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Research article

EVALUATION OF MICROBIAL CONSORTIUM FOR 'PLANT HEALTH MANAGEMENT' OF PIGEON PEA

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ABSTRACT: Diverse mechanisms are involved in the suppression of plant pathogens, which is often indirectly connected with plant growth. Plant growth promoting microorganisms (PGPM) and biological control agents (BCA) are shown to possess secondary beneficial effects that would increase their usefulness as bio-inoculants, regardless of the need for their primary function. Indeed, PGPM, such as *Rhizobium* spp., can promote plant growth and productivity (primary effect) but have now been shown to also play a role in reducing disease (secondary effect). Conversely, BCA, such as *Trichoderma spp.* and *Pseudomonas* spp., can control disease (primary effect) but have recently demonstrated stimulation of plant growth (secondary effect) in the absence of a pathogen. Based on these beneficial plant–microbe interactions, it is possible to develop microbial inoculants for use in agriculture. Dependent on their mode of action and effects, these products can be used as bio fertilizers, plant strengtheners, phytostimulators, and biopesticides. The use of microorganisms and the exploitation of beneficial plant–microbe interactions offer promising and environmentally friendly strategies for conventional and organic agriculture worldwide. Widely studied and most promising antagonists are *Trichoderma harzianum* (TH), *Pseudomonas flourescens* (PF), *Rhizobium* (Rh) and *Bacillus subtilis* (BS) have been evaluated in our study for plant health management of pigeonpea in different combinations to make consortia. T 1 (TH+PF), T2 (TH+BS), T3 (TH + Rh), T4 (PF+BS), T5 (PF+ Rh), T6 (Rh+BS) and T7 (TH+PF+ BS + R). These were inoculated by soil application under green house conditions and in reducing the activity of majority soil borne pathogens. Results in this study indicating that the consortia having PGPR (T5, T4 and T3) performed well in the seedling vigor improvement in pigeonpea. Highest fresh weight of plants at 15 and 30 DAS was recorded in treatments T5 (PF+ Rh), (1.480 g) and T4 (PF+BS) (1.323 g). Whereas Treatments T7 (TH+PF+BS+Rh) (86 %), T2 (TH+BS) (82 %) and T5 (PF+Rh) (77 %) have shown remarkable disease reduction. These results are showing role of biological consortia to play in integrated disease management and plant health management of pigeonpea.

Key words: BCA – Bio control agents, Pigeon pea, Plant Health management

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INTRODUCTION

In response to environmental and health concerns about extended use of pesticides, there is considerable interest in finding alternative approaches for use in integrated pest management strategies for crop diseases. Indiscriminate use of pesticides renders the crop products to be considered unsafe and hazardous. Therefore, biological technologies including the use of antagonistic microorganism are getting paramount importance in crop production both for domestic and export market. However, most approaches for bio control of plant diseases have been using single biocontrol agents as antagonists to counteract a single pathogen. This may partially account for the reported inconsistent performance by bio control preparation, because single bio control agents are not likely to be active in all soil environments in which they are applied or against different pathogens that attack the host plant.

Control of wide spectrum of pathogens by applied antagonists largely remains an unfulfilled goal for efficacious exploitation of biological control. The micro organisms employed in biological control of plant diseases act by different mechanisms on pathogens, antagonism is one of those mechanisms. These antagonists are natural components of plant ecosystem and interact directly when grow in association, and adversely affect pathogens. Among these antagonists, very important and promising antagonists are *Trichoderma* sp., *Pseudomonas* sp. and *Bacillus* sp. in reducing the activity of majority soil borne pathogens.

