

METARHIZIUM GRANULES FOR CONTROL OF RHINOCEROS BEETLE

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MPOB INFORMATION SERIES • ISSN 1511-7871 • JUNE 2008

MPOB TT No. 404

The fungus *Metarhizium anisopliae* var. *major* is highly effective in controlling the rhinoceros beetle, *Oryctes rhinoceros*. It has been mass produced and formulated into a powder bio-insecticide (Ramle *et al.*, 2006), and field application of it to rotting oil palm residues significantly reduced the larvae, pre-pupa and pupae populations of the insect (Ramle *et al.*, 2007). But the powder is applied as a solution, an inconvenience where there is no water. As an alternative, formulating the fungus as granules was studied – as alginate granules, incorporating a nutrient carrier to ‘kick-start’ the fungal growth on application for earlier sporulation. The alginate granules can be prepared using the mycelium or spores of the fungus, or mycelium and spores together (Fravel *et al.*, 1995).

BENEFITS

- Effective control of *O. rhinoceros* in oil palm;
- Easy to apply – just broadcast on the breeding sites of the beetle;
- Longer control as the nutrients in the granule promote the fungal growth for earlier sporulation;
- The granules serve as a source of infectious spores, obviating dependence on the dead infected host as secondary source of inoculum; and
- Safer to the environment and non-target organisms than chemical insecticides.

BASIC PREPARATION OF ALGINATE GRANULES

The fungus is cultured in a liquid medium containing dextrose, peptone and yeast extract. An alginic acid solution is prepared using absolute alcohol. The fungal mycelium is harvested then added into the alginic acid solution and mixed. Sterilized kaolin and rice bran are added. The

granulation is by dropping the mixture into a calcium chloride solution. The granules are rinsed in distilled water, dried at room temperature (Figure 1) and their quality assessed. A patent for the process has been granted for the United Kingdom (GB 2380131).



Figure 1. Granule formulation of *M. anisopliae*.

QUALITY OF GRANULES PREPARED FROM MYCELIUM WITH/WITHOUT MEDIUM

The quality of the granules made from mycelium alone (G+M) and from the mycelium and growing medium (G+MM) was determined. The latter proved to be better, with faster growth of the fungus and sporulation by 87.2% *vs.* only 48.0% by the former (Figure 2).

OPTIMIZATION OF GRANULE INGREDIENTS

Six recipes were tried in an effort to ascertain the best ingredients for the granules. Each recipe had different volumes of distilled water, and contents of kaolin and rice bran. The best was Recipe 4 (925 g kaolin and 400 g rice bran, 2 litre distilled water), closely followed by Recipe 5 (950 kaolin, 400 g rice bran, 2 litre distilled water) (Figure 3). Using Recipe 4, the fungus sporulated from 88.6% of the granules, higher, but not significantly more ($P < 0.05$), than with Recipe 5 (80.3%).

