ECO-FRIENDLY APPROACH TO CONTROL *CALLOSOBRUCHUS MACULATUS* F. IN PULSE STORAGE FOR FOOD SAFETY

UMESH CHANDRA SHARMA

CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

UMESH CHANDRA SHARMA

Centre for Rural Development and Technology

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Dedicated to

My Parents, Family

and

Sri Krishna ji

Certificate

This is to certify that the thesis entitled **"Eco-friendly Approach to Control *Callosobruchus maculatus* F. in Pulse Storage for Food Safety"** being submitted by **Mr. Umesh Chandra Sharma** to the Indian Institute of Technology, Delhi, for the award of **'Doctor of Philosophy'** is a record of bonafide research work carried out by him under our guidance and supervision in conformity with the rules and regulations of Indian Institute of Technology Delhi. The research report and results presented in this thesis have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.

Prof. Hariprasad P.

(Associate Professor)

Centre for Rural Development of Technology,

Indian Institute of Technology, Delhi

Prof. Santosh Satya

(Honorary Professor)

Centre for Rural Development of Technology,

Indian Institute of Technology, Delhi

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Umesh Chandra Sharma

ABSTRACT

Pulses (family Leguminosae) are the major source of protein (20-30 %) in the everyday Indian diet. They are also a good source of other nutrients, including fiber, vitamins, and minerals. Pulses are susceptible to insect infestation during storage, deteriorating their quality and quantity. The Coleopteran insects (Bruchidae family) are the most common insects associated with stored legumes. *Callosobruchus maculatus* is very common and highly detrimental to pulses during storage. Farmers in developing countries like India have traditionally used various non-chemical methods, including plant-based materials like leaves, spices, and essential oils, to protect their stored grains from insects. In previous studies, essential oils such as eucalyptus oil demonstrated insecticidal and repellant activity against various storage insects, including *Rhyzopertha dominica*, *Tribolium castaneum*, and *C. maculatus*. Different chemical pesticides in large-scale grain storage systems are currently employed to combat insect infestations; however, these pesticides have proven detrimental to human health and the environment. Therefore, developing eco-friendly holistic solutions for insect pest control during storage is necessary. In the present study, research is carried out to develop a metallic bin and eucalyptus essential oil-based nanoemulsion to control *C. maculatus* during pulse (*Vigna mungo*) storage.

Initially, a field study was conducted in Nandgao (Uttar Pradesh, India) to understand the current grain storage system for storing pulses domestically. It was found that almost 75 % of farmers keep their legume grain in plastic bottles or jars. Jute and polyester bags are also being used. Only 5 % of farmers (very small landholding) purchase from the market as per their needs. It was noted that the traditional storage methods/practices were insufficient to ensure complete protection during storage. The findings highlight the necessity of developing a new storage bin specially designed for pulse storage.

The *C. maculatus* infestation has a negative impact on the nutritional value of stored pulses. Additionally, changes in the amino acid profile resulted in nutritional deficiencies in the infested pulses, rendering them unfit for human consumption. In the absence of insect infestation, most nutrients in stored black gram grain remained steady. As the storage insects consumed the carbohydrates over time, the proportion of

carbohydrates decreased. It was found that the level of insect infestation was negatively correlated with the fat and carbohydrate proportions while positively correlated with the protein content.

To determine their chemical composition, every essential oil underwent a standardized investigation. The chemical composition of the chosen essential oils-*Mentha piperita, Eucalyptus globulus*, *Curcuma longa*, *Cymbopogon citratus,* and *Ocimum sanctum,* was confirmed by the results of the GC-MS data. The effectiveness of each essential oil against *C. maculatus* was evaluated using bioassays for repellent activity, contact toxicity, and fumigant toxicity. *Eucalyptus globulus* essential oil was the most effective repellent against *C. maculatus*, whereas *O. sanctum* was the least effective.

Based on the results obtained after screening essential oils for insecticidal activity, *Eucalyptus globulus* was chosen to develop a formulation against *C. maculatus.* Several combinations (F1, F2, F3, F4, and F5) were tried and optimized to get a stable nanoemulsion. The absence of phase separation, creaming, or crystallization demonstrated the kinetic stability of F4 nanoemulsion. The spherical shape of the emulsion droplets was validated by TEM imaging, and the nanoscale homogenous composition was further confirmed by the small droplet size (17.16 nm) and low PDI (0.313) values. Its stability was confirmed after six months of storage, as there was no sign of crystallization, creaming, phase separation, or sedimentation. Testing at the laboratory level verified the nanoemulsion's potency against *C. maculatus.*