REDGRAM (*Cajanus cajan L.)*

Red gram is an important pulse crop in India. It is also known as Pigeonpea or Arhar or Tur. Red gram is mainly cultivated and consumed in developing countries of the world. It is the second most important pulse crop only after pigeonpea. Though pigeon pea is grown in a wide range of agro-ecological situations, its deep rooting and drought tolerant characters make it especially useful crop in the area of low and uncertain rainfall and on the lighter soils. This crop is widely grown in India & India is the largest producer and consumer of Red gram in the world. Red gram is a protein rich staple food. Red gram is grown mainly as pure kharif monocrop or intercropped with Greengram, Blackgram , Groundnut, Sorghum (Jowar), pearlmillet (Bajra), Maize in black soils as well as in red sandyloam soils. Red gram is grown during June to July. Ideal time for sowing is second week of June to second week of July. Under delayed monsoon conditions it can be sown up to end of July. Late sown Red gram will result in low productivity. Pigeonpea being a leguminous plant is capable of fixing atmospheric nitrogen and thereby restore lot of nitrogen the soil. Wilt is the most destructive fungal disease of red-gram and is common throughout India. It is prevalent in Andhra Pradesh, Maharashtra, Madhya Pradesh, Uttar Pradesh and Bihar. Red-gram is susceptible to wilt pathogen throughout its development. However, the symptoms are more pronounced and the damage is greater at flowering and podding stage. Wilting of seedlings and grown up plants as if they have suffered from water shortage although there is plenty of moisture in the field, is the main symptom. Wilting is characterized by gradual, sometimes sudden yellowing, withering and drying of leaves followed by drying of the entire plant or some of its branches. Phytophthora blight is a devastating disease that kills young plants (1 -7 week old) leaving large gaps in plant stand. It causes seedlings to die suddenly as in damping-off disease. Dry root rot is also serious problem in late-sown or summer crops and in perennial or ratooned red-gram. Sterility mosaic is a serious problem in India and is transmitted by eriophid mite.

The genus *Trichoderma spp.* comprises a great number of fungal strains that act as biological control agents, the antagonistic properties of which are based on the activation of multiple mechanisms. *Trichoderma* strains exert biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth, induced plant defensive mechanisms and antibiosis, or directly by mechanisms such as mycoparasitism. *Trichoderma* spp. have gained wide acceptance as effective bio-control agents against several phytopathogens [1]. *Bacillus subtilis* is a Gram positive, endospore forming, rod shaped bacterium, commonly found in soil rhizosphere. It acts as potential antagonist as well as plant growth promoting rhizobacteria. Several reports have described *Bacillus* strains worthy to be used as bio-control agents for plant disease [2]. Rhizobium is a host specific symbiotic bacterium in leguminous plants producing root nodules. The bacteria can convert inactive nitrogen into its compounds i.e. atmospheric NO_2-NO_3 with the help of enzyme nitrogenase and other nitrogenous compounds are such as amino acids and polypeptides. *Pseudomonas fluorescens* is ubiquitous bacterium in agricultural soils and has got many traits that make them well suited as PGPR. The presence of *Pseudomonas fluorescence* inoculants in the combination of microbial fertilizer plays an effective role in stimulating yield and growth traits of pulse crops. Isolates of pseudomonas from roots, shoots, and rhizosphere soil provides significant increases in fresh and dry masses. The direct promotion by PGPR entails either providing the plant with plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The exact mechanisms by which PGPR promote plant growth are not fully understood, but are thought to include (a) the ability to produce or change the concentration of plant growth regulators like indoleacetic acid, gibberellic acid, cytokinins and ethylene [3], (b) symbiotic N2 fixation [4], (c) solubilization of mineral phosphates and other nutrients [5] (d) antagonism against phytopathogenic microorganisms by production of siderophores, antibiotics. Most popular bacteria studied and exploited as biocontrol agent includes the species of Pseudomonas and Bacillus. Some PGPR may promote plant growth indirectly by affecting symbiotic N2 fixation, nodulation or nodule occupancy [6].

