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Compatibility of Biocontrol Bacteria with Phyto-Extracts

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Biocontrol agents and phyto-extracts (botanicals) have a great promise as preventive measure against phyto-pathogens and till date negligible research attention has been to evaluate compatibility of these two bicontrol agents. In present investigation, compatibility of phyto-extracts *viz.* neem extract, besharam extract and mustard cake extract with native biocontrol agents (*Providencia vermicola AAU* PR1, *Pseudomonas putida AAU* PR2 *and Pseudomonas fluorescens AAU* PR3) was checked. All the cultures were found to be compatible with phyto-extracts of neem, besharam and mustard cake on solid agar medium showing no zone of inhibition. The isolates were further confirmed to be compatible with phyto-extract in liquid assay wherein there was no reduction in optical density of cultures was recorded. Moreover, neem, besharam and mustard cake extract up to 10 % concentration showed to increase the growth of all the three tested microbial strains. The results of the present investigation reveal the scope of simultaneous application of biocontrol bacteria and phyto-extract to get benefit of wider range of biocontrol naturally.

Key words: phyto-extract, botanicals, biocontrol,

World over, plants diseases are major cause of yield lose. The global market for phytosanitary products is dominated by synthetic pesticides. There are many disadvantages of using such chemical pesticides which includes accumulation of toxic residues in environment, adaptation of pathogens to such chemicals which in turn reduce its efficiency, undesirable effect on non-target organisms prevailing in the same niche. Moreover, nowadays consumers are becoming more and more concerned about pesticide free safer foods which results in emergence of an eco-friendly strategies for plant disease management i.e. biocontrol agents. Microbial biocontrol agents are having different modes of action for dealing with pathogens. Among which species of fluorescent *Pseudomonas* are capable of utilizing wide range of organic and inorganic compounds which imparts them capacity to live in varied environmental conditions. Among various *Pseudomonas* spp., fluorescent pseudomonads have received particular attention as biocontrol agent of choice. *Pseudomonas* exerts its biocontrol activity through direct antagonism of phytopathogens and induction of disease resistance in the host plant. Presence of pseudomonads in soil provides natural suppressiveness to the soil against some soil borne pathogens which also known as natural biocontrol. Besides using liquid biocontrol consortium for crop protection, recently in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides. Pyrethroids and neem products are well established commercially as botanical pesticides and recently some essential oils of higher plants have also been used as antimicrobials against storage pests because of their relatively safe status

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and wide acceptance by the consumers. In the context of agricultural pest management, botanical pesticides are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and post-harvest protection of food products in developing countries. If one can combine these two biocontrol agents than we can ensure wider range phytopathogen control. Hence efforts were made to determine the effect of phyto-extracts (neem extract and besharam extract, mustard cake) on native biocontrol agents (*Pr. vermicola AAU* PR1, *P. putida AAU* PR2 *and P. fluorescens AAU* PR3) to make their integrated application possible for wider biocntrol.

MATERIALS AND METHOD

Collection and revival of microbial cultures

Microbial strains *viz. Pr. vermicola* AAU PR1 (KJ161325), *P. putida* AAU PR2 (KJ161326) and *P. fluorescens* AAU PR3 (KJ161327) having known biocontrol capacity (Panpatte *et al.,* 2015) are collected from Department of Agricultural Microbiology, B. A. College of Agriculture, Anand Agricultural University, Anand and revived using Nutrient agar and King's B agar.

Preparation of extracts

To check the compatibility of most widely use botanicals *viz.* neem (*A. indica)* besharam *(I. carnea)* and mustard cake with biocontrol activity their extracts were prepared. 10 % aqueous extract of green leaves of neem (*A. indica*) and besharam *(I. carnea)* was prepared, after 24 h of stabilization, filtered through muscling cloth. Similarly, for preparation of 10 % extract of mustard cake, it was mixed thoroughly and allowed to stay in static condition. The supernatant was collected. The extracts were stored in refrigerator till further processing.

