

**BOTANICAL FORMULATIONS FOR THE
MANAGEMENT OF IMPORTANT
SELECTED PESTS – TERMITES, APHIDS
AND FUNGI**

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CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

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Submitted

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DEDICATED TO MY FAMILY

CERTIFICATE

This is to certify that the thesis entitled “*Botanical Formulations for the Management of Important Selected Pests – Termites, Aphids and Fungi*” submitted by **Mr. Sharad Verma** has been prepared under our guidance with the rules and regulations of Indian Institute of Technology Delhi, India. 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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(Sharad Verma)

ABSTRACT

Termites, aphids and fungal diseases cause substantial losses to the agriculture. Development of botanical pesticides for pest control, as a sustainable alternative to chemical insecticides, is an emerging arena in the present scenario. Although plants contain compendium of bioactive compounds with bio-pesticidal value, adaptability of plant based pesticides in the form of commercial product is needed to establish the applicability at ground level. The present study attempted to design a discrete approach for effective utilization of botanicals in a deliverable product form with the accomplishment of employment generation facet.

The study began with the evaluation of termiticidal value of potent botanicals. Various aqueous and solvent extracts of different plant parts of *Jatropha* (*Jatropha curcas*) and *Datura* (*Datura stramonium*) as well as Orange (*Citrus sinensis*) and Marigold (*Tagetes erecta*) essential oils were tested against two species of termites (*Odontotermes obesus* and *Microtermes incetoides*). *Jatropha* root methanolic extract, *Datura* leaf hexane extract and Marigold oil were obtained with best anti-termitic activity showing comprehensive termite mortality in considerable time. Among these, Marigold oil showed excellent termite control with 100 % mortality in 16 hours at 0.1 mL mL⁻¹ concentration. Comparable results were obtained with *M. incetoides* however this species showed more resistance than *O. obesus*. Chemical composition analytical study of these botanicals suggested the presence of terpenoids and fatty acids in *Jatropha*; alkaloids and terpenoids in *Datura* and terpenoids in Marigold essential oil, responsible for termiticidal activity. Based on best results, aforesaid botanicals were selected to bring about advancement, in the form of deliverable product through formulation development. The unstable crude botanicals were converted into stable and storable form of micro-emulsions (ME) and emulsifiable concentrate (EC) formulations. Two ME, from *Jatropha* root (JRF) and *Datura* leaf extracts (DLF) each and; one EC from Marigold oil, formulations were developed. Stability, storability and intactness of developed formulations were confirmed through physical and chemical characterization. Rigorous testing of the formulations was done to establish their termiticidal potential at lab and field level. Lab results of formulations showed significant efficacy against both the species of termite. Total (100 %) *O. obesus* mortality was achieved in 4 hours with MOF dilution comprising 0.03 mL mL⁻¹ Marigold oil. However, same mortality of termite (*O. obesus*) was observed in 40 and 44 hours with JRF and DLF (dilutions containing 0.0175 g mL⁻¹ active crude extract), respectively. *M. incetoides* showed some resistance and delayed mortality in comparison to *O. obesus* termites. In field experiments (conducted at Micro-model, IIT Delhi) also, the formulations proved their potency with significant wood protection. However, dip treatment provided higher wood preservation for longer duration than surface coating treatment. Diluted (1:1 ratio with water) and undiluted JRF and MOF dip treated wooden blocks were recorded with no weight loss for the test period of six months whereas in case of DLF, 5.87 (1:1 dilution) and 2.36 % (no dilution) wood was consumed by termites in the same duration. Moreover, 78.47 % weight loss occurred in control. Deep impregnation and proper absorption of formulations inside the wooden blocks through dip treatment might

