physiollogical and pathological studies onuromyces faba the causal of broat bean rust in vitro

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1.Isolation trials on the tissue culture Murashige and Skoog's (MS-) medium and other media resulted in different non-sporulated mycelia! growths but all except one isolate could be sporulated will on PDA medium. On PDA medium Alternaria sp., Bottytis sp., Cladosporium sp., Epicoccum sp., Stemphylium sp., Tuberculina sp. could be identified.2.However some isolated fungal growths were grown only on MS-medium producihg whitish colonies 25-40 mm in diameter with septate hyphae and dicaryotic cells. Some dicaryotic enlarged, rounded cells like-spores were also observed. The latter isolates formed like-rust fungus pustule on glass slides in vitro and subjected to further studies. These fungal growths produced scarcely, pale, very week and not sporulated colonies when inoculated onto honeybee agar medium and produced brownish, superficially, profusely branched mycelia) growth with very dense sporulation on honeybee agar medium supplemented with pollen grains. These characters were improved with increasing amount of pollen grains up to 6.0 g/l. Enormous numbers of Uredospores and few of teletosi5ores like-structures were clearly seen on the latter medium. Based on measurements and characters of uredospore and teletospore, the obtained isolates could be identified as Uromyces fabae.3.The basal MS-medium containing pollen grains alone or combined with inisitol were better for Uromyces fabae linear growth. The media diluted to 1N, 'AN or 1/4N was best for growth of Uromyces fabae than the full strength of the basal medium (2N). However, the highest rate of linear growth of Uromyces fabae was obtained by adding both pollen grains and myo-inositol to basal medium diluted to %N. After 11 days of incubation, the lowest Uromyces fabae linear growth was observed on the 2N strength basal medium supplemented with myo-inisitol.4.The addition of myo-inisitol alone to most of tested dilutions of the basal medium decreased formation of Uromyces fabae-uredospores, while, basal medium with pollen grains only or combined with myo-inisitol produced the highest numbers of uredospores. The number of uredospores on medium with 1N strength was relatively larger than that produced on media diluted to %N or 'AN strength. In general, the non diluted (2N) basal medium supplied with pollen graines only, 1N supplied with both pollen grains and myo-inisitol followed by the same dilution provided with pollen grains only were promising for formation of Uromyces fabae-uredospores in axenic culture. In contrast, the lowest number of Uromyces fabae-uredospores was produced on the non-diluted (2N) basal medium supplemented with myo-inisitol only.5.The wall thickness of Uromyces fabae-uredospore produced in vitro was ranged between 2.15 and 2.88 pm. However the highest wall thickness was associated with dilution's of %N and 'AN provided with both pollen grains and myo-inositol compared with the non diluted medium (2N) provided with same amendments. The length and width of these Uromyces fabae uredospore were ranged between 20.56-23.23 pm and 19.14 - 21.20 pm, respectively. The higest length and width were produced on the non diluted (2N) and 'AN strength of the basal medium, respectively, while the lowest values for both mesurements were associated with uredospores formed on IAN strength of the basal medium provided with both pollen grains and myo-inisitol. The average of width/length ratio was ranged between 0.895 in case of %N diluted medium provided with myo-inisitol only to 0.994 in case of 1/4N diluted medium supplemented with both pollen grains and myo-inisitol.6.The obtained results proved that both

Qalubia and Beheira isolates (i.e. A and B, respectively) of U. fabae were significantly varied. Isolate A produced the highest values of growth rate and number of uredospores. Removing stock solution D (contained CaC12.2H20) from composition of the normal MS- medium improved rate of growth and uredospores production in both isolates A and B compared with the complete (control) medium. On the other hand, removing of the stock solution E" (contained MnSO4.4H20, ZnSO4.7H20, and CuSO4.5H20) resulted in the highest reduction in both rate of growth and uredospores production especially in isolate A while removing stock solutions E, G, H or Inositol showed intermediate effects in this respect. It was interested to state that the uredospores production was very tiny and was not affected by removing of any stock solution if compared with the normal MS- medium in case of isolate B. 7. On MS-medium containing NH4NO3 at ra e of 1. 