Abstract

Fourteen highland herbs species were collected from 4 locations of Royal Project station for isolation of antagonistic microorganisms by tissue transplanting technique. From this technique, 113, 135 and 27 isolates of fungi, bacteria and actinomycetes were isolated, respectively. The isolated microorganisms were used for screening of antagonistic activity in control of chrysanthemum white rust disease (CWRD) caused by *Puccinia horiana* with detached leaf method. The results showed that 2 isolates of antagonistic bacteria, AK-MCL5 and KW-CAR17, could control CWRD with 82.66 and 88.33% of inhibition, respectively and 2 isolates of antagonistic actinomycetes, LEPE04 and LEPE18, had 83.33% and 88.89%, respectively. While all of isolated fungi could not control CWRD. Moreover, five chemicals including salicylic acid, jasmonic acid, chitosan, ethylene and petroleum oil were used for control CWRD and the results showed only 2% and 5% of petroleum oil could control CWRD with 100% of inhibition. Results of the screening test indicated that 2 isolates of antagonistic bacteria, 2 isolates of antagonistic actinomycetes and 2 concentrations of petroleum oil were selected to use in the final test. In the final test, 5 concentrations consist of 10^4, 10^5, 10^6, 10^7 and 10^8 cfu/ml of antagonistic bacteria and actinomycetes in ratio 1 ml/leaf were carried out. The results showed that antagonistic bacteria isolates AK-MCL5 and KW-CAR17 at concentration of 10^7 – 10^8 cfu/ml could control CWRD with 100% of inhibition at 5 days post-treatment (dpt) while antagonistic bacteria isolates LEPE04 and LEPE18 at the concentration of 10^8 cfu/ml had 89.00% and 91.66% of inhibition at 7 dpt, respectively. The highest inhibition results were given from 2% and 5% of petroleum oil which had 100% of inhibition from 3 dpt. The morphology of *P. horiana* spores were observed under compound microscope after treatments and the results showed that the tested spores showing wilt-shaped and lack of cytoplasm in the spores. In summary, the use of antagonistic bacteria, isolates AK-MCL5 and KW-CAR17, antagonistic actinomycetes, isolates LEPE04 and LEPE18, and petroleum oil at concentration of 2% and 5% had an *in vitro* efficiency for control chrysanthemum white rust disease caused by *P. horiana.* The study on the method for mass production of antagonistic microorganisms is figured out. There are 2 factors used for determination of mass production including factor 1 (media), factor 2 (time for cultivation) and finally, the relationship between factor 1 and factor 2 was determined as well. For cultivation of antagonistic bacteria, factor 1 was medium number 3 (soybean meal residue) pH 7.2 with the conditions 150 rpm, light for 12 h and dark for 12 h at 25-28°C could give the highest bacterial concentration at 4.5×10^9 and 1.6×10^{10} cfu/ml for isolate AK-MCL5 and KW-CAR17, respectively. The factor 2 (time for cultivation) showed

that the optimum days for cultivation is 7 days with the highest bacterial concentration at 1.2×10^{9} and 1.1×10^{10} cfu/ml for isolate AK-MCL5 and KW-CAR17, respectively. The results of relationship between factor 1 (media) and factor 2 (time for cultivation) found that for isolate AK-MCL5 the medium number 3 (soybean meal residue) had the highest bacterial concentration at 1.0×10^{10} cfu/ml and at 1.2×10^{11} cfu/ml for isolate KW-CAR17 when cultured for 7 days. The cost for medium number 3 was 2.25 Baht per litter. Moreover, antagonistic actinomycetes with the conditions 200 rpm, light for 12 h and dark for 12 h at 25-28°C were used for cultivation. The factor 1 (media) was medium number 6 (ISP-2) showed the highest actinomycetes concentration for isolate LEPE04 with 1.0×10^{9} cfu/ml and for isolate LEPE18 found that medium number 2 (soybean seed) had the highest of actinomycetes concentration at 7.4×10^{8} cfu/ml. The factor 2 (time for cultivation) showed that 5 days after cultivation was suitable for isolate LEPE04 with antinomycetes concentration at 2.9×10^{8} cfu/ml, but for isolate LEPE18 after 7 days of cultivation gave the highest of actinomycetes concentration at 3.4×10^{8} cfu/ml. The results of relationship between factor 1 (media) and factor 2 (time for cultivation) found that the medium number 6 (ISP-2) was suitable for isolate LEPE04 when cultured for 5 days with actinomycetes concentration at 2.3×10^{9} cfu/ml, while the medium number 2 (soybean seed) was suitable for isolate LEPE18 with the concentration of 1.2×10^{9} cfu/ml after 5 days of cultivation. The cost for medium number 2 and 6 were 38.6 and 68 Baht per litter, respectively.