

DEVELOPMENT OF MICROBIAL FORMULATIONS FOR PRODUCTION OF COMPOST TO BE USED FOR PLANT GROWTH AND DISEASE MANAGEMENT

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**DEVELOPMENT OF MICROBIAL
FORMULATIONS FOR PRODUCTION OF
COMPOST TO BE USED FOR PLANT
GROWTH AND DISEASE MANAGEMENT**

by

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Submitted

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Dedicated

To

The Almighty

&

My family

CERTIFICATE

This is to certify that the thesis entitled “**Development of microbial formulations for production of compost to be used for plant growth and disease management**” submitted by **Ms. Ritika Pathak** to Indian Institute of Technology Delhi India for the award of **Doctor of Philosophy** has been prepared under our guidance with the rules and regulations of this institute. The research report results presented in this thesis have not been submitted for any degree or diploma in any other institute or university.

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ABSTRACT

Rapid industrialization and urbanization has resulted into a drastic escalation in waste generation, disposal of which has become a major concern. Moreover, it has amplified the burden on agriculture sector for increased crop productivity, resulting which farmers have aggravated the use chemical pesticides, and fertilizers to increase their productivity and protect crops from disease causing pathogens. This modern farming practice has resulted into serious human health and environmental impacts. It has therefore become essential to switch over to environmental friendly methods for waste disposal and plant nutrition and disease management. Acknowledging the ubiquitous nature of microorganisms and their importance in natural environments, utilizing their potential to mitigate these problems can be an encouraging approach. Composting is a microbiological process for converting waste into a nutrient rich product (humus/compost). However, a rapid composting method that employs the use of bioinoculants for compost production in short duration is need of the hour. Waste generated from oil industries (non edible oil cakes) also opens great avenues in the field of composting and agriculture. In this context, the current work investigated the potential of some efficient microorganisms (*Trichoderma asperellum*, *Paecilomyces variotii*, *Azotobacter chroococcum*, *Pseudomonas fluorescens* and *Lactobacillus casei* Shirota), earthworms and non edible oil cakes in rapid composting of kitchen waste and plant disease management.

Interaction studies between the selected microbial consortia revealed that all the three bacteria viz., *L. casei* Shirota, *P.fluorescens* and *A. chroococcum*, showed positive interaction with the fungi (*P. variotii* and *T. asperellum*). However negative interaction was seen between the two fungi viz., *T.asperellum* and *P.variotii*, which clarified that these two fungi couldn't be employed together for rapid composting. The interaction of earthworms with microbes and non edible oil cakes were also found to be positive. Microbial consortium involving the lignocellulolytic fungi *P.variotii* and *T.asperellum* separately along with three bacteria *L. casei* Shirota, *P.fluorescens* and *A. chroococcum*, earthworms and jatropha cake (1%) produced quality compost within a reduced time span (60 days). The nutrient contents (TKN,

P_{av} , K_{av}) and the degradation components (cellulose, hemicellulose) were found to be far better than the other treatments involved. *L.casei* Shirota played an important role in eliminating foul odor during composting and also reduced the *E.coli* count in the final compost generated. Application of talc based formulations of the selected microorganisms also resulted in production of quality compost. All the microbes (bacteria and fungi) recorded a count of 10^6 cfu/g till 6 months after which the count declined.

Amongst the non edible oil cakes (karanja, neem, mahua and jatropha cake), jatropha cake emerged as the best non-traditional solid substrate for culturing microorganisms and producing lignocellulolytic enzyme (cellulase and xylanase) by solid state fermentation. Optimization studies under controlled lab conditions revealed that *P.variotii* recorded highest cellulase (Fpase: 27.37 IU/g; CMCCase: 383.95 IU/g) and xylanase (4842.93 IU/g) activities on deoiled jatropha cake. Combination T3 i.e. *P.variotii* as the lignocellulolytic fungus along with the three bacteria (*L. casei* Shirota, *P. fluorescens*, and *A. chroococcum*) gave the highest xylanase activity (10.656 IU/g) whereas, T4 with *T. asperellum* as the lignocellulolytic fungi along with the three bacteria gave highest FPase (6.52 IU/g) and CMCCase (7.71 IU/g) activities during the vermicomposting of kitchen waste. Emergence and role of black soldier flies (BSF) larvae in waste decomposition disclosed that they have an important role in solid waste decomposition but generate a low nutrient content product. The earthworms also did not survive in the presence of these larvae.

