ROLE OF PAECILOMYCES IN RAPID COMPOSTING AND PLANT DISEASE MANAGEMENT

by

KALPANA ARORA

Centre for Rural Development and Technology

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CERTIFICATE

This is to certify that the thesis entitled *"Role of Paecilomyces in Rapid Composting and Plant Disease Management"* submitted by Ms. Kalpana Arora has been prepared under my guidance with the rules and regulations of Indian Institute of Technology Delhi, India. The research report and results presented in this thesis have not been submitted for any degree or diploma in any other institute or university.

Date **(Prof. Satyawati Sharma)**

Place Centre for Rural Development and Technology
Indian Institute of Technology Delhi
Hauz Khas, New Delhi 110016

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ABSTRACT

There has been a rapid industrialization and urbanization in India in the last few decades, which has led to improvement in the living standards and change in the production and consumption patterns. Consequently, the nature and volume of the Municipal solid waste has changed considerably. Thus demands a suitable and cost effective technology for the environmentally safe treatment and disposal of MSW. Composting is a promising method of waste management to stabilize solid waste not only to produce compost but also keep environment pollution free. Bioinculants play an important role in nutrient recycling, rapid composting and also act as biocontrol agent in crop production. Since, modern farming practices involve extensive usage of chemical fertilizers and pesticides which result in land and water pollution and also affect the human health and other life forms severely., these practices should be substituted by alternative agricultural practices, which vitally reliant upon the proper and regular addition of organic manure and utilizing the biofertilizers and biopesticides. So it was considered of interest to explore the possibility of using lignocellulolytic fungus Paecilomyces variotii, nitrogen fixing and phosphate solubilizing bacteria along with the earthworms to fasten the rate of MSW stabilization and improve the quality of compost also having biopesticidal value.

Therefore, in search of an integrated system for rapid composting of MSW and plant disease management, present study was taken up with following specific objectives:

- *To screen and identify cheaper and easily available C and N sources for the multiplication of P.variotii.*

- *To assess the potential of P. variotii along with efficient micro organisms (Pseudomonas and Azotobacter) and earthworms in rapid composting.*

- *To study the role of P.variotii as biocontrol agent against Fusarium and Verticillium causing wilt diseases in tomato.*

- *To prepare the formulations for rapid composting and controlling the selected pathogen.*

Prior to the composting experiments, the growth parameters of the P. variotii were studied and interaction among all the bioinoculants (Efficient microbes and earthworms) and selected pathogens was tested. The optimum temperature, pH and relative humidity (RH) for P. variotii's

best growth on MSW were identified as 30°C, 5 and 70% respectively. The existence of 'functional compatibilities' among all the three microbes (P. variotii, A. chroococcum and P. fluorescence) and earthworms (Eisenia fetida) made them suitable to employ in rapid composting of MSW. Also, the antagonistic activity of P. variotii against F. oxysporum and V. dahilae (visible by inhibition zone) provided the possibility to use it as biocontrol agent against Fusarium and verticillium wilt of tomato.

P. variotii alongwith Pseudomonas and Azotobacter with earthworms improved the quality and quantity of compost over other treatments and decreased ~50% time of composting. The reduction in percentage of C:N ratio (25.48 to 7.01), cellulose (up to 6.38%), hemicelluloses (18.46%) and lignin content (16.46%) and increase in percentage of TKN (up to 2.5 %), phosphorus (up to 1.7%) and potassium (up to 1.8%), were noted within 60 days of composting. FTIR studies revealed the decrease in TOC content by showing the decrease in peaks of different C- moieties present in the compost.

The doubling time of P. variotii was found to be 8.2h and 90mg/L of bioactive metabolite, Dipicolinic acid (DPA) was produced. The growth analysis of fungus P. variotii on nitrogen rich non edible oil cakes (Jatropha, Karanja, Neem and Mahua) showed better results than Potato Dextrose Agar (PDA), the growth media commonly used. The growth rate of P. variotii was 0.42 cm/ day on Jatropha cake while on PDA it was 0.26 cm/day. Similarly, spore count was also enhanced from 2.5 x 10^9 of control to 5.1 x 10^{10} of Jatropha cake. In this context, it may be noted that Jatropha, Karanja and Neem oil cakes when amended with dextrose were found superior to PDA. However, the performance of Mahua cake was not found satisfactory. The mass cultivation of the P. variotii on the non edible oil cakes with their various combinations with saw dust proved the feasibility of these oil cakes as a substrate in SMF. The best treatment was Karanja oil cake (70%) with saw dust producing the spores up to 7.2 x 10^{12} per gram of substrate. The pot trials to control F.oxysporum infection in tomato plants with the formulated product were efficient.

Possibilities were explored to increase the DPA production by utilizing these non edible oil cakes and different carbon sources by experimental designing for medium optimization with Response Surface Methodology (RSM). The DPA production has increased by 2.7 times on

optimized the medium consisted of non edible oil cakes as N sources (Jatropha and Karanja cakes optimized at 20g/L and 45g/L respectively) and dextrose (10g/L).

The non edible oil cake based formulation of P. variotii significantly reduced the % of disease incidence of tomato plants as compared to control. The treatment improved plant health by reducing wilt symptoms, vascular invasion and sporulation of the pathogen and decreasing the disease incidence (17.2% for F. oxysporum and 18.83% for V. dahilae). Minimum Inhibitory Concencentrations (MIC) of the fungal extract (P. variotii) was observed with the 10^{-3} dilution for both F. oxysporum and V. dahilae. MIC of DPA was detected as 0.25 mg/mL for F. oxysporum and 0.21 mg/mL for V. dahilae.

Real time PCR studies provided an insight to the molecular mechanism involved in the biocontrol of F. oxysporum. As P. variotii produces certain bioactive compounds eg. DPA, which might have inhibited FOW 1 expression in F. oxysporum. Real Time analysis for the FOW1 gene which is responsible for the colonization of the F. oxysporum showed that it was down-regulated linearly on a time course. The attenuation of PG1 gene (responsible for endopolygalactouronase) was also seen. Enhancement in the expression of CRE1 showed that P. variotii produce some bioactive substance (DPA) which may have inhibited FOW1 gene expression in F. oxysporum. Also, in concert with the over expression of CRE 1 gene and PG1 gene is down regulated which further decrease the FOW1 expression.

The present study clearly reveals the possibilities to use the P. variotii with other bioinoculants successfully to enhance the rate of biodegradation and improve the quality of compost from MSW. Moreover, as far as the economics of this system is concerned it is obviously much cheaper as compared to the other composting processes which involve mechanical turning, aeration and so on. The experimental findings to exploit the antagonistic activity of P. variotii against Fusarium and Verticillium wilts proved it as promising biocontrol agent as an efficient, environmentally safe alternative.

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Chapter III: Materials and Methods

Chapter IV: Results and Discussion