Microbial consortium

Plant-associated microorganisms fulfill important functions for plant growth and health. Direct plant growth promotion by microbes is based on improved nutrient acquisition and hormonal stimulation. Diverse mechanisms are involved in the suppression of plant pathogens, which is often indirectly connected with plant growth. Plant growth promoting microorganisms (PGPM) and biological control agents (BCA) are shown to possess secondary beneficial effects that would increase their usefulness as bio-inoculants, regardless of the need for their primary function. Indeed, PGPM, such as *Rhizobium* spp., can promote plant growth and productivity (primary effect) but have now been shown to also play a role in reducing disease (secondary effect). Conversely, BCA, such as *Trichoderma spp.* and *Pseudomonas* spp., can control disease (primary effect) but have recently demonstrated stimulation of plant growth (secondary effect) in the absence of a pathogen. Whereas members of the bacterial genera *Bacillus*, *Pseudomonas*, *Rhizobium* well-studied examples for plant growth promotion, and *Trichoderma* are model organisms to demonstrate influence on plant health. Based on these beneficial plant–microbe interactions, it is possible to develop microbial inoculants for use in agriculture. Dependent on their mode of action and effects, these products can be used as biofertilizers, plant strengtheners, phytostimulators, and biopesticides. The future success of the biological control industry will benefit from interdisciplinary research, e.g., mass production, formulation, interactions, and signaling with the environment, as well as on innovative business management, product marketing, and education. As the separate seed treatment and application of biopesticides has been the regular practice in the farmers for managing the different pests and diseases, which not only increase the cost of cultivation but also reduce the synergistic effect of these organisms for overall plant health management. Altogether, the use of microorganisms and the exploitation of beneficial plant–microbe interactions offer promising and environmentally friendly strategies for conventional and organic agriculture worldwide. Visualizing the success stories of mixed inoculants (combination of microorganisms that interact synergistically) over single bio inoculants, consortiums are being currently devised for crop management. The extreme complexity of interactions occurring is highlighted, and some potential areas and shortcomings required to overcome for future researches in this area are discussed briefly. Looking into the benefits of microbial consortia for overall plant health management compared to the single and separate application of biopesticides, the present study is aimed to mainly emphasize in finding suitable microbial consortium for plant health management of pigeonpea which include fungal antagonists like ***Trichoderma* sp.**, bacterial antagonist ***Pseudomonas* sp.** and ***Bacillus*** in collaboration with ***Rhizobium*** in different combinations. Objectives of present study include

- To test the compatibility among fungal, bacterial antagonists and Rhizobium *In vitro*
- To study the effect of microbial consortia on germination, plant vigor and plant biomass.
- To evaluate different microbial consortia against soil borne pathogens of pigeonpea in terms of Disease incidence reduction in green house conditions.

MATERIALS AND METHODOLOGY

The present experiment was conducted during Post Graduate Diploma in plant Health Management 2013-2014 at NIPHM Rajendranagar, Hydrabad (A.P.). The all mother cultures of microorganisms were procured from centre for biological control (CBC), NIPHM. All the laboratory equipment was provided by CBC. Green house experimentation was carried out in the same institute with required facilities.

Compatibility test among microbial agents:

The microbial to be used were tested for compatibility *invitro* by dual culture technique. To carry out the consortium studies we should know about compatibility of selected microorganisms because incompatibility of the co inoculants can arise because biocontrol agents may also inhibit each other as well as the target pathogen or pathogens [7]. Thus, an important prerequisite for successful development of strain mixtures appears to be the compatibility of the co inoculated microorganisms [8]. The selected microorganisms comprises one fungus i.e. *Trichoderma harzianum* and three Bacterial organisms i.e. *Pseodomonas flourescens*, Rhizobium, *Bacillus subtilis*. Dual culture technique is one of the useful methods to study compatibilities. Two micro organisms were grown together on PDA media (Potato Dextrose Agar Medium) in a Petri dish to study compatibility. PDA medium was prepared by mixing 39gms of readymade dehydrated PDA powder in 1000ml of distilled water followed by autoclaving at 121°C, 15lb pressure for 15 min. The same is poured into 90 mm Petri dishes in aseptic conditions (20ml each). The selected micro organisms from mother cultures have been transferred with inoculation loop on to the media plate. Compatibility studies were carried out in the laboratory of NIPHM, center for biological control.

To study compatibility among microbial cultures i.e. fungal culture as well as bacterial cultures, initially we have maintained subcultures of *Trichoderma harzianum, Pseudomonas flourescens*, *Bacillus subtilis*, Rhizobium from the available mother cultures in the same laboratory. Three replicates were prepared for each pairing, in a total of 21 replications were examined. For the fungal, bacterial combination 8mm disc of *Trichoderma harzianum* was placed in the middle of half radius, and bacteria streaked out in the rest of the portion. For bacterial combination, two cultures were streaked out in such a manner to give plus symbol in the plates. Immediately after inoculation, the plates were sealed with plastic film and incubated at 24°C, 70% RH for 1 week period. Observations were recorded after 3, 5, 7 days of inoculation on growth of individual antagonist in presence of its co inoculant. Pairs of co inoculants were considered as compatible if they grow without any inhibition zone in the culture plate.