Finally, based on the findings, a new metallic bin capacity of 20 kg was designed for pulse storage to test the bio-efficacy of the nanoemulsion under simulated field settings (in artificial infestation conditions). It was found that insect infestation caused the moisture content (MC) of black gram stored in the negative control (NC) bins to increase from 7.91 to 8.54 %. In contrast, insect feeding caused a significant decrease in the 100-grain weight of infected samples (from 3.86 to 3.34 g). It was found that the untreated bins had a higher population of insect pests and egg counts as compared to untreated storage bins. This may be due to the development of favorable conditions for insect multiplication in untreated bins. Both weight loss (less than 1 %) and grain damage (less than 1 %) were minimal in treated bins (T) due to the arrested growth of insects at earlier stages in the treated bins. Insect trap also effectively captures the insect, enhancing the effectiveness of the newly designed bin. Thus, an integrated system based on a newly

designed storage bin and developed nanoformulation showed effective control of *C. maculatus* during the storage period of three months. It indicated its feasibility for field application. Also, the insect traps in the new design can act as insect monitoring devices in the grain storage system.

शोध-सारांश

दालें भारतीय दैनिक आहार में प्रोटीन (20-30 %) का प्रमुख स्रोत हैं। वे फाइबर, विटामिन और खनिजों सहित अन्य पोषक तत्वों का भी एक अच्छा स्रोत हैं। भंडारण के दौरान दालें कीटों के संक्रमण के प्रति संवेदनशील होती हैं, जिससे उनकी गुणवत्ता और मात्रा पर प्रतिकूल प्रभाव पडता है। कोलॉप्टेरान कीट (Bruchid family) दाल भंडारण से जुड़े सबसे आम कीट हैं, जिनमें *कैलोसोब्रुचस मैक्युलेटस* (*Callosobruchus maculatus*) प्रमुख रूप से भंडारित-दालों के लिए हानिकारक है। भारत जैसे विकासशील देशों में, किसान भंडारित अनाज को कीटों से बचाने के लिए विभिन्न पारंपरिक तरीकों (गैर-रासायनिक) का इस्तेमाल करते हैं, जिनमें पेड़-पौधों से प्राप्त सामग्री जैसे पत्ते, मसाले और इसेंशियल ऑयल आदि शामिल हैं। कई अध्ययनों में नीलगिरी (*यूकेलिप्टस*) जैसे इसेंशियल ऑयल ने विभिन्न भंडारण-कीटों (जैसे *राइजोपर्था डोमिनिका, ट्राइबोलियम कैस्टेनम* और *सी. मैक्युलेटस*) के विरुद्ध कीटनाशक-गुण प्रदर्शित किये हैं। वर्तमान परिवेश में दाल-भंडारण के दौरान कीटों से निपटने के लिए विभिन्न रासायनिक कीटनाशकों का उपयोग किया जाता है; परंतु, ये कीटनाशक मानव स्वास्थ्य और पर्यावरण के लिए हानिकारक सिद्ध हुए हैं। इसलिए, भंडारण के दौरान कीटों को नियंत्रित करने के लिए गैर-रासायनिक समाधान विकसित करना आवश्यक है। हमने प्रस्तुत शोध में, घरेलु स्तर पर दाल-भंडारण के दौरान *सी. मैक्युलेटस* को नियंत्रित करने के लिए एक धातु-निर्मित बिन (Metallic bin) और *यूकेलिप्टस*-इसेंशियल ऑयल आधारित नैनोइमल्शन विकसित करने का प्रयास किया है।

वर्तमान में घरेलु स्तर पर दाल-भंडारण प्रणाली को समझने के लिए नंदगाँव (उत्तर प्रदेश, भारत) में एक क्षेत्रीय अध्ययन किया गया। हमने पाया कि लगभग 75 % किसान अपनी उपज को प्लास्टिक की बोतलों या जार में रखते हैं। कुछ किसान जूट

और पॉलिएस्टर बैग का भी इस्तेमाल करते हैं। केवल 5 % किसान (छोटी जोत वाले) दालों का भंडारण नहीं करते हैं; बल्कि अपनी जरूरत के हिसाब से बाजार से खरीदते हैं। अध्ययन में, पाया गया कि भंडारण के दौरान पूर्ण सुरक्षा सुनिश्चित करने के लिए पारंपरिक भंडारण विधियां/प्रणालियां अपर्याप्त हैं। अत:, एक विशेष स्टोरेज-बिन विकसित करने की आवश्यकता है, जोकि घरेलू स्तर पर दाल-भंडारण के लिए उपयुक्त हो।