Compatibility of native biocontrol bacterial isolates with phyto-extracts possessing biocontrol properties

Compatibility of bacterial biocontrol agents and phyto-extract on solid agar media

For checking compatibility of microbial cultures with phyto-extract the cultures were grown in Nutrient broth media for 24 h and spreaded on the surface of sterile nutrient agar plates. Phyto-extract were filled up in the 3 mm sized cup bored in the center of plate, allow it to diffuse for half an hour in refrigerator and incubated for 24 h at 28±2°C and observe for inhibition zone if any.

Compatibility of bacterial biocontrol agents and phyto-extract in liquid media

After testing the compatibility of microbial cultures on solid agar media, their compatibility was further confirmed in liquid culture medium. For that 10 ml of nutrient broth was prepared and inoculated with 0.5 ml of microbial culture as well as 0.2, 1.0, 5.0, 10.0, 15.0 and 20.0 % extracts of neem (*A. indica*), besharam *(I. carnea)* and mustard cake keeping uninoculated control. The tubes were incubated on shaker at 100 rpm and 28±2°C. The optical density was measured initially and at 3 days and 5 days. Treatments T_1: Phyto-extract @ 0.2 %, T_2: Phyto-extract @ 0.5 %, T_3: Phyto-extract @ 1 %, T_4: Phyto-extract @ 10.0 %, T_5: Phyto-extract @ 15 %, T_6: Phyto-extract @ 20 %, T_7: Only *Pr. vermicola* AAU PR1, T_8: T_1 + *Pr. vermicola* AAU PR1, T_9: T_2 + *Pr. vermicola* AAU PR1, T_{10}: T_3 + *Pr. vermicola* AAU PR1, T_{11}: T_4 + *Pr. vermicola* AAU PR1, T_{12}: T_5 + *Pr. vermicola* AAU PR1, T_{13}: T_6 + *Pr. vermicola* AAU PR1, T_{14}: only *P. putida* AAU PR2, T_{15}: T_1 + *P. putida* AAU PR2, T_{16}: T_2 + *P. putida* AAU PR2, T_{17}: T_3 + *P. putida* AAU PR2, T_{18}: T_4 + *P. putida* AAU PR2, T_{19}: T_5 + *P. putida* AAU PR2, T_{20}: T_6 + *P. putida* AAU PR2, T_{21}: only *P. fluorescens* AAU PR3, T_{22}: T_1 + *P. fluorescens* AAU PR3, T_{23}: T_2 + *P. fluorescens* AAU PR3, T_{24}: T_3 + *P. fluorescens* AAU PR3, T_{25}: T_4 + *P. fluorescens* AAU PR3, T_{26}: T_5 + *P. fluorescens* AAU PR3, T_{27}: T_6 + *P. fluorescens* AAU PR3 were tried to find out the threshold concentration of the phyto-extracts. Three separate sets of the treatments were kept for three phyto-extracts. The optical density was measured initially, at 3 and 5 days after inoculation (DAI). The initial OD was subtracted from the OD measured at 3 and 5 DAI.

RESULTS AND DISCUSSION

Extracts of plants such as neem (*A. indica),* besharam *(I. carnea)* and mustard cake are more effective against crop pests and the bacteria mailnly belonging to the genera *Pseudomonas* and *Providencia* are found to effectively control fungal pests and nematodes. So, if one can combine both phyto-extracts and these biocontrol bacteria than we can get maximum

benefit of the natural entities in the field of biocontrol and get wider spectrum of pest management.