Consortia with fungal and bacterial antagonists and PGPR

A total of eight treatments combinations were designed to study the plant health of pigeonpea in green house condition.

T 1 = *Trichoderma harzianum* + *Pseudomonas flourescens*

T2 = *Trichoderma harzianum* + *Bacillus subtilis*

T3 = *Trichoderma harzianum* + Rhizobium

T4 = *Pseudomonas flourescens* + Bacillus *subtilis*

T5 = *Pseudomonas flourescens*+ Rhizobium

T6 = Rhizobium + *Bacillus subtilis*

T7 = *Trichoderma harzianum*+ *Pseudomonas flourescens* + Bacillus + Rhizobium

To make Consortium, at first giant cultures were prepared in laboratory. *Trichoderma harzianum* has been mass produced by using sorghum grain media (solid fermentation). Per tray (accommodating 2.5 kg pot mixture) 15 gm of Trichoderma powder was mixed. All bacterial preparation were carried out in potato dextrose broth and used in pot mixture at the rate 50ml/tray.

Giant culture preparation

Trichoderma harzianum has been mass multiplied on sorghum grain media. Hi Dispo poly bags filled with 500gms of sorghum grain have been autoclaved for 15 min. at 121°C temp, 15 lb Pressure. After autoclaving the bags cooled down to room temperature. 8mm discs from mother culture of *Trichoderma* have been transferred aseptically to these sorghum bags and shake vigorously. Leave these bags as such for fungal growth development at room temperature. These grains were grinded after 10 days of inoculation in a blender to make powder form. For mass cultures of bacterial microbial, nutrient broth has been prepared by mixing 13gm of readymade dehydrated nutrient broth powder form in 1000 ml of distilled water. This thoroughly mixed broth is filled into Hi-dispo poly bag, with support of cotton plug as well as rubber band and autoclaved for 15 min at 121°C temperature, 15lb pressure. These bags were cooled down for room temperature for a while and inoculated aseptically. Bacterial inoculated bags were kept under room temperature up to 3-4 days for the development of bacterial growth on broth medium. These culture is been utilized as such in making consortium. Even Rhizobium and bacillus also been prepared by using this nutrient broth medium only and utilized the same for doing experiment.

Green house study

Experiment was carried out in green house at National Institute of Plant Health Management, Rajendranagar, Hyderabad. Coco peat trays have been selected of size 70 cm x 30 Cm size comprises 50 pits to plant selected crop seed. Once after preparation of microbial cultures at laboratory level in required quantities, they were mixed in pot mixture as per the treatments designed earlier. Soil and FYM mixture been prepared by mixing 1.5Kg FYM with 1 kg soil by pouring on plastic bag to mix thoroughly along with microbial consortium of different treatments and transferred the same into selected coco peat trays. Each treatment is replicated thrice. Experiment was carried out by using local variety of pigeonpea for sowing by dibbling. Sowing depth was maintained uniformly to ensure uniform germination. Irrigation was given in every alternate day interval. Treys were supervised daily for germination of seeds. After 5- 7 days observations were recorded and germination percentage was obtained thereafter. Other plant growth parameters such as plant height, root length and volume, plant biomass etc. and vigor index were obtained thereafter. Growth parameters were recorded at 15 DAS and 30DAS for comparison in different treatments for which 2 sets were maintained as measuring root length is destructive method. Fresh weight is recorded in analytical balance immediately after uprooting and oven dry weights were recorded thereafter. Control was maintained with only pot mixture without any inoculations. Natural disease incidence was recorded to screen the microbial consortia.

Statistical analysis

Experiment was conducted in CRBD and results were analyzed by analysis of variance technique (ANOVA) for comparison of treatments, least significant difference test was used.

RESULT AND DISCUSSION

Compatibility of microbials:

The microbial antagonists were found to be compatible in all the consortia combinations where no inhibition was noticed between any pair of co-inoculants. In the treatment of tetra consortium (T7) also there was no inhibition of any microbial agent hence all were compatible in mixture. Results were in agreement with other studies. Vidhyasekaran and Muthamilan [9] in their experiment found that *Rhizobium and P. fluorescens* were not inhibiting growth of each other and compatible in pigeonpea rhizosphere.