5 g/ , h li ear growth was significantly increased with increasing KNO3 from 1.9 gIL to 5.9 g/L and myo-inisitol from 0.1 gIL to 0.2 g/L. However, MS-media contained NH4NO3 at rate of 0.825 g/L ,in most cases, produced the highest linear growth particularly when KNO3 was added at rate of 6.85 gIL. When myo-inisitol was added at rate of 0.2 g/L to MS-media contained NH4NO3 an KN 3 at rates of 0.825 and 6.85 gIL, respectively both linear growth and number of uredospores produced were significantly lower than the corresponding values in case of the same media with 0.1 g/L of myo-inisitol. from these results it could be concluded that the MS-7medium (which contained 0.825; 6.85 and 0.1 g/L of NH4NO3; KN 3 and amyo-inisitol, respectively) was the best for linear growth and production ofuredospores of U. fabae.8. The linear growth of isolate B was greatly enhanced on MS-7 mediumprovided with broad bean leaf extracts compared with the same medium without broad bean leaf extracts. As for isolate A the same trend was observed with 10 days-old culture only, while no variations were detected in both 15 and 21 days-old cultures. The mycellial dry weight of both isolates A and B was increased with aging and greatly stimulated by broad bean extracts added to MS-7 medium. Uredospores production was similarly affected, however, the highest numbers of uredospores was produced by 21 days-old cultures of both isolates particularly in MS-7 medium containing broad bean leaf extracts. 9.U. fabae-uredospores production was greatly enhanced on the modified MS-7 medium supplemented with broad bean leaf extracts compared with MS-7 medium alone particularly in case of isolate B which produced few numbers of uredospores when grown on the latter medium. Percentage of germination of uredospores was inversed proportionally with spore concentration. It was conspicuously increased as uredospores concentration was decreased and higher in case of uredospores produced on MS-7 medium alone than those produced on the same medium provided with broad bean leaf extract, however the opposite behavior was noticed in case of isolate B. In most cases percentage oftredospores germinated was time elapsed up to 24 hours from incubation.10.Percentages of U. fabae-uredospores germination were sharply decreased with increasing age of cultures. The highest % uredospores germination was associated with 5 dyas old cultures then decreased with aging of cultures up to 21 days particularly in case of uredospores produced from cultures grown on MS-7 medium with broad bean leaf extract. At different ages of cultures, % spore germination was higher in isolate B than isolate A.11.The highest percentage of uredospores germination were obtained when 2% sucrose or glucose solutions were used as substrate for germination. Germinability of uredospores of both isolates A and B was slightly decreased with increasing concentration of both sucrose and glucose up to 6% particularly in case of uredospores produced on MS-7 medium containing broad bean extract. In contrast, percentage of germination of uredospores formed on MS-7 medium without broad bean extract, was higher on 4 and 6% glucose solution than the same concentrations of sucrose.12.When distilled water was used as a substrate for uredospore germination the resultant germ tubes as well as mycellial fragments changed to yeast like sprouted mycelium. The cell of this mycelium were fragmented and large numbers of one-celled structures like oidiospores were formed. When these structures germinated in sucrose or glucose solution, germ-tube likeconidiophores wereproduced.13.Both linear growth and uredospores formation were significantly affected by kind of the tested carbon sources (xylose, arabinose, glucose, galactose, fructose, maltose, lactose, sucrose, starch and mannitol and citric acid). Except citric acid (not utilized) all other tested carbon sources were utilized better by U.fabae isolate A than isolate B. In this regard the best linear growth and uredospores production were obtained when sucrose and starch were used as sole sources of carbon for U. fabae isolae A and B,

respectively. 14.The linear growth of both isolate A and B of U. fabae was increased significantly by increasing concentration of sucrose in MS-7 medium up to 30 g/I then decreased by elevating concentration up to 60 g/I., however production of uredospores in case of isolate A only was significantly affected. Adding sucrose to MS-7 medium at rate of 30, 45 or 15 g/I resulted in the highest numbers of uredospores /ml without significant differences in between, however uredospores production in case of isolate A only was decreased significantly by increasing sucrose up to 60 g/1. Uredospores production in case of isolate B was not significantly affected by sucrose concentrations added to MS-7 medium.15.The isolates A and B of U. fabae responded differently against tested nitrogen sources (Sodium nitrate, Potassium nitrate, Ammonium nitrate, Sodium nitrite, Ammonium sulfate, Urea, Aspargine , Gelatin, Yeast extract, Beef extract, Casin and Peptone). The isolate A could utilize most tested nitrogen sources while isolate B could utilize asparagine, yeast and beef extracts only. The highest linear growth