Encapsulation of biopesticide *Metarhizium Anisopliae*: Improved soil stability.

Badgujar M. D[1], Bhaskar C[2+], Shukla P.G[2]*
[1] Praj Industries Ltd , Pune 411 021, India (Present address)
[2] Poly. Sci. Eng. Division, National Chemical Laboratory, Pune 411 008, India
* pg.shukla@ncl.res.in
[+]Retired scientist

INTRODUCTION

Pesticides whether chemical or biological are considered to be very important and necessary to prevent crop losses both at the time of raising the crop and the time of storage after harvest. It is estimated that on an average one third of the food production in the world is destroyed by the pests every year. The world's pesticide industry has grown to enormous limits to contain this pest problem, by producing highly toxic and broad spectrum insecticides , the use of which has become hazardous to man and animals by polluting the environment. The lack of specificity of these insecticides kill desirable species , while insects develop resistance due to injudicious use and also raising more problems of disposal of often toxic residues and byproducts. The increasing pressure from the Environmental Protection Agency (EPA) and global regulations, has led to the banning of many toxic and highly persistent pesticides like Organochlorine. So the obvious solution now is to develop new pesticides which are safer to use, more specific to insects, weeds, plant diseases and also non polluting to environment.

Biopesticides have great potential in agriculture for the control of many pests (Rodgers, 1993). Biological control using fungi can play important role in the Integrated Pest Management, but their poor persistence and soil stability have stood in the way of their use. These agents are susceptible to the UV fraction of sunlight and other environmental stresses such as higher levels of ambient temperatures, dry conditions, etc and are not persistent, necessitating repeated applications. Therefore it was necessary to develop the microencapsulated / encapsulated formulations which can impart stability and also improves the utility of the agents. There would be a commercial interest and greater acceptance of encapsulated biopesticides by the farmers because of increase in the productivity of crops without causing harm to man and the environment. The Entomopathogenic Fungus *Metarhizium anisopliae* is reported to be very effective biopesticide (Cloyd, Raymond A., 1999). We have shown that fungal spores of *Metarhizium anisopliae* can be encapsulated using natural polymers such as starch. The present paper describes preparation and performance of starch encapsulated fungal spores with special reference to shelf life and soil stability.

MATERIALS AND METHODS

A highly virulent isolate of *M. anisopliae*, a hyphomycetes fungus infecting whitegrubs was obtained in the form of conidial powder from Agricultural Research Station, Durgapura, Jaipur, India. Starch from the different sources (potato, maize and tapioca) were used for encapsulation.
Encapsulated formulation of *M. anisopliae* was prepared as follows. A starch slurry was prepared by mixing 180 g of maize starch in 2000 ml distilled water and cooked it over boiling water bath till the starch got gelatinized. This gelatinized starch was then cooled to room temperature and 20 g of *M. anisopliae* conidiospores was added, mixed well and kept for retrogradation in fridge for 48 hours. This mixture was then spread on a polyethylene sheet and dried in an air-draft oven at room

temperature. The obtained mass was then ground in a mixer to give encapsulated product in the powder form.

Viability studies: Free and encapsulated spores were analyzed as follows. . Aqueous suspension of each sample was diluted serially and a 0.1 ml of the final dilution was spread onto Veen's agar surface on each Petri plate. Observations for colony counts were made and mean colony forming units (cfu) g^{-1} of the formulation were computed.

Soil stability studies were conducted as follows. Garden soil was taken for the stability study of the fungal spores. Two pits were prepared in the soil of depth 30 cm two hollow plastic tubes of 20 cm length with sample point at each 5 cm length (Fig 1). One tube was labeled as control while other was labeled as test. Garden soil was filled in the tube.

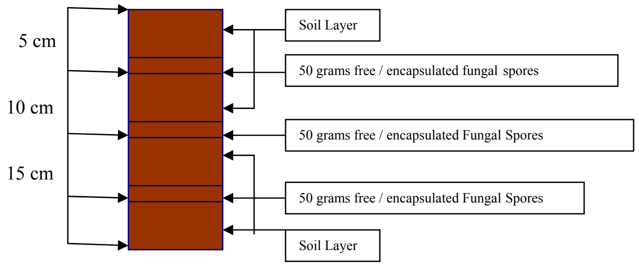

Fig 1: **Soil stability study setup**

Both tubes were kept in pits prepared in the garden 5 grams of sample was taken from both tubes from three sample points 5 cm, 10 cm, 15 cm length after every 15 days to check the viability of the encapsulated and unencapsulated (free) fungal spores.

RESULTS AND DISCUSSION

Starch is an effective encapsulating polymer to prepare controlled release formulation of variety of chemical pesticides. However as the methods employed to prepare these formulations are based on chemical crosslinking (Shasha 1981, Stout 1979, Trimnell 1982, Shukla 1991, 1992, 1993), these methods can not be employed to prepare formulation containing biopesticides. Starch contains mainly amylose and amylopectin and their ratio varies depending on the starch source. Maize starch contains less amount of amylose as compared to root starches like tapioca. Starch undergoes retrogradation when gelatinized starch is cooled. This is the retrogradation of starch caused by recrystallization of amylose through cooling. We explored this property of starch to prepare encapsulated formulation of fungal spores where encapsulation takes place by physical means and not through chemical crosslinking.

