade gelatin and edible gum from local market, Tilak Bazar, Delhi. All chemicals were of highest grade commercially available. Double distilled water was used for the preparation of slurry of bio-polymer and essential oil.

**Formulation Preparation**: Slurry was prepared by dissolving bio-polymers in boiling hot water under vigorous stirring. After the complete homogenization, the slurry is cooled to 35-40$^{O}$ C and 1g of Tween -80 mixed with Turmeric essential oil was added to the slurry. Different ratios of Biopolymer (fixed as 5%) and turmeric essential oil were used to prepare slurry. The slurry (3 gms) thus prepared was poured onto Petri-plate (9 cm diameter) and then spreaded uniformly. Concentration of turmeric essential oil in different formulations in g/cm$^{2}$ was 0.004718, 0.002359 , 0.001571, 0.00118, 0.000944, 0.00059, 0.000472. Each Petri-plate was left in incubator (30$^{O}$ C) for drying for 24 hrs. Control of each bio-polymeric coating was also prepared.

**Bioassay Experiments:** Newly emerged 10 unsexed adults were released into each dried petri-plate. All seven formulation based on different bio-polymer to essential oil of each bio-polymer as well as control was replicated 3 times. The insect mortality was recorded after 1 DAT, 2 DAT and 3 DAT (DAT-Day After Treatment).

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

## Results and Discussion

Bio-polymeric coatings of essential oils are shown in Figures 1-3. Bioefficacy results of bio-polymeric coatings entrapped with turmeric essential oil against *Callosobruchus maculatus* are presented in Figures 4-6. It is clearly evident from the figures that the developed coatings of different formulations of different bio-polymer to essential oil ratio, entrapped with essential oil showed excellent contact toxicity against *Callosobruchus maculatus.* The results are corroborated with earlier work (Agular et al, 1994) where turmeric essential oil has shown excellent bio-efficacy against this pest.

Sodium alginate and gum entrapped coating of 1:1 (ratio) furnished 100 % mortality, however in gelatin coating 1:2 (ratio) of bio-polymer to essential oil as well gave 100 % mortality. This behavior can be attributed to loose physical adsorption of essential oil onto the gelatin bio-polymer. X-ray diffraction (Ramchandran 1957), IR (Robinson 1953) and optical rotation (Coops, 1974) studies of gelatin films have revealed that in films cast at room temperatures, the gelatin macromolecules have mainly a collagen-like helical structure. At the same time, in gelatin films prepared from aqueous solutions by evaporating the solvent at temperatures above 35°C, gelatin macromolecules assume the conformation of a statistical coil with no indications of ordering (Jolly, 1970). In our study the water was evaporated at 30$^{O}$C therefore the structure taken by gelatin molecules may be helical and among those helices, adsorption of essential oil might have taken placetook place. Since the adsorption of essential oil was of physical natures therefore the essential oil was immobilised in the helices loosely resulting into its easy escape and hence high mortalty was observed in 1:2 ratio as well. At 1:5 ratio of bio-polymer to essential oil we observed more or less equivalent mortality ~50% on 1DAT. This can be attributed to lower concentration of essential oil embedded deep into the helices of gelatin and holding the essential oil with physical bonds. Similar trend was also observed for sodium alginate and gum. So the concentration of essential oil becomes a limiting factor at this concentration and therefore this trend was observed. However, in sodium alginate coating at 3 DAT, produced considerably higher mortality in comparison to gelatin and gum coatings. This can be due to delayed effect of essential oil on the *Callosobruchus maculatus* insect.

