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# Pathological and biochemical studies on microorganisms isolated from faba bean

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Some of fungicides have been successfully used for controlling some plant disease but due to their harmful effect in environmental pollution, their toxic effect on human body and carcinogenic diseases, it became dangerous on man health for using. In the last periods more efforts have been made for producing new substances instead of these chemical substances (pesticides) via using some microorganisms which have the ability to produce some antibiotic for controlling some plant diseases. It was found that, the biological control, is the best mean that replace the chemical control, but it was needed more investigation. The present work deals with isolation of some microorganisms from Egyptian soil and study its potentiality for producing some antibiotics which affect on the growth of some fungi that causes some disease to faba bean plants. The examination of the ability of these microorganisms on growth in the presence of certain concentrations from the fungicide (kocide 101) which used for controlling some foliar fungal disease of some agricultural plants as faba bean which used by 0.20 % was also studied. In this research some microorganisms were isolated from three regions of Egyptian soil and purified, and also from faba bean plant which cultivated in different regions of Egypt. The causal agent of chocolate spot disease was also identified as it is a type of fungi. The study also extended to make the pathogenicity test for the isolated fungi on three faba bean cultivars Giza 40, G.3 and G.716. The produced antibiotic was also extracted, purified and identified. Also tested for recognized their effect on controlling chocolate spot disease. The following results can be obtained: (1) One hundred and fifty six colonies of microorganisms were isolated from some Egyptian soil. The identified as 94 bacterial isolates, 35 actinomycete isolates, 22 fungal isolates and only 5 isolates of yeast. (2) It was found that in the presence of the fungicide the microorganisms was reduced of which only 8 fungal isolates and 6 actinomycete isolates were succeeded to grow. (3) The results revealed the some isolates of actinomycetes have the ability for growing in the presence of some concentrations of the applied fungicide while the isolate TII8 of actinomycete could grow with all the used concentrations during incubation period 3-5 days at 30°C. (4) The causal agent of chocolate spot disease have been isolated from infected faba bean leaves and it was identified as two species of fungi related to the genus *Botrytis*, namely *B.fabae* and *B.cinerea*. (5) It was found that *Botrytis fabae* was the more pathogenic (virulent) fungus than *B.cinerea* on the three tested cultivars (Giza 40, G.3 and G.716) it was also observed that GAO was the highly sensitive for

the disease infection. (6) It was found that some isolated microorganisms from soil have the ability to produce substances that have anti-microbial activity against some tested microorganisms (Gram positive and negative bacteria, fungi and yeast) but the isolate T118 of actinomycete was shown a wide anti-microbial effect against all the tested isolates. (7) The isolate T118 of actinomycete also was the best one for reduction of the mycelium growth of *B. fabae* which is considered the most destructive agent on faba bean plants. (8) Taxonomical (Biological & physiological) characters shown that the isolate T118 of actinomycete related to the genus *Streptomyces* which called *S. violaceus* T118. (9) The antibiotic was extracted from the culture filtrate of *S. violaceus* T118 after 6 days incubation, 30°C, pH 7.0 on starch nitrate medium. It was also found that, n-Butanol (immiscible water solvent) was the most effective one to extract the most part from the culture broth, it was also obvious from the bioautograph that, the antibiotic is one compound and the highest R<sub>f</sub> value appeared with ethyl acetate and ~1 (3% in water). (10) It was found that the extracted antibiotic from *S. violaceus* T118 had a clear effect for controlling chocolate spot disease by concentration 85 and 90 mg/ 100 ml, it reduced both mycelium growth and disease severity (on detached leaves) and 100 mg / 100 ml on plants in pots (under greenhouse conditions). (11) The physicochemical properties of the antibiotic showed that, it was yellow colour, without characteristic odour, its melting point -198°C, soluble in chloroform, butanol and dimethyl sulfoxide, while sparingly soluble in water and insoluble in acetone, cyclohexane, petroleum ether and carbon tetrachloride. All of non-reducing sugar (glucose), free NH<sub>2</sub> !, 'TOU' and aromatic amines were present, while tyrosine, diketones or enolic group, free aldehyde, nitro !, 'TOU' and amino acids containing sulphur were not present. The elemental and spectroscopic analysis (IR., U.V. NMR and Mass spectrum) represented that, the compound may related to formula C<sub>32</sub>H<sub>35</sub>N<sub>2</sub>O<sub>7</sub> with molecular weight 562 gm, which is related to microlide. n 136 '90 compounds especially Anthracycline, so it identify as Anthracycline T118. According to both chemical and physical analysis it was classified as Anthracycline compound. OH 0 OH Anthracycline T118 • e€ > 137 '90 ATCCCFUc.v.DPAEYFBLAf.sp.HNMRt.eIRISP1.Z.MBAMBCMICM.PNCIBNCTCPDARFRHS BMSSRTEMTSA.YDY.M.ABBREVIATIONS American Type Culture Collection Cell Forming Unit Cultivated Variety Diaminopimelic Acid Egg Yolk Faba Bean Leaf Agar Special Form Proton Nucleus Magnetic Resonance That is Infrared International Streptomyces Project Inhibition Zone Modified Bennet Agar Minimum Bactericidal Concentration Minimum Inhibitory Concentration Melting Point The National Collection of Industrial and Marine Bacteria National Collection of Type Culture Potato Dextrose Agar Rate of Flow Relative Humidity Soy Bean Meal Stuart, Van Stratum and Rustigian Transmission Electron Microscope Tryptic Soy Agar U.V. Ultra Violet Yeast Extract Dextrose Yeast Extract-Malt extract.