Fig 2 shows SEM picture of encapsulated fungal spores in starch matrix. As the final product obtained in film form which was then ground to get particles, few spores got exposed to the surface due to the breaking of film. These spores are seen on the surface of starch particle.

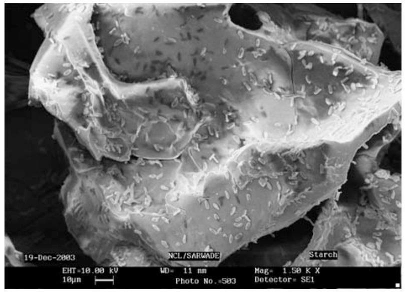

Fig 2: *Metarhizium anisopliae* spores encapsulated in Starch Matrix

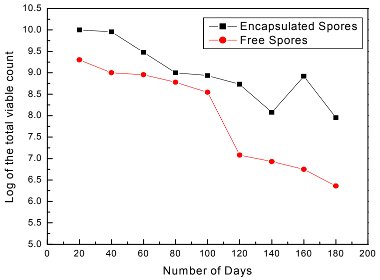

Fig 3: Survival of spores stored at room temperature as a function of time

Encapsulated spores and free spores were stored at room temperature and their viability was checked as a function of time. From Fig 3 it can be seen that after 180 days encapsulated spores show more viability as compared to free spores and thus are more stable.

Formulation→	**SP-II**		**ST-II**		**SM-II**		**Free spores Talc powder + M.a. conidia**	
Dose	D-1	D-2	D-1	D-2	D-1	D-2	D-1	D-2
*Percent grub mortality after 40 days of incubation	80	100	80	100	100	100	60	80

D-1 = 2.5×10^7 cfu/g soil; D-2 = 5.00×10^7 cfu/g soil;
*Number of replications = 5

Table 1: Effect of different encapsulated formulations of *Metarhizium anisopliae* for larval infectivity against whitegrubs (*Holotrichia consanguinea*).

Encapsulated formulations of fungal spores SP-II, ST-II and SM-II prepared with potato starch, tapioca starch and maize starch respectively were evaluated for grub mortality (Table 1). It can be seen that encapsulated formulations have better control over conventional fungal spore formulations. Maize starch formulation(SM-II) showed 100% grub mortality at lower dose (D1) whereas at the same dose conventional fungal spore formulation showed only 60% grub mortality. This better performance can be attributed to better soil stability of encapsulated fungal spore formulation.

To understand the soil stability at different soil depth experiment was carried out as described in material and methods section. It can be seen from Fig 4 and Fig 5 that encapsulated spores have better soil stability at all the depths (5, 10 and 15 cm) and there is significant improvement in soil stability at 15cm depth as compared to free spores. Thus the coating of polymer around the spores protects the spores from different environmental impact such as temperature.

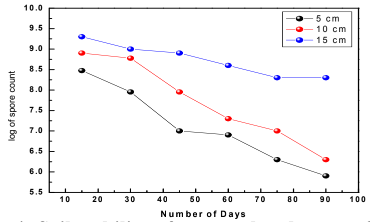

Fig 4 :Soil stability of encapsulated spores for survival of spores at different depths

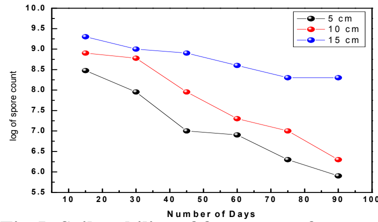

Fig 5: Soil stability of free spores for survival of spores at different depths

CONCLUSION:

Encapsulation of *Metarhizium anisopliae* spores in starch matrix was carried out by retrogradation without any use of chemical agent. Encapsulated *Metarhizium anisopliae* spores have potential application in the control of soil borne pests. Encapsulated spores showed greater viability and soil stability in comparison with the free spores. The improved stability and viability of this bioactive agent will have the long term effect in the soil to control the pests effectively.

REFERENCES

Rodgers P. B. (1993) *Potential of biopesticides in agriculture* Pesticide Science 39(2) 117-129
Cloyd, Raymond A. (1999). *The Entomopathogenic Fungus Metarhizium anisopliae* . Midwest Biological Control News VI (7).
Shasha B.S. et al. (1981*) Encapsulation of in a Starch –Calcium Adduct*. J.Polym. Sci. Polym. Chem 19 1891-1899
Stout E. L. et al. (1979) *Pilot-Plant Process for Starch Xanthide Encapsulated Pesticides* J.Appl. Polym. Sci. 24 153-159
Trimnell D. et al (1982) *Pesticide encapsulation using a starch-borate complex as wall material* J. Appl. Poly. Sci. 27(10) 3919-3928
Shukla P. G. et al.*(1991) Crosslinked starch-urea formaldehyde (St-UF) as a hydrophilic matrix for encapsulation: Studies in swelling and release of carbofuran* J. Controlled Release, 15 153-166
Shukla P.G. et al. (1992) *Structure of carbofuran in crosslinked starch matrix by* ^{13}C *NMR: Correlation of release and swelling kinetics with the dynamic behaviour of polymer chains* Polymer 33 3611-3615
Shukla P.G. et al. (1993***)** Starch urea-formaldehyde matrix encapsulation. IV. Influence of solubility and physical state of encapsulant on rate and mechanism of release* J. Appl. Poly.Sci. 48 (7) 1209-1222