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APPLICATION OF NANOMATERIALS TO RICE FOR DISEASE RESISTANCE

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IN

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6- Summary

Rice (*Oryza sativa*), a monocot graminae plant, is one of the world's most significant food crops. Rather, it is the primary source of food for half of the world's population, particularly in Asia's southeastern areas. Rice is one of Egypt's most significant major grain crops, ranking second after wheat in terms of importance as a source of food for Egyptians. It is also one of the most important summer crops. This is owing to rice's nutritional value, as well as the fact that it is one of the most profitable crops for farmers. Rice is affected by many pathogens, the most important of which are brown spot disease caused by the fungus *Bipolaris oryzae* and leaf blight caused by the bacteria *Xanthomonas oryzae*. The following are the most important obtained results of the current work:

1- Ten Egyption rice cultivars Sakha 101, Sakha 104, Sakha 106, Sakha 107, Giza 176, Giza 177, Giza 178, Giza 182, Egyptian hybrid and Egyptian jasmine were obtained from the Rice Technology Research Center - Sakha, Kafr El-Sheikh / Egypt and screened for pathogen association.

2- The obtained fungi were initially identified in terms of the morphological shape of the fungal cultures and the shape of the fungal spores as *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Bipolaris hawaiiensis, Cephaliophora tropica, Curvularia lunata, Curvularia tuberculate, Fusarium chlamydosporum, Fusarium verticillioides, Negrospora sp., Penicillium sp., Rhizopus oryzae,* and *Trichoderma sp.* as well as *Bipolaris oryzae* (10 isolates) that causes brown leaf spot disease on rice.

3- The fungal identifcation was confirmed using molecular method. Genomic DNA of the isolates were extracted, and a pair of primers for the internal transcribed spacer (ITS) regions (ITS1, ITS4) were used to amplify the ITS2 region 5.8 ITS1s - from the rRNA (rDNA) genes using the Polymerase chain reaction (PCR). The nucleotide sequences were determined using a sequencer and using the Basic Local Alignment Search Tool (BLAST) in GenBank, and by comparing the sequences obtained for the tested isolates in isolates of each isolate separately with the isolates recorded in the gene bank, it was found that there is a similarity rate of up to 100% For *Alternaria alternata*, 100% for *Curvularia lunata*, 100% for

Cephaliophora tropica, 99% for *Fusarium chlamydosporum*, 98.5% for *Fusarium verticillioides*, and a percentage ranging between 92 and 99% for *Bipolaris oryzae* (10 isolates recorded with global isolates). Using the MEGA 6 program, a genetic tree was created to study the phylogeny of the different isolates based on the similarity in the nucleotide sequence.

4- Two bacterial isolates were associated with varities Giza 178 and Sakha 104 isolated and the initial identifcation using semiselective medium indicated that they are *Xanthomonas oryzae* the causal agent of rice bacterial leaf blight (BLB).

5- The isolated bacteria were initially identified in terms of cell shape, their interaction with Gram stain and the shape of the developing colonies on some differential environments, including nutrient agar, yeast extract and Peptone Sucrose Agar, in addition to the physiological and biochemical tests adopted to identify the genera that cause leaf blight on rice, including the tests that performed: Indole formation, starch hydrolysis, hydrolysis of Tween 80, fermentation of some sugars such as acid production from lactose, glucose, fructose, sorbitol, mannitol, and sucrose, growth in 5% NaCl.

6- the isolated bacteria genomic DNA was extracted for their use in PCR identification experiments, and accordingly: the obtained isolates were identified by using the 16S rRNA gene using a pair of global primers (P0, P6) and then showing its sequence of nucleotides using Basic Local Alignment Search Tool (BLAST) search in GenBank. It was found that 2 isolates belong to the bacterium *Xanthomonas oryzae* and these isolates were recorded with an accession number. MZ714131 and MZ714557.

7- The pathogenicity of *Bipolaris oryzae* isolates on 21 days old rice cultivars (Sakha 108, Sakha Super 300, Giza 177, and Giza182 and Egyptian hybrid) were carried out. Disease severity was estimated using a numerical scale from 0 to 9 as a criterion for differentiating between isolates. The results showed significant differences between the different isolates in their pathogenicity, as isolates BOS104 and BO2G177 were the most virulent isolates for cultivar Giza 177 and the isolate BO2G177 was the highest on Giza 182. The cultivar Giza 177 had the highest susceptibility to infection and it was found that Sakha 108 had the lowest susceptibility to infection, while the cultivars Sakha Super 300, Giza 182 and the Egyptian hybrid were moderate of susceptibility.

8- The pathogenicity of *Xanthomonas oryzae* isolates on rice cultivars (Sakha104 and Giza 178), was conducted by Cassette Holder Method. The obtained results indicated that typical BLB symptoms were observed after rice plants were inoculated with bacteria.