Plant growth parameters

Observations were taken regarding no. of days taken to germinate all plants in each treatment along with control. Some observations were recorded in view of germination percentage as well as of about plant growth and development i.e. Root length, shoot length and fresh weight and dry weight 15 days and 30 days after sowing.

Data regarding germination Percentage was calculated by using formula i.e.

$$\frac{\text{Total no. of plants germinated in a tray X 100}}{\text{Total no. of seed sown in that tray}}$$

After 15 days and 30 days of sowing observations were recorded about plant growth and development i.e. root length and shoot length by uprooting the plants from the trays. Slight irrigation was given before uprooting the plants for better upliftment of plants from the trays without causing any damage to roots. Details of calculations like plant vigor index and percent disease reduction and biomass parameters were discussed in chapter experimental findings.

The Plant vigor index (PVI) was calculated by using the formula PVI= Percent germination x (Average root length + Average shoot length).

Percent Plant germination

Per cent germination was recorded highest in T7 (TH+PF+BS+Rh) (88 %) and T5 (PF+Rh) (86%) followed by T2 (TH+BS) with 84 per cent and T6 (Rh+BS) with 83 per cent germination. It was observed that all the treatments have shown effect on germination compared to control. This percent plant germination was utilized in calculating plant vigor index at 15 DAS as well as at 30 DAS. Results indicated the positive effect of microbial mixtures on germination and seedling vigor. Though the exact mechanism is not understood similar results were obtained in many studies in this regard. Pradeepkumar *et al.* [10] evaluated various rhizosphere fungi in pigeonpea, wherein seeds treated with *T. viride* significantly increased seed germination per cent (96.6 %) compared to control.

Average root length and shoot length:

After uprooting the plants randomly from the treated and control trays, length of root and shoot were measured by using scale and observations were taken at 15 DAS and 30 DAS and average root length as well as shoot length were calculated for analysis. Remarkable root growth was recorded after 15 DAS in T4 (PF+BS) (13.7 cm) which is significantly superior over others; next best is T5 (PF+Rh) (12.7cm) followed by T3 (TH+Rh) (11.7cm) are significantly differing with rest of the treatments and control (7.7 cm). Even after 30 DAS the same trend remained showing highest root growth in T4 (PF+BS) (23.7 cm) followed by T5 (PF+Rh) (21.4cm) and T3 (TH+Rh) (20.2 cm). All the treatments were showing significant effect on root growth when compare with control. It is noticed that control tray with 13.9 cm without any coiling growth of roots. Effect of consortia on shoot length was also realized as remarkable growth of shoot with healthy leaves was observed in almost all the treatments. Treatments T4 (19.0 cm), T5 (18.57 cm) and T3 (17.4 cm) have shown good results which are statistically on par with each other but significantly better over the rest of treatments as well as control. It was noticed that in control plants the plant height is less (14.0 cm) with less foliage compared to the consortia treated plants where in plants look lush green with luxuriant foliage. Observations of shoot length at 30 DAS showed highest with T4 (24.2 cm) and T5 (22.6 cm) which are on par and followed by T3 (21.5 cm). These are significantly better over the rest of treatments as well as control. These results are similar to other studies; Appa Rao Podile & G. Krishna Kishore in their study they found that bacteria of diverse genera were identified as PGPR of which *Bacillus* and *Pseudomonas* spp. are predominant and exert a direct effect on plant growth by production of phytohormones, solubilization of inorganic phosphates, increased iron nutrition through iron-chelating siderophores and volatile compounds that affect the plant signaling pathways.

The PGPR can influence plant growth directly through the production of phytohormones and indirectly through N_2 fixation and production of biocontrol agents against soil-borne phytopathogens [11].