ISSN 1511-7871



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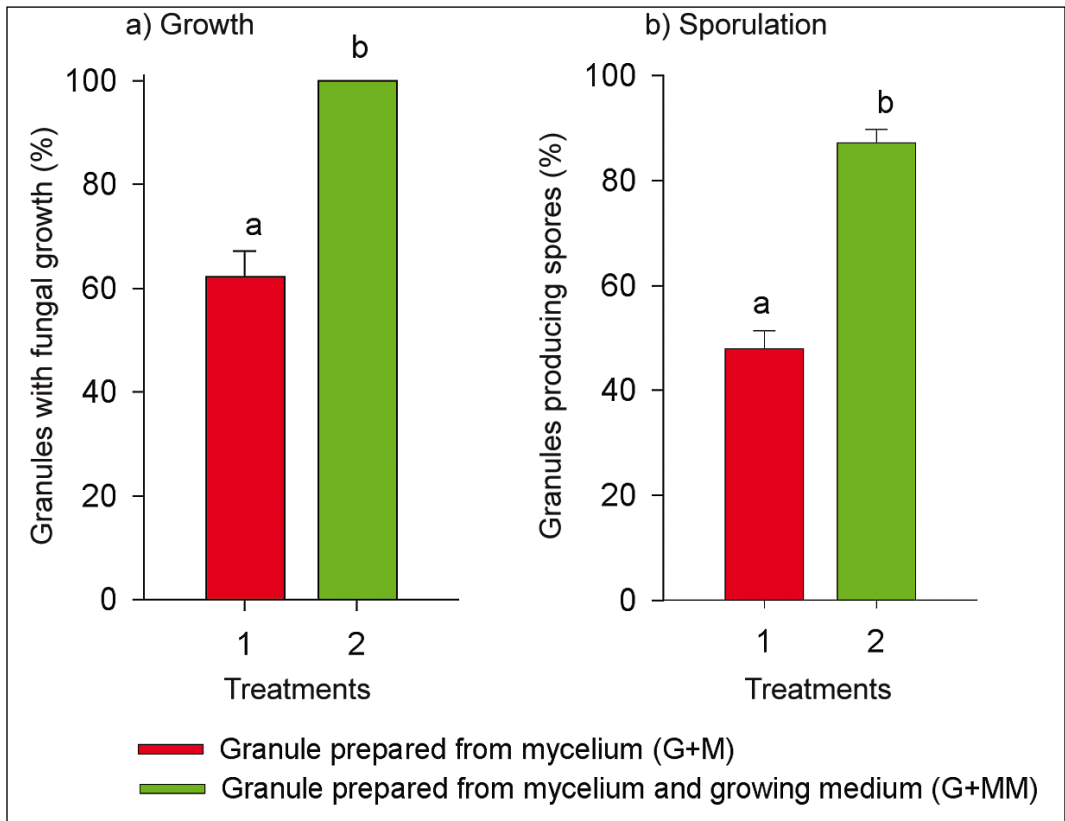


Figure 2. Quality of granules made from mycelium alone and with medium. a) Growth of fungus, b) sporulation. Bars in the same group with the same letter are not significantly different at $P < 0.05$.