and uredospores production of isolate A was obtained in control MS-7 medium (contained potassium nitrate + ammonium nitrate) followed by media contained beef extract, yeast extract, peptone, or asparagine as sole source of nitrogen. However, the highest linear growth of isolate B wasproduced on MS-7 media containing beef extract, yeast extract, or asparagine but yeat extract was the best nitrogen source for its uredospores production.16. The linear growth and sporulation of U. fabae isolate A was significantly affected by wavelengths. Under controlled temperature (25 °C) the continues hyaline light produced the highest linear growth followed by red, blue, yellow and green light conditions. Meanwhile under normal daylight and room temperature conditions the blue followed by red and hyaline wavelengths, in respective, gave the best results. Darkness (black) in the first case and green light and yellow light in the later case produced the lowest values of linear growth. As for uredospores production, the red, blue and hyaline wavelengths under continues illumination and blue wave length followed by darkness, red and hyaline light waves underdiscontinues light (daylight) conditions produced the highest numbers of uredospores 17, The temperature range required for linear growth and uredospores formation was wider in isolate A of U. fabae (17-32 °C) than isolate B (17-23 °C). However, the best linear growth of isolate A grown on MS-7 medium without. The range of temperature regime required for uredospores production was wider (17 — 30 °C) in case of isolate A than isolate B (17-25 °C). The highest number of uredospores was produced at 25 and 23 °C for isolates A and B, respectively. 18. Growing colonies of U. fabae isolate A under controlled relative humidity conditions (14-100% R.H.) resulted in significantly lower values of both linear growth and uredospores production compared with colonies grown under uncontrolled relative humidity conditions (Control). In case of isolate B the highest values of linear growth was obtained in control treatment followed by treatments of 74 and 50% R.H.%, respectively, however treatments of 14 and 100% R.H. produced the lowest linear growth values of this isolate.19.The best linear growth of isolates A and B of U, fabae was produced at pH values of 8.6 and 9.0, respectively, however, linear growth of isolate B was significantly decreased by pH values below 6.6 and approximately stopped at 4.6 and 4.0 pH values. As for uredospores production, similar trend was alsoobserved particularly in isolate A.20.Both isolates A and B can hydrolize starch (Amylase test) and liquefy gelatin (gelatinase test). Isolate A secret starch hydrolytic and gelatin liquefying enzymes faster than isolate B. Amylase and gelatinase enzymes activities expressed in term of percentage of halo-zone was slightly decreased with aging of culture in case of isolate A, however the opposite trend was observed in caseof isolate B.21.The activities of the induced pectulolytic (PG) and cellulolytic (Cx) enzymes were higher in U. fabae isolate A than isolate B. Activities of these enzymes were slightly increased by increasing pH values in substrate reaction from 4.0 to 6.0 for PG enzymes and from 4.o to 6,6 for Cx enzymes in both isolates A and B of U. fabae then decreased again by raising pH values above these limits. from these results it could concluded that the pH values 6.0 and 6.6 were the best for highest activitiese of PG and Cx enzymes, respectively.22.The activities of both PG and Cx enzymes were steadily progressed by increasing time reaction from 5 to 240 minutes. The Cx enzyme activity was very higher in isolate A after 5 minutes than isolate B.23.Both constitutive and inductive PG and Cx enzymes were more active in cultural filtrates of isolate A than isolate B. Activities of these enzymes were steadily increased by increasing age of cultures. However, increasing age of cultures from 14 to 21 days resulted in sharp increase in Cx enzymes activity frorn

26.1% to 75.6% expressed as loss in viscosity of substrate reaction. The ratios between activity of inductive and constitutive PG enzymes of both isolates A and B were narrow in filtrates of 14 and 21 days old cultures compared with filtrates of 7 days old cultures. Regarding with ratios between activity of inductive and constitutive Cx enzymes, the same trend was noticed also in case of isolate A only but reversed manner was associated with isolate B. 24. The uredospores of both isolates A and B of U. fabae which produced in axenic cultures were able to infect callus tissues, detached and intact leaves of broad bean plants. In callus tissues, intercellular mycelium and oidiospores like structures were clearly observed. The oidiospore like structures were also observed in epidermal tissues of inoculated detached leaves.The typical rust pustules were formed on leaves of inoculated broad bean plants which grown under greenhouse conditions.