Plant vigor index

Plant vigor index was calculated by using the formula; Percent germination x (Average root length + Average shoot length). Pigeonpea plants responded well to all the given treatment as it has shown remarkable difference in plant vigor index when compared with untreated control (910). The value of vigor index was found better in treatments T7 (TH+PF+BS+Rh) (3113), T5 (PF+Rh) (3106), T4 (PF+BS) (3007) over others and all consortia were superior over control at 15 DAS. The same trend was observed in vigor index even after 30DAS in the treatment combinations; T5 (4102), T4 (3855), T7 (3727) which showed considerable results when compared to untreated control (2293) at 30 DAS. Results in this study indicating that the consortia containing Rhizobium and pseudomonas were performing well in the seedling vigor improvement in pigeonpea. Similarly combined inoculation of Rhizobium and phosphatesolubilizing bacteria in pigeonpea has been reported to enhance nodulation and plant growth [12, 13].The highest vigour index was observed in chickpea pot inoculated with *T.viride* (21863.3) in a study carried by Pradeep kumar *et al.* [10]. Singh *et al.* [14] found that a combined application of *Pseudomonas fluorescens, Trichoderma harzianum and Mesorhizobium* enhanced seedling vigour as well as phenol contents in roots and leaves there by reduced the infection by root rot fungus *Sclerotium rolfsi.*

Effect of consortia on plant biomass of pigeonpea

Plant biomass was taken in to consideration in view of plant health, observations of fresh weight and dry weight of pigeonpea plants at 15 DAS and 30 DAS in analytical balance. Average fresh weight of plants in all treatments were recorded and analyzed, at 30 DAS highest fresh weight was recorded in treatments T5 (PF+Rh) (1.48 g) T4 (1.32 g) which are on par with each other. Treatments T3 (1.30 g) and T1 (TH+PF) (1.1 g) also had significant effect on plant fresh weight which were superior over the rest of treatments and control. The PGPR isolates used did not antagonize the introduced *Rhizobium* strain and the dual inoculation with either *Pseudomonas putida, P. fluorescens* or *Bacillus cereus*resulted in a significant increase in plant growth, nodulation and enzyme activity over *Rhizobium*-inoculated and uninoculated control plants. The nodule occupancy of the introduced *Rhizobium* strain increased from 50% (with *Rhizobium* alone) to 85% in the presence of*Pseudomonas putida*. Dual inoculation of seed with *Rhizobium* and *Pseudomonas putida, P. fluorescens* and*Bacillus cereus* enhanced plant biomass as well as yields of pigeon pea [15].

Percent Disease reduction

Pigeonpea is very much sensitive to fusarium wilt and *Sclerotium rolfsi* rot in initial days of plant growth. Per cent disease incidence was recorded after 15 DAS and per cent disease reduction for every treatment and percent disease reduction values were obtained by calculation. Percent disease reduction was compared with untreated control plants and considerable results were shown by all the treatments. Treatments T7 (TH+PF+BS+Rh) (86%), T2 (TH+BS) (82 %) and T5 (PF+Rh) (77 %) have shown remarkable disease reduction which were statistically superior over other treatments. After 30 days of sowing percent disease reduction was compared and found to follow the same trend. Under greenhouse conditions, the application of *Trichoderma harzianum* (ANR-1) exhibited the least disease incidence (by 15.33%). Also tomato plants treated with *Trichoderma harzianum* (ANR-1) isolate showed a significant stimulatory effect on plant height (by 73.62 cm) and increased the dry weight (by 288.38 g) of tomato plants in comparison to other isolates and untreated control. [16]. Soil amendment, with ***T. harzianum*** at 10 and 20 g gave 42.9% and 61.5% disease control, respectively and Even at the highest pathogen density (log 5.34), soil amendment with ***T. harzianum*** at 10 g gave about 30% disease reduction. [17]. These results were comparable with some studies in different experiments. It was observed from the results that *Trichoderma harzianum* has significant role in reducing the disease incidence probably because it's multiple mechanism of antagonism. In a green house study conducted by Raupach *et al.* [18], Plant growth-promoting rhizobacteria (PGPR) strains INR7 (*Bacillus pumilus*), GB03 (*Bacillus subtilis*), and ME1 (*Curtobacterium flaccumfaciens*) were tested singly and in combinations for biological control against multiple cucumber pathogens. The three-way mixture of PGPR strains (INR7 plus ME1 plus GB03) as a seed treatment showed intensive plant growth promotion and disease reduction to a level statistically equivalent to the synthetic elicitor Actigard applied as a spray. Similarly tetra-inoculants (*Mesorhizobium-Azotobacter-Pseudomonas-Trichoderma*) have shown significant growth attributes, yield and phytopathogen growth inhibition followed by tri-inoculants than control when applied as seed treatment in chickpea [19]. Results of a study indicated that *B. subtillis and T. harzianum* effectively suppress the *Fusarium* wilt and increasing the protein content and β-1, 3-glucanas enzyme activity might have contributed to inducing systemic resistance after treatment with bio-control agents [20].