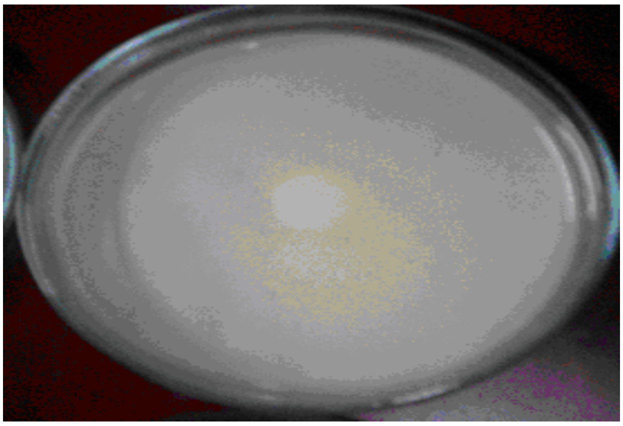

Figure 1: Showing Alginte-TEO Coating

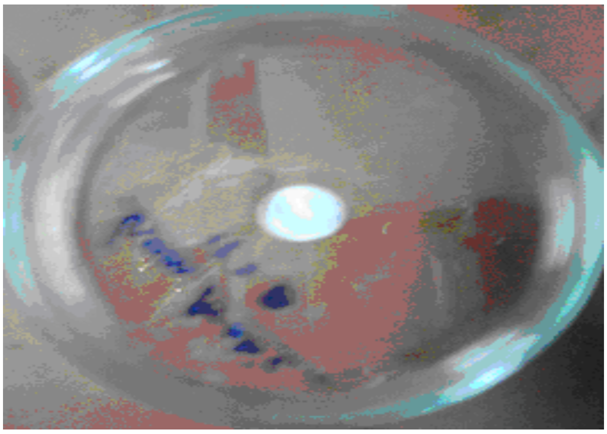

Figure 2: Showing Gum-TEO Coating

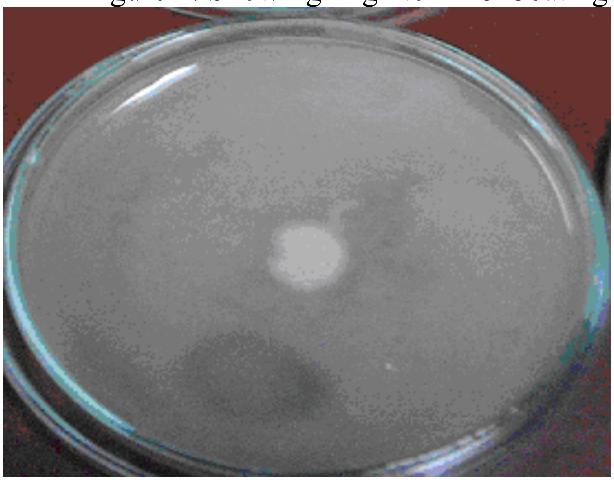

Figure 3: Showing Gelatin-TEO Coating

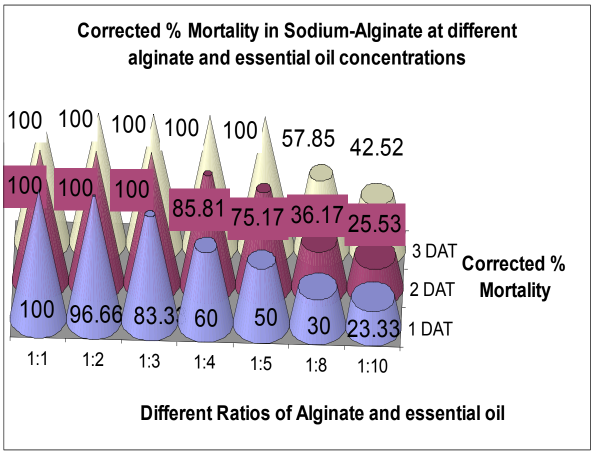


Figure 4

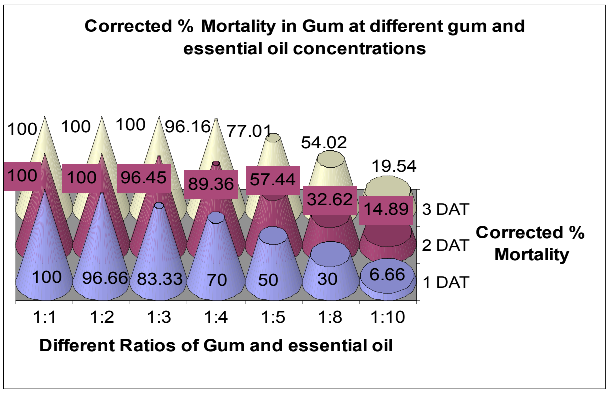


Figure 5

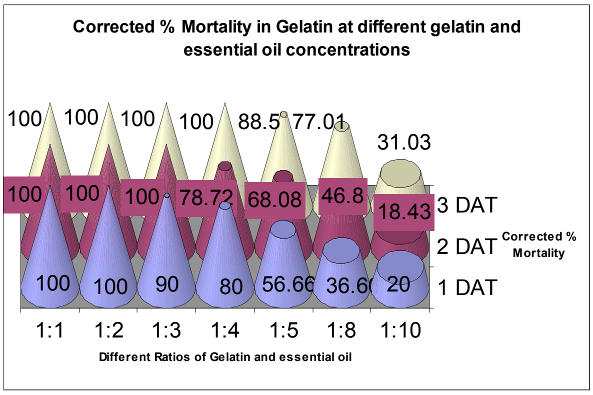


Figure 6

Overall, the data reveals that all the bio-polymeric coatings of turmeric essental oil upto 1:4 ratio are found effective in controlling *Callosobruchus maculatus*. At this concentration 100 % mortality can be achieved with- in 3 DAT reducing the population and checking the population build up of insect *Callosobruchus maculatus.*

## Conclusion

It can be concluded from the data and above discussion that bio-polymeric entrapment of essential oil is an effective stratagem to control insect infestation especially the stored grain pest *Callosobruchus maculatus*. For application of a formulation in agrochemical industry and specifically for stored grains, it is obligatory that the ingredient should be non-toxic to humans as the exposed grains are generally consumed by humans directly without any further processing. Therefore, employing food grade ingredients for such applications is very essential. In this study all the bio-polymers used, are of food grade therefore their application in this sector is highly appreciable. These coating formulations with excellent bioefficacy for stored food grains would provide food safety, as these are non-toxic to the humans.

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