

**NANO-BIOPESTICIDES DEVELOPMENT USING
COW DUNG AND COW URINE AGAINST
Oligonychus coffeae Nietner**

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COW DUNG AND COW URINE AGAINST
Oligonychus coffeae Nietner**

by

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Submitted

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CERTIFICATE

This is to certify that the thesis entitled “**Nano-Biopesticides Development Using Cow Dung and Cow Urine Against *Oligonychus coffeae* Nietner**”, being submitted by **Ms. Mansi Mishra** to the Indian Institute of Technology Delhi for the award of the degree of Doctor of Philosophy is a record of bonafide research work carried out by her. She has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis, which has attained the standard required for a Ph.D. degree at this Institute. The results represented in this thesis have not been submitted elsewhere for the award of any degree or diploma.



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ABSTRACT

The red spider mite or *Oligonychus coffeae* Nietner is a significant pest of tea (*Camellia sinensis* L.) in the majority of tea-producing nations. In Northeast India, it is among the most devastating pests that affect tea plants. Currently, synthetic acaricides are used to control the pest. Excessive use of which causes a variety of problems, including pesticide resistance, impact on species not intended to be affected, environmental pollution, and residues in tea. Alternative pest management techniques are thus being used more frequently; however, the continued use of synthetic pesticides on food and beverage crops is raising concerns around the globe due to their possible impacts on human health and the environment. A promising approach for controlling several pests has been made possible by recent advancements in nanotechnology. In the current work, biological synthesis of zinc oxide nanoparticles and iron oxide nanoparticles using cow urine and cow dung extract and acaricidal effect on *O. coffeae* was reported. UV-vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy dispersive X-ray (EDX) analysis were used to bio-physically characterize the biosynthesized iron oxide nanoparticles and zinc oxide nanoparticles using extract of cow dung and cow urine. An absorption peak at 410 nm was observed in the UV-Vis spectra of cow dung-mediated zinc oxide nanoparticles, whereas an absorption peak at 365 nm was observed in the cow urine-mediated zinc oxide nanoparticles. UV-Vis spectral analysis revealed an absorption peak at 290 nm, and 293 nm for cow dung extract and cow urine-mediated zinc oxide nanoparticles. The biophysical characterization of the synthesized zinc oxide nanoparticles using cow dung extract had rod structures with particle sizes ranging from 44 nm to 60 nm, while synthesized zinc oxide nanoparticles using cow urine have spherical structures with diameters ranging from 24 nm to 40 nm. Iron oxide nanoparticles synthesized using cow dung extract and cow urine were spherical in shape, with particle sizes ranging from 50 nm to 55 nm and 46.5 nm and 73.5 nm, respectively. The experiments carried out under laboratory and field conditions

significantly demonstrated the efficacy of biologically synthesized zinc oxide nanoparticles and iron oxide nanoparticles using cow urine and cow dung extract for the control red spider mites. Using the leaf dip and spray method, the acaricidal effect of biologically synthesized zinc oxide and iron oxide nanoparticles at different concentrations of 100 ppm, 250 ppm, 500 ppm, 750 ppm, and 1000 ppm, was tested against *O. coffeae* at various stages (adults, nymphs, and eggs) at the laboratory. The mortality percentage of the pest was recorded for the different concentrations of nanoparticles tested. The mortality was observed to be dose- and time-dependent, i.e., mortality increases with an increase in the concentration of nanoparticles. The mortality of 71.67 % and 86.67 % against cow dung-mediated zinc oxide nanoparticles, 83.33 % and 98.33 % against cow urine-mediated zinc oxide nanoparticles was observed for adults and nymphs of *O. coffeae* at 1000 ppm for the leaf dip and spray method. Mortality of 63.33 % and 93.33 % was observed against cow dung mediated iron oxide nanoparticles, and, 93.33 % and 96.67 % mortality against cow urine mediated iron oxide nanoparticles was observed for adults and nymphs of *O. coffeae* at 1000 ppm for the leaf dip and spray method. The ovicidal activity for leaf dip and spray method was observed to be 61.11% and 64.44% for cow dung-mediated zinc oxide nanoparticles, 95 % and 96.67 % for cow urine-mediated zinc oxide nanoparticles, 66.67 % and 70 % for cow dung-mediated iron oxide nanoparticles, and 51.67 % and 98.33 % for cow urine-mediated iron oxide nanoparticles on eggs of *O. coffeae* at 1000 ppm. Deposition of eggs by adult mites on treated leaf surfaces decreased significantly, and the viability of eggs was also significantly reduced. Field bio-efficacy results indicated that the cow dung-mediated zinc oxide and iron oxide nanoparticles and cow urine-mediated zinc oxide and iron oxide nanoparticles at 750 ppm and 1000 ppm concentrations were highly effective against *O. coffeae* and were superior to the commonly used pesticides like Azadirachtin 0.03% EC and Propargite 57% EC. Different dosages of the zinc oxide and iron oxide nanoparticle solution and cow urine-mediated zinc oxide and iron oxide nanoparticle solution were sprayed onto tea bushes, but no phytotoxic effects were observed. The appearance of liquor, flavour, and