Amongst all the selected efficient microorganisms, both the fungi (*P.variotii* and *T.asperellum*) emerged as potent candidates as biocontrol organisms against wilt causing pathogens viz., *Fusarium oxysporum* and *Verticillium dahliae*. *T.asperellum* showed better antagonism with inhibition percentage of 72 and 66% in dual plate assay followed by 63.23 and 56.48 % in non volatile assay and 33.38 and 53.38% in volatile assay against *F. oxysporum* and *V.dahliae* respectively. High cell wall degrading enzymes viz., chitinase (202.68 μ moles/ml/min) and β -1, 3 glucanase (381.48 μ moles/ml/min) were produced by *T. asperellum*. GC-MS studies also revealed the production of volatile antifungal metabolites by both the biocontrol fungi (*T.asperellum* and *P.variotii*).

Amid all the vermicomposts produced, vermicompost IV (Derived from *A. chroococcum*, *P. fluorescens*, *L. casei* Shirota, *T. asperellum* and 1% jatropha cake) gave excellent results for growth parameters of tomato plant in terms of plant height (66.33 cm), biomass (48.00 g) and fruit yield (118.04 g). Highest disease reduction against *Fusarium* and *Verticillium* wilt was also attained by vermicompost IV.

This current study revealed that the integration of bioinoculants and jatropha cake played a pivotal role in rapid composting of kitchen waste and produced quality compost in 60 days. The compost produced had dual role of providing plant nutrients and as a biopesticide against *Fusarium* and *Verticillium* wilt.

सार

तेजी से औद्योगिकीकरण और शहरीकरण के कारण कचरे के उत्पादन में भारी वृद्धि हुई है, जिसका निपटान एक बड़ी चिंता का विषय बन गया है। इसके अलावा, कृषि क्षेत्र में फसल की उत्पादकता को बढ़ाने के लिए बोझ बढ़ा है, जिसके परिणामस्वरूप किसानों ने रासायनिक कीटनाशकों और उर्वरकों को अपनी उत्पादकता बढ़ाने एवं बीमारियों से फसलों की रक्षा करने के लिए इसके प्रयोग में बढ़ोत्तरी की है। इस आधुनिक कृषि पद्धति का परिणाम गंभीर मानव स्वास्थ्य और पर्यावरणीय प्रभावों के रूप में हुआ है। इसलिए अपशिष्ट निपटान, संयंत्र पोषण और रोग प्रबंधन के लिए पर्यावरण के अनुकूल तरीकों पर स्विच करना आवश्यक हो गया है। सूक्ष्मजीवों की सर्वव्यापी प्रकृति और प्राकृतिक वातावरण में उनकी महत्व को स्वीकार करते हुए, इन समस्याओं को कम करने के लिए उनकी क्षमता का उपयोग एक उत्साहजनक दृष्टिकोण हो सकता है। खाद एक अपशिष्ट को एक पोषक तत्व से भरपूर उत्पाद (खाद मिट्टी / वानस्पतिक खाद) में परिवर्तित करने के लिए एक सूक्ष्मजीवविज्ञानी प्रक्रिया है। हालांकि, एक तीव्र खाद विधि जो कि कम अवधि में खाद उत्पादन के लिए बायोइनोकुलेंट्स का इस्तेमाल करते हैं, समय की आवश्यकता है। तेल उद्योगों (गैर खाद्य तेल केक) से उत्पन्न अपशिष्ट भी खाद और कृषि के क्षेत्र में जबरदस्त अवसर प्रदान करता है। इस संदर्भ में, वर्तमान समय में इस कार्य के लिए रसोई के कचरे के तेजी से खाद और पौधे रोग प्रबंधन में कुछ कुशल सूक्ष्मजीवों (ट्रायकोडर्मा एस्परेल्मम, पेसिलोमिअस विलियोटी, एज़ोटोबैक्टर क्रोकोस्कम, स्यूडोमोनस फ्लोरसेन्स और लैक्टोबैसिलस केसिनी शिरोटा), केंचुए और गैर खाद्य तेल केक की क्षमता की जांच की गई है।