Compatibility of bacterial biocontrol agents and phyto-extract on solid agar media

Microbial strains *viz. Pr. vermicola* AAU PR1 (KJ161325), *P. putida* AAU PR2 (KJ161326) and *P. fluorescens* AAU PR3 (KJ161327) having biocontrol activity against phyto-pathogenic fungi were evaluated for their compatibility with botanicals *viz.* neem (*A. indica)* besharam *(I. carnea)* and mustard cake. All the bacteria were found to be compatible with all the three tested phyto-extracts showing no zone of inhibition. The results showed that *Pr. vermicola* AAU PR1 (KJ161325) showed no zone of inhibition surrounding the well filled with phyto-extract rather they showed growth enhancement in the near

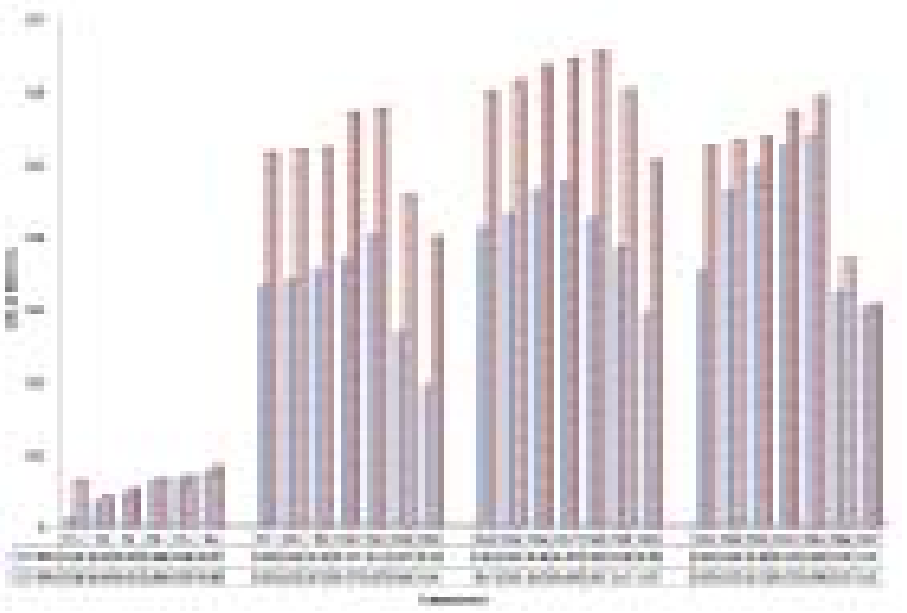

Fig. 1. Compatibility of *I. carnea* extract with biocontrol bacteria in liquid media