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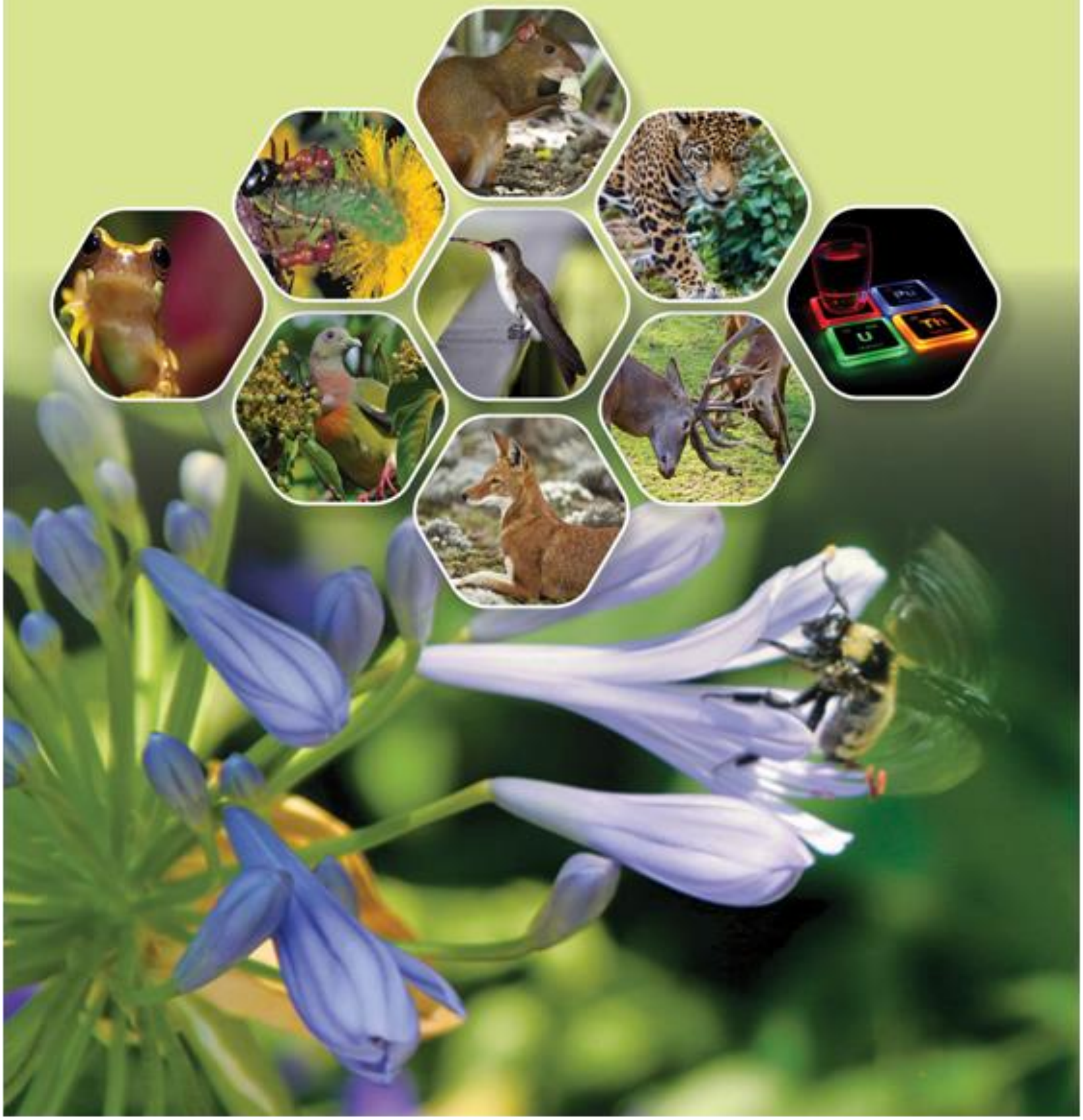

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