चयनित माइक्रोबियल कंसोर्टियम के बीच इंटरएक्शन के अध्ययन से पता चला कि सभी तीन बैक्टीरिया यथा एल केसि शिरोटा, पी.फ्लूरोरेसेन्स और ए. क्राकोकोक्यूम, कवक के साथ सकारात्मक बातचीत (पी. विविधताई और टी एस्परेल्म) हुई है। हालांकि, दो कवक, टी एसपीरेल्म और पी वारियती के बीच नकारात्मक इंटरएक्शन को देखा गया, जिससे स्पष्ट किया है कि इन दोनों कवकों को तेजी से खाद बनाने के लिए एक साथ नहीं जोड़ा जा सकता है। सूक्ष्म जीवों और गैर खाद्य तेल केक के साथ केंचु का संपर्क सकारात्मक भी पाया गया। माइक्रोबियल कंसोर्टियम जिसमें तीन बैक्टीरिया यथा एल केसी शिरोटा, पी.फ्लूरोरेसेन्स और ए.

क्राकोकोक्यूम, केंचुए और जेट्रोफा केक (1%) के साथ अलग-अलग लिग्नोकोल्लोलीटिक कवक पी वोरियोती और टी एसपीरेल्म से कम समय अवधि (60 दिन में विशेष वानस्पतिक खाद तैयार हुआ)। पोषक तत्व सामग्री (टीकेएन, पाव, काव) और गिरावट घटकों (सेल्यूलोज, हेमीलेलोस) को अन्य उपचारों से कहीं बेहतर पाया गया। एल सी सी शिरोटा ने खाद के दौरान खराब गंध को खत्म करने में महत्वपूर्ण भूमिका निभाई और अंतिम खाद में उत्पन्न ईकोली गिनती को भी कम किया। चयनित सूक्ष्मजीवों के टैंक आधारित फार्मूलेशन के उपयोग के कारण गुणवत्तायुक्त खाद का उत्पादन भी हुआ। सभी रोगाणुओं (बैक्टीरिया और कवक) ने 6 महीने तक 106 सीएफयू / ग्राम की गिनती दर्ज की जिसके बाद गणना में गिरावट आई।

गैर खाद्य तेल केक (कर्नाजा, नीम, महुआ और जेट्रोफा केक) में, जटरोफा केक, सूक्ष्मजीव संवर्धन के लिए सबसे अच्छा गैर-पारंपरिक ठोस सब्सट्रेट के रूप में उभरा और ठोस अवस्था किण्वन द्वारा लिगोनोक्लोलॉलायटीक एंजाइम (सेल्युएस और जियालेनस) का उत्पादन किया है। नियंत्रित प्रयोगशाला परिस्थितियों के तहत अनुकूलन अध्ययन से पता चला है कि पी वोरीओटी ने सबसे अधिक सेल्यूलस दर्ज किया है (एफपीएस: 27.37 आईयू / g; सीएमसीज: 383.95 आईयू / g) और जीलैनेस (4842.93 आईयू / g)। कॉम्बिनेशन टी 3 यानी लिग्नोकोलेलोलीयटीक कवक पी.वाराओतिई के साथ तीन जीवाणुओं (एल केसी शिरोटा, पी. फ्लोरोससेंस और ए. क्रोकोस्कैम) उच्चतम जियानैस गतिविधि (10.656 आईयू / g) जबकि टी 4, लाइगनोसेलुलाइटिक कवक टी एस्परेल्म के साथ तीन बैक्टीरिया के साथ रसोई कचरे के वर्मीकंपोस्टिंग के दौरान उच्चतम एफपेज (6.52 आईयू /g) और सीएमसीकेस (7.71 आईयू /g) की गतिविधि प्रदान करता है। कचरे के अपघटन में उभरती हुई काली मिलाप मक्खियों (बीएसएफ) लार्वा की भूमिका ने खुलासा किया कि ठोस अपशिष्ट अपघटन में उनकी एक महत्वपूर्ण भूमिका है लेकिन इससे कम पोषक तत्व सामग्री उत्पाद उत्पन्न होता है। इन लार्वा की उपस्थिति में केंचुए भी जीवित नहीं रह सकते थे।