have been the reason for better protection of wood. Further, onsite testing (at two sites inside the IIT Delhi) of formulations (1:1 dilution) offered complete protection of wooden structures for six months when applied through sprayer at the rate of 9 ml / sq. feet twice (second time after one month of the first application). To accomplishment of vision of botanical pesticide propagation from *Lab to Land*, bio-efficacy testing of formulation was carried out at agricultural field at Farrukhnagar, Gurgaon, Haryana. During crop (*Chrysanthemum*) season of 6 months, no termite infestation was noticed in the test crop field as healthier plants, high plant density and no crop loss was observed. However, 47.5 % crop loss was recorded with untreated control field. Farmers were satisfied with crop protection offered by **Marigold oil formulation** applied at the rate of 200 mL / acre. In another field testing of formulation under different climatic condition at Institute of Wood Science and Technology (IWST), Bangalore, the MOF formulation (undiluted) presented 88.5 % wood protection (through pressure treatment) during 6 months. Formulations were found to be cost effective in economics calculation. On the basis of providing the employment to a person on salary basis (INR 20,000 / month), cost of one liter JRF, DLF and MOF was estimated to be INR 444.0, 392.0 and 1239.0 respectively. To establish the multi-targeted approach of the developed bio-formulations, broad range pesticidal potential was also evaluated by testing its efficacy against Mustard aphid (*Lipaphis erysimi*) and fungi (*Fusarium oxysporum*, *Rhizopus oryzae*, *Rhizoctonia solani* and *Aspergillus niger*). Formulations showed significant aphidicidal activity also, with complete adult mortality in 72 hours (JRF and DLF with 0.025 g mL⁻¹ extract) and 8 hours (MOF with 0.1 mL mL⁻¹ oil). Nonetheless, the susceptibility of nymphs and alates was found higher than adults. Moreover, in case of fungal testing, MOF completely inhibited the growth of all four fungi at all concentrations except 0.001 mL mL⁻¹ while DLF and JRF totally restricted *F. oxysporum*, *R. solani* and *R. oryzae* at 0.00375 and 0.005 g mL⁻¹ tested concentrations. *A. niger* was limited by DLF at highest concentration (0.005 g mL⁻¹) while JRF failed to control *A. niger* at any of the tested concentration. Overall, MOF exhibited excellent results against termites, aphid and fungi suggesting it to be most effective among all the three formulations. It is hoped that the current research findings in the form of extensively tested multi-targeted botanical formulations, would find a significant place in providing solution to the complex problems of multiple pest attack on crops by providing a single sustainable alternative to the cocktail of chemical insecticides.

संक्षेप

दीमक, एफिड्स और फंगल रोग, कृषि के लिए पर्याप्त नुकसान के कारण हैं। वर्तमान परिदृश्य में, रासायनिक कीटनाशकों के स्थायी विकल्प के रूप में, वानस्पतिक कीटनाशकों का विकास एक उभरता हुआ क्षेत्र है। यद्यपि पौधों में जैव-कीटनाशक मूल्य के साथ बायोएक्टिव यौगिकों का संग्रह होता है, फिर भी वनस्पति आधारित कीटनाशकों की वाणिज्यिक उत्पाद के रूप में प्रयोज्यता स्थापित करने की आवश्यकता है। वर्तमान अध्ययन में, रोजगार प्राप्ति के पहलुओं की उपलब्धि के साथ वितरण योग्य कीटनाशक उत्पाद के रूप में वनस्पति के प्रभावी उपयोग के लिए असतत दृष्टिकोण तैयार करने का प्रयास किया गया है।