taste of black tea manufactured from tea shoots treated with these solutions was unaffected by the treatments, and the tea was free from any taint. Further, the cow dung-mediated zinc oxide and iron oxide nanoparticle solution and cow urine-mediated zinc oxide and iron oxide nanoparticle solution had no significant effect on nontarget natural enemies and soil microbiota. Cow dung and cow urine-mediated zinc oxide and iron oxide nanoparticles showed no significant antibacterial effects against *Escherichia coli*, *Bacillus paranthracis*, and *Bacillus thuringiensis*. This study concludes that cow dung-mediated zinc oxide and iron oxide, as well as cow urine-mediated zinc oxide and iron oxide nanoparticles, are both efficient against *O. coffeae* and may be employed as a substitute pest control agent in the future to manage tea pests.

सारांश

Red spider mite or *Oligonychus coffeae* Nietner अधिकांश चाय उत्पादक देशों में चाय (*Camellia sinensis* L.) का एक महत्वपूर्ण कीट है। पूर्वोत्तर भारत में, यह चाय के पौधों को प्रभावित करने वाले सबसे विनाशकारी कीटों में से एक है। फिलहाल, कीट को नियंत्रित करने के लिए सिंथेटिक एसारिसाइड्स का उपयोग किया जाता है। जिसके अत्यधिक उपयोग से कई तरह की समस्याएं पैदा होती हैं, जिनमें कीटनाशक प्रतिरोध, प्रभावित न होने वाली प्रजातियों पर प्रभाव, पर्यावरण प्रदूषण और चाय में अवशेष शामिल हैं। वैकल्पिक कीट प्रबंधन तकनीकों का इस प्रकार अधिक बार उपयोग किया जा रहा है; हालाँकि, खाद्य और पेय फसलों पर सिंथेटिक कीटनाशकों का निरंतर उपयोग मानव स्वास्थ्य और पर्यावरण पर उनके संभावित प्रभावों के कारण दुनिया भर में चिंता पैदा कर रहा है। नैनोटेक्नोलॉजी में हालिया प्रगति से कई कीटों को नियंत्रित करने के लिए एक आशाजनक दृष्टिकोण संभव हो गया है। वर्तमान कार्य में, गोमूत्र और गाय के गोबर के अर्क का उपयोग करके जिंक ऑक्साइड नैनोपार्टिकल्स और आयरन ऑक्साइड नैनोपार्टिकल्स के जैविक संश्लेषण और *O. coffeae* पर एसारिसाइडल प्रभाव की सूचना दी गई थी। UV-vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy dispersive X-ray (EDX) analysis का उपयोग जैव-भौतिक रूप से जैवसंश्लेषित and iron oxide nanoparticles using cow urine and cow dung extract का उपयोग करके चिह्नित करने के लिए किया गया था। गाय के गोबर का उपयोग करके संश्लेषित zinc oxide nanoparticles के UV-Vis spectra में 410 nm पर एक अवशोषण शिखर देखा गया, जबकि गोमूत्र का उपयोग करके zinc oxide nanoparticles में 365 nm पर अवशोषण शिखर देखा गया। UV-Vis spectral विश्लेषण ने 290 nm और 239 nm पर गाय के गोबर के अर्क और गोमूत्र का उपयोग करके iron oxide nanoparticles के लिए एक अवशोषण शिखर का खुलासा किया गया। गाय के गोबर के अर्क का उपयोग करके संश्लेषित zinc oxide nanoparticles के बायोफिजिकल लक्षण वर्णन में 44 nm से 60 nm तक कण आकार के साथ रॉड संरचनाएं थीं, जबकि गोमूत्र का उपयोग करने वाले संश्लेषित zinc oxide nanoparticles में 24 nm से