सी. मैक्यूलेटस संक्रमण का भंडारित-दालों के पोषक-तत्वों पर भी प्रतिकूल प्रभाव पड़ा; साथ ही, अमीनो-एसिड प्रोफ़ाइल में भी परिवर्तन पाया गया, जो उसे मानव-उपभोग के लिए अनुपयुक्त बनाता है। कीटों की अनुपस्थिति में, भण्डारित उरद-दाल के अधिकांश पोषक-तत्वो पर कोई प्रतिकूल प्रभाव नहीं पाया गया। भंडारण के दौरान, कीटों द्वारा सेवन के कारण कार्बोहाइड्रेट तथा वसा का स्तर कम हुआ। यह भी पाया गया कि कीट-संख्या, वसा और कार्बोहाइड्रेट स्तर के साथ नकारात्मक, जबकि प्रोटीन स्तर के साथ सकारात्मक रूप से सहसंबद्धित थी।

प्रत्येक इसेंशियल-ऑयल के प्रमुख रासायनिक-अवयवों को जानने के लिये जी.सी.-एम.एस. द्वारा जांच की गई। जी.सी.-एम.एस. डेटा के परिणामों से इसेंशियल-ऑयल (*मेंथा पिपेरिटा, यूकेलिप्टस ग्लोब्युलस, कुरकुमा लोंगा, सिंबोपोगोन साइट्रेटस* और *ओसिमम सैंक्टम*) के प्रमुख रासायनिक-अवयवों की पुष्टि की गई। *सी. मैक्युलेटस* के विरुद्ध प्रत्येक इसेंशियल-ऑयल की प्रभावशीलता का मूल्यांकन विभिन्न बायोएसेज़ (Contact toxicity, Repellence, and Fumigant toxicity) द्वारा किया गया। *ई. ग्लोब्युलस* इसेंशियल-ऑयल, *सी. मैक्युलेटस* के विरुद्ध सबसे अधिक प्रभावी पाया गया।

प्राप्त परिणाम के आधार पर, *ई. ग्लोब्युलस* इसेंशियल-ऑयल को कीटनाशक विकसित करने के लिए चुना गया। विभिन्न संयोजनों (F1, F2, F3, F4 और F5) को आजमाकर, एक स्थिर-नैनोइमल्शन तैयार किया गया। F4-नैनोइमल्शन में पृथक्करण, क्रीमिंग या क्रिस्टलीकरण की अनुपस्थिति उसकी स्थिरता को दर्शाता है। नैनोइमल्शन

की बूंदों के गोलाकार को टी.ई.एम. इमेजिंग द्वारा सत्यापित किया गया। बूंदों का छोटा आकार (17.16 nm) और कम पी.डी.आई. (0.313) ने नैनोस्केल-संरचना और समरूपता की पुष्टि की। छ: माह के भंडारण के बाद क्रिस्टलीकरण, क्रीमिंग, पृथक्करण या अवसादन का कोई संकेत नहीं पाया गया, जोकि नैनोइमल्शन की स्थिरता की दर्शाता है। प्रयोगशाला-परीक्षण *सी. मैक्युलेटस* के विरुद्ध नैनोइमल्शन की प्रभावशीलता को दर्शाते हैं।

अंत में, निष्कर्षों के आधार पर, दाल-भंडारण के लिए 20 कि.ग्रा. क्षमता का एक नया धातु-निर्मित स्टोरेज-बिन तैयार किया गया, तदोपरांत कृत्रिम कीट-संक्रमण करके (Simulated field settings) में नैनोइमल्शन की जैव-प्रभावशीलता का परीक्षण किया गया। परिणामों में पाया गया कि कीट-संक्रमण के कारण NC-बिन में भण्डारित उरद-दाल में नमी की मात्रा (MC) 7.91 % से बढ़कर 8.54 % हो गई, और 100-ग्रेन-वेट 3.86 से 3.34 ग्रा. हो गया, जोकि उल्लेखनीय है। कीटों और अंडों की संख्या अनुपचारित-बिन में उपचारित-बिन से अधिक पायी गायी, जिसका मुख्य कारण अनुपचारित-बिन में कीटों के लिए अनुकूल परिस्थितियों विकसित होना माना जा सकता है। शुरुआत में ही नैनोफॉर्मुलेशन के प्रभाव से कीटों की वृद्धि रुक जाने के कारण, उपचारित-बिन (T) में वेट-लौस (<1 %) और ग्रेन-डैमेज (<1 %) दोनों ही न्यूनतम पाये गये। Insect-trap भी कीटों को पकड़ने में प्रभावी पाया गया, जो इस बिन की प्रभावशीलता को बढ़ा देता है। इसलिए कहा जा सकता है कि नए स्टोरेज-बिन और नैनोफॉर्मुलेशन ने सामूहिक रूप से *सी. मैक्युलेटस* का प्रभावी रुप से नियंत्रण किया और भण्डारित-दाल की क्षति को कम किया। अत: यह स्टोरेज-बिन एवं नैनोफॉर्मुलेशन संयुक्त रुप से घरेलू स्तर पर दाल-भण्डारण के लिए एक उपयुक्त समाधान सिध्द हो सकता है।

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