सभी चुने हुए कुशल सूक्ष्मजीवों के बीच, कवक (पी.वाराओतिय और टी एस्परेल्म) दोनों ही शक्तिशाली उम्मीदवारों के रूप में उभरा, जैसे कि विषाणुओं के कारण जैव-नियंत्रक जीव, फ्यूसरियम ऑक्सीसोरम्म और वर्टिसिलियम डाहलिया टी सस्परेल्म ने गैर-वाष्पशील परख में 63.23 और 56.48% और क्रमशः एफ ऑक्सीरोमोरम और वी डैह्लिया के खिलाफ अस्थिर

आवरण में 33.38 और 53.38% के बाद दोहरी प्लेट परख में 72 और 66% के निषेधात्मक प्रतिशत के साथ बेहतर विरोध दिखाया। हाई सेल वाल डिग्रेडिंग एंजाइम यथा काइटिनेस (202.68 μ मोल्स / मिली / मिनट) और β -1, 3 ग्लुकानेस (381.48 μ मोल्स / एमएल / मिन) टी एस्परेल्म द्वारा उत्पादित किए गए और जीसी-एमएस अध्ययनों से भी पता चला कि बायोकंट्रोल कवक (टी एस्परेल्म और पी वाराओती) द्वारा अस्थिर रोधी चयापचयों का उत्पादन होता है।

सभी वर्मी कंपोस्ट्स के उत्पादन में, वर्मीकंपोस्ट IV (ए. क्राकोकोक्यूम, पी. फ्लोरोससेंस, एल कैसी शिरोटा, टी. एस्परेल्म और 1% जेट्रोफा केक से प्राप्त) ने पौध की ऊँचाई (66.33 सेंटीमीटर) के संदर्भ में टमाटर प्लांट के विकास मापदंडों के लिए उत्कृष्ट परिणाम प्राप्त हुए हैं जो इस प्रकार हैं), बायोमास (48.00 ग्राम) और फलों की उपज (118.04 ग्राम)। फूसरियम और वर्टिसिलियम विल्ट के खिलाफ सबसे ज्यादा बीमारी में कमी भी वर्मीकॉम्प IV द्वारा प्राप्त की गई।

वर्तमान अध्ययन से पता चला है कि जैवोनोकुलंट्स और जेट्रोफा केक के एकीकरण ने रसोई के कचरे के तेजी से खाद में एक महत्वपूर्ण भूमिका निभाते हुए 60 दिनों में गुणवत्तायुक्त खाद का उत्पादन किया है। उत्पादित वानस्पतिक खाद में पौधे के पोषक तत्वों को प्रदान करने के साथ-साथ फ़्यूसरियम और वर्टिसिलियम विल्ट के खिलाफ जैव कीटनाशक के रूप में प्रयोग करने की दोहरी भूमिका थी।

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ABBREVIATIONS

ANNOVA	Analysis Of Variance
C/N	Carbon to Nitrogen ratio
cm	Centimeter
PDB	Potato Dextrose Broth
PDA	Potato Dextrose Agar
^o C	Degree centigrade
EC	Electrical conductivity
Fig.	Figure
g	Gram
ha	Hectare
l	Liter
ml	Milliliter
kg	Kilogram
min	Minute
μ mol	Micro moles
FTIR	Fourier transform infrared
cfu	Colony forming units
mS/cm	Milli Siemens per centimeter
TOC	Total organic carbon
TKN	Total kjeldahl nitrogen
dia	Diameter
IU	International Units
kDa	Kilo Dalton
Cel	Cellulase
Xyl	Xylanase
EMB	Eosin methylene blue
MPN	Most Probable Number
BSF	Black Solder fly
cm ⁻¹	Per centimeter
MSW	Municipal solid waste
GC	Gas chromatography
MS	Mass spectroscopy
FPU	Filter paper Unit
CMCase	Carboxy Methyl Cellulase
%	Percentage
SSF	Solid State Fermentation
DNS	3,5-Dinitrosalicylic Acid
K	Potassium
N	Nitrogen
P	Phosphorus
Av	Available
w/w	Weight by weight