40 nm तक के व्यास के साथ गोलाकार संरचनाएं होती हैं। गाय के गोबर के अर्क और गोमूत्र का उपयोग करके संश्लेषित iron oxide nanoparticles आकार में गोलाकार थे, जिनके कण आकार क्रमशः 50 nm से 55 nm और 46.5 nm और 73.5 nm थे। प्रयोगशाला और क्षेत्र की स्थितियों के तहत किए गए प्रयोगों ने जैविक रूप से संश्लेषित zinc oxide nanoparticles और iron oxide nanoparticles की प्रभावकारिता में गोमूत्र और गाय के गोबर के अर्क का उपयोग किया। लीफ डिप और स्प्रे पद्धति का उपयोग करते हुए, जैविक रूप से संश्लेषित zinc oxide nanoparticles और iron oxide nanoparticles के 100 ppm, 250 ppm, 500 ppm, 750 ppm, और 1000 ppm के विभिन्न सांद्रणों पर एसारिसाइडल प्रभाव का विभिन्न चरणों में *O. coffeae* के खिलाफ परीक्षण किया गया (वयस्क, निम्फ और अंडे) प्रयोगशाला में। मृत्यु दर प्रतिशत की गणना नैनोकणों की विभिन्न सांद्रता के साथ की गई थी। मृत्यु दर को खुराक पर निर्भर और समय पर निर्भर देखा गया, यानी nanoparticles की एकाग्रता में वृद्धि के साथ बढ़ता है। गाय के गोबर की मध्यस्थता वाले zinc oxide nanoparticles 71.67% और 86.67%, गोमूत्र-मध्यस्थ zinc oxide nanoparticles के खिलाफ 83.33% और 98.33%, गाय के गोबर की मध्यस्थता वाले iron oxide nanoparticles के खिलाफ 63.33% और 93.33%, और गोमूत्र-मध्यस्थ iron oxide nanoparticles के खिलाफ 93.33% और 96.67 % लीफ डिप और स्प्रे विधि के द्वारा 1000 पीपीएम पर *O. coffeae* के वयस्कों और अप्सराओं खिलाफ मृत्यु दर देखी गई। गोबर-मध्यस्थ zinc oxide nanoparticles के लिए ओविसाइडल गतिविधि 61.11% और 64.44%, गोमूत्र-मध्यस्थ zinc oxide nanoparticles के लिए 95% और 96.67%, गाय के गोबर-मध्यस्थ iron oxide nanoparticles के लिए 66.67% और 70%, गोमूत्र-मध्यस्थ iron oxide nanoparticles के लिए 51.67% और 98.33% लीफ डिप और स्प्रे विधि के द्वारा 1000 ppm पर *O. coffeae* के अंडों पर मृत्यु दर देखी गई। उपचारित पत्ती की सतहों पर वयस्क घुनों द्वारा अंडों का जमाव काफी कम हो गया, और अंडों की जीवनक्षमता भी काफी कम हो गई। क्षेत्र जैव-प्रभावकारिता के परिणामों ने संकेत दिया कि 750 ppm और 1000 ppm सांद्रता पर गाय के गोबर-मध्यस्थ zinc oxide और iron oxide nanoparticles और गोमूत्र-मध्यस्थ zinc oxide और iron oxide nanoparticles, *O. coffeae* के खिलाफ अत्यधिक प्रभावी थे और आमतौर

पर उपयोग किए जाने वाले कीटनाशक जैसे Azadirachtin 0.03% EC और Propargite 57% EC से बेहतर थे। चाय की झाड़ियों पर गाय के गोबर-मध्यस्थ zinc oxide और iron oxide nanoparticles घोल और गोमूत्र-मध्यस्थता वाले zinc oxide और iron oxide nanoparticles घोल की अलग-अलग खुराक का छिड़काव किया गया, लेकिन कोई फाइटोटॉक्सिक प्रभाव नहीं देखा गया। इन घोलों से उपचारित चाय की कलियों से निर्मित काली चाय की शराब, स्वाद और स्वाद उपचार से अप्रभावित था, और चाय किसी भी कलंक से मुक्त थी। इसके अलावा, गाय के गोबर-मध्यस्थ zinc oxide और iron oxide nanoparticles सॉल्यूशन और गोमूत्र-मध्यस्थ zinc oxide और iron oxide nanoparticles सॉल्यूशन का गैर-लक्षित प्राकृतिक दुश्मनों और मिट्टी के माइक्रोबायोटा पर कोई महत्वपूर्ण प्रभाव नहीं पड़ा। गाय के गोबर और गोमूत्र-मध्यस्थ zinc oxide और iron oxide nanoparticles ने *Escherichia coli*, *Bacillus paranthracis*, और *Bacillus thuringiensis* के खिलाफ जीवाणुरोधी प्रभाव दिखाया। इस अध्ययन का निष्कर्ष है कि गाय के गोबर-मध्यस्थ zinc oxide और iron oxide nanoparticles, साथ ही गोमूत्र-मध्यस्थ zinc oxide और iron oxide nanoparticles, दोनों *O. coffeae* के खिलाफ कुशल हैं और भविष्य में प्रबंधन के लिए एक विकल्प कीट नियंत्रण एजेंट के रूप में नियोजित किए जा सकते हैं।

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ABBREVIATIONS

%	=	Percentage
°C	=	Degree centigrade
ml	=	Milliliters
cm	=	Centimeters
mm	=	Millimeters
cm ⁻¹	=	Per centimeters
ml ⁻¹	=	Per millimeter
M	=	Molar
mM	=	Millimolar
g	=	Grams
rpm	=	Rotations per minute
nm	=	Nanometers
θ	=	Theta
±	=	Plus minus
Ha	=	Hectare
ppm	=	Parts per million
RH	=	Relative humidity
TV	=	Tocklai variety
UV	=	Ultraviolet
Vis	=	visible
µm	=	micrometer
ANOVA	=	Analysis of variance
CD	=	Critical difference
CV	=	Critical variation

l/ha	=	Liter per hectare
SE	=	Standard error
hrs	=	hours
Fig	=	Figure
min	=	minute
ml/min	=	Milliliters per minute
EC	=	Emulsifying concentrate
IPM	=	Integrated pest management
RSM	=	Red spider mite
MRL	=	Minimum residual limit
psi	=	Pound force square inch
RBD	=	Random block design
No.	=	Number (s)
EPA	=	Environment Protection Agency
WHO	=	World Health Organizations
FAO	=	Food and Agriculture Organizations
EEC/EC	=	European Economic Comission