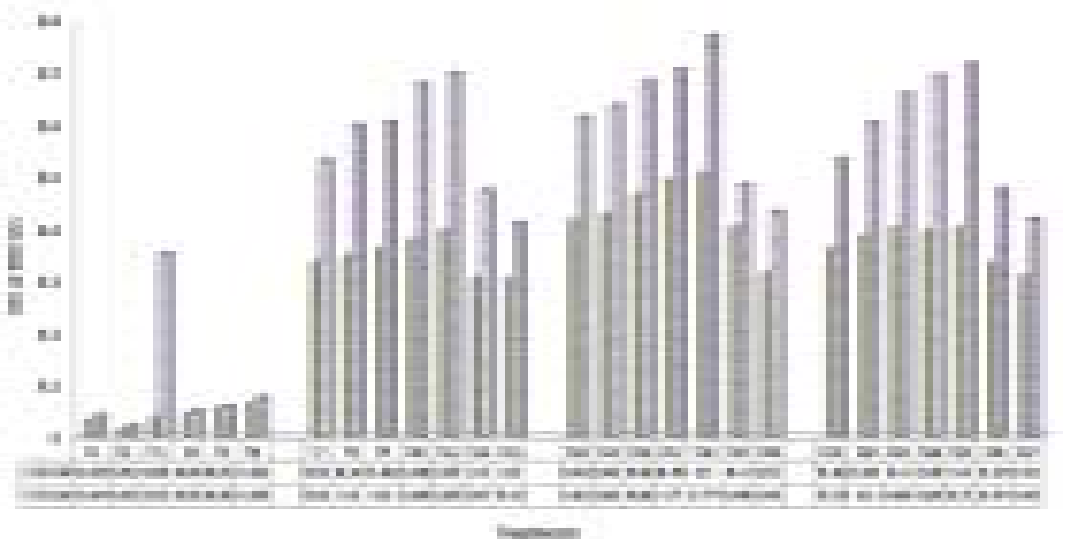

Fig. 2. Compatibility of *A. indica* extract with biocontrol bacteria

vicinity of agar well filled with the extracts. Similar type of trend was observed for *P. putida* AAU PR2 (KJ161326) and *P. fluorescens* AAU PR3 (KJ161327). In present study we have evaluated compatibility of Microbial strains *viz. Pr. vermicola* AAU PR1 (KJ161325), *P. putida* AAU PR2 (KJ161326) and *P. fluorescens* AAU PR3 (KJ161327) having capacity phytopathogenic fungal biocontrol (Panpatte *et al.*, 2015) with extracts of neem (*A. indica*), besharam *(I. carnea)* and mustard cake. In solid agar plate assay all the combinations were found to be compatible with each other showing no inhibition of microbial growth by any of the tested extracts.

Compatibility of bacterial biocontrol agents and phyto-extract in liquid media

The compatibility of the biocontrol bacteria and phyto-extracts was further confirmed in liquid media wherein different concentration of all the three phyto-extracts was separately evaluated for its effect on growth of test bacterial cultures. All the phyto-extracts showed no inhibitory effect up to 10 % concentration rather they showed increase in bacterial growth.

Treatments T_8 – T_{11} receiving *I. carnea* extract @ 0.2 %, 0.5 %, 1.0 % and 10.0 % along with *Pr. vermicola* AAU PR1 showed increase in O.D. at 3 and 5 DAI as compared to T_7 receiving *Pr. vermicola* AAU PR1 alone. The highest O.D. (0.401 & 0.574 at 3 and 5 DAI) was measured in T_{11} receiving *I. carnea* extract @ 10 % + *Pr. vermicola* AAU PR1 inoculation. T_{12} and T_{13} receiving *I. carnea* extract @ 15 % and 20 % inhibition of *Pr. vermicola* AAU PR1 showing reduction in O.D. at 3 and 5 DAI as compared to T_7 comprising *Pr. vermicola* AAU PR1 only. Similarly T_{15} to T_{18} receiving inoculation of *P. putida* AAU PR2 along with *I. carnea* extract @ 0.2 %, 0.5 %, 1.0 % and 10.0 % showed increase in O.D. at 3 & 5 DAI as compared to T_{14} comprising *P. putida* AAU PR2 alone. Highest cell density was observed in T_{18} having combination of *P. putida* AAU PR2 and *I. carnea* extract @ 10 % showing O.D. 0.421 at 3 DAI and 0.652 at 5 DAI. Similar trend was observed for *P. fluorescens* AAU PR3 (Fig. 1)

The same set of treatments was repeated with *A. indica* extract (Fig. 2) and mustard cake extract (Fig. 3) and the results obtained showed similar trend wherein tretaments receiving combined inoculation of bacterial biocontrol agents and either *A. indica* extract or mustard cake extract showed increase in cell density of bacteria up to 10 % concentration and showed decrease in optical density at 15 and 20 % concentration of extract for all the three test cultures *viz. Pr. vermicola* AAU PR1, *P. putida* AAU PR2 and *P. fluorescens* AAU PR3.