अध्ययन, अनेक वनस्पतियों के दीमकनाशक मूल्यांकन के साथ शुरू हुआ। जट्रोफा (*Jatropha curcas*) और धतूरा (*Datura stramonium*) पौधों के अनेक भागों के विभिन्न जलीय एवं सॉल्वेंट अर्कों के साथ-साथ ऑरेंज (*Citrus sinensis*) और मैरीगोल्ड (*Tagetes erecta*) के सगंध तेलों का दीमक की दो प्रजातियों (*Odontotermes obesus* एवं *Microtermes incetoides*) के विरुद्ध परीक्षण किया गया। मेथनोलिक जट्रोफा जड़ तथा हेक्सानॉलिक धतूरा पत्ती अर्क और मैरीगोल्ड सगंध तेल, व्यापक उच्छृंखल मृत्यु दर के साथ श्रेष्ठ दीमक विरोधी पाए गए। इनमें से, मैरीगोल्ड तेल ने, 16 घंटे में 0.1 मि.ली./मि.ली. सांद्रता पर 100% मृत्यु दर के साथ अति उत्कृष्ट दीमक नियंत्रण किया। दीमक की दोनों प्रजातियों के साथ तुलनात्मक परिणाम प्राप्त हुए किन्तु *O. obesus* के अपेक्षा में *M. incetoides* अधिक प्रतिरोधी पायी गयी। पौधों के रासायनिक संरचना विश्लेषणात्मक अध्ययन द्वारा यह पता लगाया गया कि, जट्रोफा में टरपेनोइड्स और फैटी एसिड्स, धतूरा में अल्कॅलॉइड्स तथा टरपेनोइड्स एवं मैरीगोल्ड सगंध तेल में टरपेनोइड्स यौगिक, दीमकनाशक गतिविधि के कारण हो सकते हैं। श्रेष्ठ परिणामों के आधार पर, पूर्वोक्त वनस्पतियों को सूत्रीकरण के लिए चयनित किया गया। अस्थिर वानस्पतिक अर्क तथा सगंध तेल को स्थिर और संग्रहणीय रूप, माइक्रोएमुल्सिओन् (एमई) तथा एमुल्सिफ़िअब्ले कंसन्ट्रेट (ईसी) में परिवर्तित किया गया। प्रत्येक, जट्रोफा जड़ और धतूरा पत्तियों के अर्कों से दो एमई क्रमशः जेआरएफ तथा डीएलएफ एवं मैरीगोल्ड तेल से एक एमओएफ नामक ईसी योगों का निर्माण किया गया। भौतिक और रासायनिक लक्षण वर्णन के माध्यम से निर्मित फार्मूलों की स्थिरता और अखंडता की पुष्टि की। योगों की दीमकनाशक क्षमता की जाँच के लिए प्रयोगशाला और क्षेत्र स्तर पर कठोर परीक्षण किये गए। योगों की प्रयोगशाला परिणामों में दीमक की दोनों प्रजातियों के विरुद्ध महत्वपूर्ण प्रभावकारिता पायी गयी। मैरीगोल्ड तेल से बनी फार्मूलेशन के 0.03 मि.ली./मि.ली. जलमिश्रित तरल विलयन द्वारा *O. obesus* की 4 घंटे में 100% मृत्यु दर प्राप्त हुई। जबकि, जेआरएफ तथा डीएलएफ के 0.0175 ग्रा./मि.ली. अर्क युक्त तनु विलयन के साथ दीमक (*O. obesus*) की समान मृत्यु दर, क्रमशः 40 और 44 घंटों में देखी गयी। *O. obesus* दीमक की तुलना में, *M. incetoides* की प्रतिरोध के साथ विलम्बित मृत्यु दर प्राप्त हुई। फ़ील्ड (माइक्रो-मॉडल, आईआईटी दिल्ली में आयोजित) प्रयोगों में भी, योगों ने लकड़ी के सार्थक संरक्षण में अपनी क्षमता साबित की। हालांकि, सतह लेप उपचार की तुलना में आप्लावन उपचार विधि द्वारा, लंबी अवधि तक लकड़ी संरक्षण प्राप्त हुआ। जलमिश्रित (1:1 अनुपात) तथा विशुद्ध जेआरएफ एवं एमओएफ आप्लावन उपचारित, छह महीनों के