9- Nano-emulsions were used for three natural oils, which are cinnamon, mint and tea tree, as one of the modern methods used to combat rice diseases, as follows:
An analysis of the three oils was done using Gas chromatography-mass spectrometry (GC-MS) to determine the active compounds and the percentage of their presence in each oil. revealed that the following components, cinnamaldehyde, eugenol, benzyl benzoate, methyl eugenol, eugenol, linalool, β-caryophyllene and estragole were identified as major components in *Cinnamomum zeylanicum* essential oil. *Melaleuca alternifolia* essential oil chemical composition indicated that Terpinenol-4, Aromandendrene , alpha-Terpineol as major components were the major components in *Mentha piperita* essential oil are Pulegone (D) , Mint Furanone, Isomenthone, Levomenthol, Eucalyptol , and Caryophyllene .
The oils were converted to a nano-formulation using a sonicator probe to connect to nano-sized particles. Particle characterization was done using a droplet size device and confirmed using a Transmission Electron Microscopy (TEM) and the diameter of the particles ranged from 17 to 60 nm.

10- Emulsion and nano-emulsions of (*Cinnamomum zeylanicum*, *Melaleuca alternifolia* and *Mentha piperita*) oils were used with different concentrations of 12.5, 25, 50, 100, 200, 375, 750 and 1500 µg/ml against ten isolates of the fungus *Bipolaris oryzae* and two isolates of bacteria *Xanthomonas oryzae* in the laboratory. The results showed that the use of oils in the nano form was more effective. Compared to the raw image, there was a difference in the used concentrations, where cinnamon nanoemulsion showed the best effect at a concentration of 200 µg/ml compared to emulsion at 375 µg/ml, followed by nano-emulsion of tea tree oil and then nano-emulsion of peppermint oil.

11- The abovementioned essential oils emulsion and nanoemulsion were used as grains treatment for Giza 177 cultivars as susceptible to infection and Sakha 108 as resistant cultivars by soaking for 12 hours to study their effect on germination rate the obtained data indicated that cinnamon **(***C. zeylanicum***)** essential oils nanoemulsion formulation concentrations were more efficient than Tea tree and Mentha in decreasing the percent of rice grain infection compared to control. The percentage of infection in control grains (0 µg/ml) was 34% in Giza 177 and 25.75% in Sakha 108, while the percentages of infection were declined after rice seeds were treated with *C. zeylanicum* at concentrations 350 µg/mL and 750 µg/ml up to 0%. On the parallel, the same concentration of
M. alternifolia decreased the infection percent up to 8% in Giza 177 and 8.5 in Sakha 108 and *M. piperita* to an inhibition of 2.5% in Giza177 and 5.25% in Sakha108.

12- Cinnamon oil nanoemulsion was used as the best treatment to study its effect on the mycelial growth and spores of one of the isolates of the fungus *Bipolaris oryzae* by examining by scanning electron microscope. The microscopic observations showed the images of conidia and mycelia of the control for *B. oryzae* with typical net structure and smooth surface. While the nanoemulsion treatment clearly damaged the hyphae and conidia of *B. oryzae* as fungal mycelia and conidia were sunken, wrinkled and damaged after 24 h.

13- the efficacy of *C. zeylanicum* nanoemulsions against brown spot disease under greenhouse condition was determined. The disease severity was significantly reduced by the treatments compared with the untreated control for two rice cultivars Giza 177 (susceptible) and Sakha 108 (resistant). The lowest disease severity percentages, being 0.6 and 0.01% were detected with nanoemulsion treatment in cultivar Giza 177 and Sakha 108, respectively followed by 1.44 and 0.15 in case of plants infected with isolate 10 and nano emulsion with cultivars Giza177 and Sakh108, respectively.

14- This was followed by a scanning electron microscope imaging of the prepared and untreated leaves of cinnamon oil nanoemulsion. In the inoculated control, *B. oryzae* succeeded to infect and colonize rice leaf segments. Conidia germinated and produce hyphal filaments that adhered to the host surface. Small oval lesions began to appear, and long hyphal strands were visible at lesion margins. In rice leaves segments treated with *Cinnamomum zeylanicum* nanoemulsion, conidia did germinate, the hyphae failed to penetrate the rice leaves and no appressoria were formed some of the appressoria were deformed, and most were not able to penetrate the rice leaf epidermis.

15- The relationship between the activity of enzymes associated with resistance and the accumulation of phenolic substances and resistance in rice leaves was studied. The enzymatic activity of peroxidase and polyphenol oxidase enzymes and phenolic compounds were estimated after 6, 12 and 24 hours of infection in the case of treatment with cinnamon nanoemulsion 24 hours before infection. Spray on the leaves compared to the untreated spray for the Giza 177 and Sokha 108 items. Sakha 108 cultivar plants infected with *B. oryzae* isolate 10 and treated with nanoemulsion had highest total phenolic content (0.505 mg/g

fresh weight) compared with control and plants of the same cultivar infected with another isolate. The same trend was noticed with plants of Giza 177 cultivar when infected with *B. oryzae* isolate 10 and treated with nanoemulsion (0.5840 mg/g fresh weight). In two cultivars total phenolic content continued to increase gradually over the test period, as the highest was recorded after 24 hrs. of treatments.