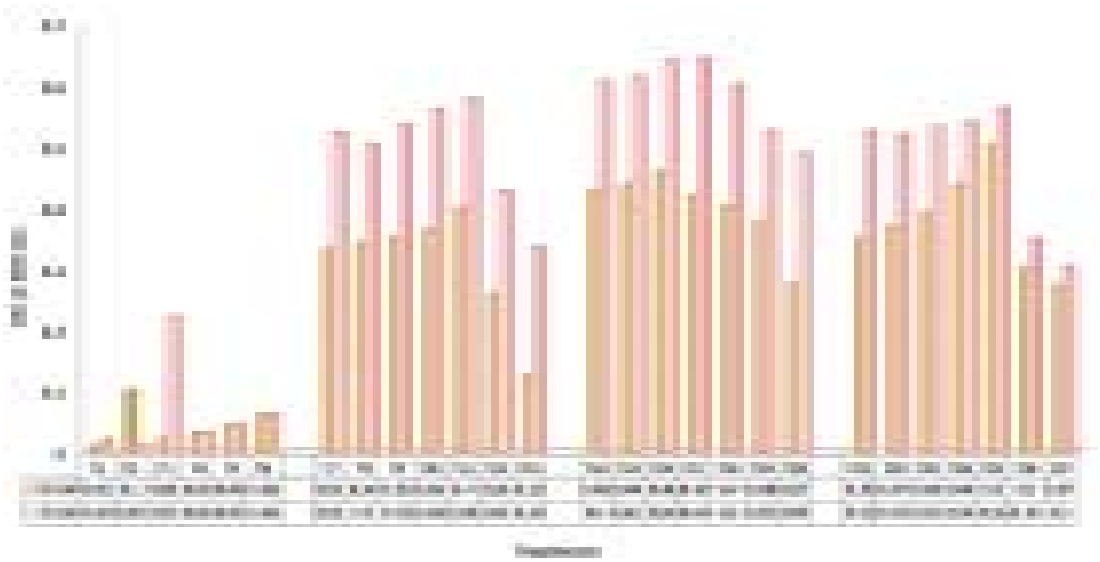

Fig. 3. Compatibility of mustard cake extract with biocontrol bacteria

The results showed that the phyto-extracts showed increase in O.D. of all the test cultures *viz. Pr. vermicola* AAU PR1 (KJ161325), *P. putida* AAU PR2 (KJ161326) and *P. fluorescens* AAU PR3 (KJ161327) up to 10 % concentration as the plant extracts contains carbohydrates, proteins, fatty acids, minerals and many more biochemical constituents which are served as source of nutrition for beneficial micro-organisms (growth promoting and biocontrol agents) in crop cultivation (Tomer *et al.*, 2015). The plant extracts are observed to support the microbial growth as the nutrients required by microorganisms for their growth are directly supplied when they are mixed with the phyto-extracts. This property of the plant products can be exploited for mass multiplication of beneficial bacteria on plant waste as reported by Tomer *et al.* (2015) wherein they tried deoiled cakes of Neem and Jatropha for mass multiplication of biocontrol bacteria *P. flourescens.* The plant cakes served as source of diversified nutrition for *P. flourescens* when used as substrate for mass culturing of antagonist. Here they have reported that deoiled cakes of Neem and Jatropha served as the ideal source for mass multiplication of *P. fluorescence* and ensure highest recovery of antagonistic bacteria after 60 days of inoculation and found that deoiled cake of Jatropha was preferable over neem cake for mass multiplication of *P. fluorescence.* Same way Ramanujam *et al.* (2011) studied the compatibility of weed, *Parthenium hysterophorus* (Congress grass) with *Pseudomonas fluorescens* wherein hot water extract of *Parthenium hysterophorus* was found to be compatible with *P. fluorescens* in various concentrations whereas highest growth and secondary metabolite production was observed at 20 % concentration of congress grass extract.

Same way our results also indicated that the plant extracts were not inhibiting the bacterial growth up to 10 % concentration and increase in concentration of phyto-extracts up to 15 and 20 % inhibit the growth of bacteria due to higher concentration of allelochemicals which may be inhibiting the growth of microorganisms a higher concentration

The overall results showed that the phyto-extracts *viz.* neem (*A. indica)* besharam *(I. carnea)* and mustard cake with known biocontrol properties are not showing inhibitory effect on *Pr. vermicola* AAU PR1, *P. putida* AAU PR2 and *P. fluorescens* AAU PR3 up to 10 % concentration. Increase in concentration of phyto-extracts up to 15 and 20 % showed decrease in cell density showing microbial growth inhibition due to increased concentration of phyto-extract.

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