परीक्षण में, लकड़ी खंडों में कोई वजन क्षति दर्ज नहीं की गयी अपितु समान समय अवधि और समान तरलता एवं विशुद्ध डीएलएफ के साथ क्रमशः 5.87 तथा 2.36% भार क्षति पायी गयी। इसके अतिरिक्त, अनुपचारित लकड़ी खंडों में 78.47% भार क्षति प्राप्त हुई। आप्लावन उपचारित परीक्षण विधि में लकड़ी द्वारा योगों का गहरी संसेचन और उचित अवशोषण, बेहतर संरक्षण के कारण हो सकते हैं। इसके अतिरिक्त, ऑनसाइट (आईआईटी दिल्ली में दो साइटों पर) परीक्षण में, फॉर्मूलेशनों (1:1 जलमिश्रित अनुपात) का स्प्रेयर द्वारा 9 मि.ली./वर्ग फुट की दर से दो बार (पहले आवेदन के एक महीने के बाद दोबारा) आवेदन करने पर लकड़ी की संरचनाएं, छह महीनों तक पूर्णतः सुरक्षित पायी गयीं। वानस्पतिक कीटनाशकों का, प्रयोगशाला से भूमि तक फैलाव के दृष्टिकोण से, फरुखनगर, गुड़गांव, हरियाणा में एक कृषि भूमि पर फार्मूलेशन का जैव-प्रभावकारिता परीक्षण किया गया। इस परीक्षण में मेरीगोल्ड तेल निर्मित फार्मूलेशन का 200 मि.ली./एकड़ की दर से गुलदाउदी की फसल पर छह माह की फसल अवधि के लिए उपचार किया गया। परिणामस्वरूप, निरोग एवं स्वस्थ पौधों के साथ, उच्च सघनता पायी गयी और दीमक द्वारा फसल में कोई हानि नहीं देखी गई। तथापि, अनुपचारित क्षेत्र में 47.5% फसल हानि दर्ज की गई। किसान, मेरीगोल्ड तेल निर्मित फार्मूलेशन द्वारा फसल संरक्षण से संतुष्ट थे। निर्मित फार्मूलेशन का, विभिन्न जलवायु परिस्थिति परीक्षण के तहत एक और प्रयोग (लकड़ी खंड का दबाव उपचारित संरक्षण), लकड़ी विज्ञान और प्रौद्योगिकी संस्थान (आईडब्ल्यूएसटी), बेंगलुरु में किया गया। इस परीक्षण में विशुद्ध एमओएफ द्वारा 6 महीने के दौरान 88.5% लकड़ी संरक्षण प्राप्त हुआ। अर्थशास्त्र गणना में निर्मित फॉर्मूलेशन किफायती पायी गयीं। एक व्यक्ति को 20,000 रुपये प्रति माह की दर से रोजगार प्रदान करने के आधार पर, एक लीटर जेआरएफ, डीएलएफ और एमओएफ बनाने की कीमत क्रमशः 444.0, 392.0 और 1239.0 रुपये परिकलित की गयी। विकसित जैव-फार्मूलस के बहु-लक्षित दृष्टिकोण को स्थापित करने के लिए, इनकी कीटनाशक प्रभावकारिता का मूल्यांकन, मस्टर्ड एफिड (*Lipaphis erysimi*) एवं कवक (*Fusarium oxysporum*, *Rhizopus oryzae*, *Rhizoctonia solani* और *Aspergillus niger*) के विरुद्ध भी किया गया। परीक्षण में, फॉर्मूलेशन की एफिडनाशक प्रभावकारिता सार्थक पायी गयी। जेआरएफ और डीएलएफ (0.025 ग्रा./मि.ली. अर्क) द्वारा वयस्कों का पूर्ण उन्मूलन 72 और एमओएफ द्वारा 8 घंटों में निरीक्षित हुआ जब की वयस्कों की तुलना में निम्फ तथा आलाते अधिक संवेदनशील पाए गए। इसके अतिरिक्त, कवक परीक्षण में, एमओएफ की 0.001 मि.ली./मि.ली. सांद्रता के अलावा सभी सांद्रताओं ने चारों कवकों के विकास को रोक दिया। जबकि, जेआरएफ और डीएलएफ की 0.00375 और 0.005 ग्रा./मि.ली. सांद्रताओं पर *F. oxysporum*, *R. solani* और *R. oryzae* कवकों का विकास पूर्णतः प्रतिबंधित पाया गया। *A. niger* की वृद्धि को जेआरएफ रोक पाने में असमर्थ रही और डीएलएफ की केवल सर्वोच्च सांद्रता (0.005 ग्रा./मि.ली.) ही *A. niger* को रोक पायी। संपूर्णतया, एमओएफ के दीमियों, एफिड और कवकों के विरुद्ध उत्कृष्ट परिणाम पाए गए जिससे यह निष्कर्ष निकलता है कि सभी तीनों फार्मूलों में से सबसे प्रभावी मेरीगोल्ड तेल से बनी फार्मूलेशन रही। मौजूदा शोध निष्कर्ष से यह उम्मीद की जाती है कि बहु-लक्षित बोटैनिकल फार्मूलों के रूप में, रासायनिक कीटनाशकों के लिए एक स्थायी विकल्प प्रदान करके फसलों पर कई कीटों के हमले की जटिल समस्याओं का समाधान प्रदान करने में सहायक होगा।

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ABBREVIATIONS

ANOVA	Analysis of Variance
ME	Microemulsion
EC	Emulsifiable Concentrate
°C	Degree Centigrade
g	Gram
L	Litre
mg	Milligram
ml	Millilitre
/	Per
g mg⁻¹	Per milligram
g ml⁻¹	Per millilitre
%	Percentage
rpm	rotation per minute
JRF	Jatropha root formulation
DLF	Datura leaf formulation
MOF	Marigold oil formulation