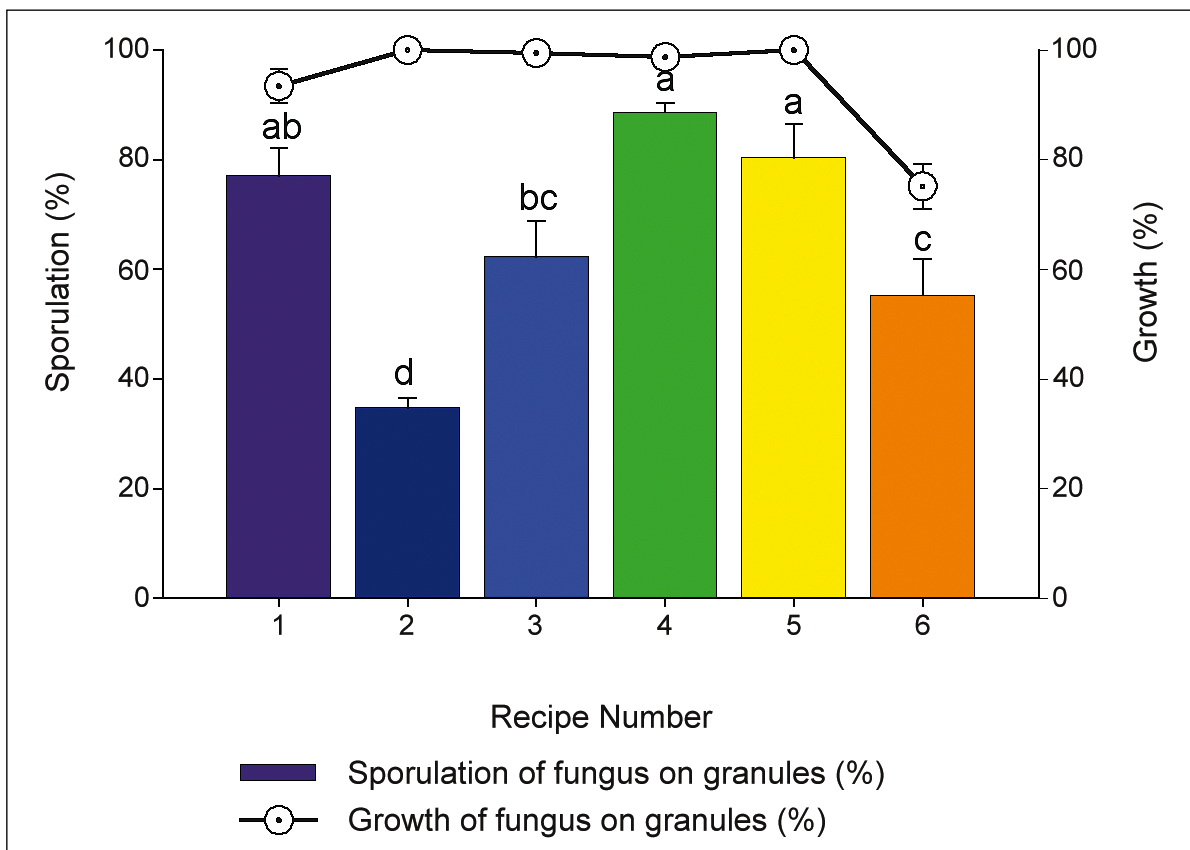


Figure 3. Growth and sporulation of *M. anisopliae* from granules formulated to different recipes. Bars with the same letter are not significantly different at $P < 0.05$.

GRANULE PATHOGENICITY AGAINST *O. rhinoceros* LARVAE

The granules made from mycelium (G+MM) and spores (G+Sp) were tested against the L3 larvae of rhinoceros beetle. The G+Sp granules were prepared as per the G+MM granules. A box was used to contain the larvae and rotting oil palm residues, and one of three rates of the granules – 3 g, 6 g and 9 g – mixed in. The control box had granules without any fungus. Both types of granules at all rates killed 100% the larvae as early as 18 days after treatment (DAT).

Percentage of dead larvae killed by the *M. anisopliae* infection increased as the application rates increased (Figure 4). At the highest rate,

application of G+MM and G+Sp granules caused the highest infection of 93.3% (Figure 5).

CONCLUSION

The granules made from fungal mycelium and growing medium were better than those made with just the mycelium alone. The best formulations for the granules were Recipes 4 (925 g kaolin, 400 g rice bran) and 5 (950 g kaolin, 400 g rice bran), as they produced the highest fungal sporulation of 88.6% and 80.3%, respectively. The granules made from mycelium or spores were equally effective for controlling *O. rhinoceros*. Both killed 100% larvae as early as 18 DAT and caused infection as high as 93.3%.



Figure 4. Dead larvae killed by *M. anisopliae* infection.

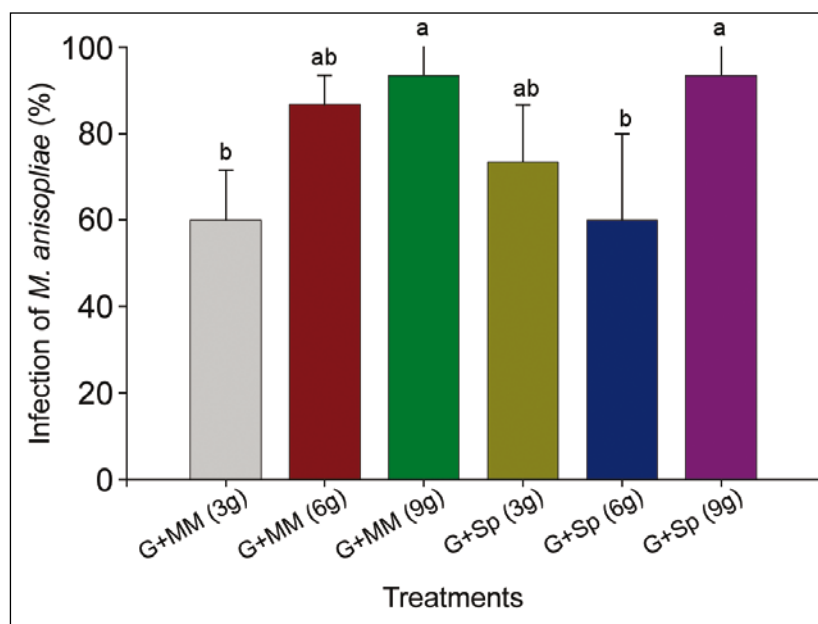


Figure 5. Patogenicity of granule formulation against the larvae of *O. rhinoceros*. G+MM - granules made from mycelium and medium, G+Sp - granules made from spores. Bars with the same letter are not significantly different at $P < 0.05$.

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