STUDY OF BACTERIAL ADHERENCE AND SURVIVAL ON INOCULATED MAIZE SEED SURFACE AND ITS IMPACTS ON NATURALLY ADHERED MICROORGANISMS, GERMINATION, SEEDLING DEVELOPMENT OF MAIZE CROP

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ABSTRACT

The objectives of the study is to evaluate adherence of bacterial inoculants on seeds surface, the action of chemical and bacterial seed treatment on germination, emergence of seedling of maize under laboratory condition and to identify effect of bacterial inoculants along with the chemicals to be used for pre sowing seed treatment of maize. Three treatments-two bacterial inoculants fresh, old culture and one bacterial inoculants with 0.5% hgcl2 for study of germination of seeds treated with inoculants was placed on petriplates such three replication was made for avoiding errors. Maize African tall seeds were utilized. The tried bacterial inoculants upgraded the germination and development of maize seeds when contrasted with untreated.

Key words: bacterial inoculants, HgCl2, Pre-sowing, seed treatment, maize.

INTRODUCTION

“Bio-fertilizers” include supplements that direct the regular procedures of “nitrogen” uptake, Solubilise “phosphorus”, and invigorating plant development through using combination of developed advancing component. Bio-fertilizers diminish the consumption of compound compost and chemical. Plants can be developed and improved by enhancing maintainability and the soundness of the dirt by using bio-fertilizers

Adherence is a fundamental advance in bacterial pathogenesis or disease required for colonizing another host. Numerous microorganisms in the indigenous habitat exist in multi cell totals commonly portrayed as bio-films, related with strong surfaces and in close link with other micro-organism electric cell. Cells stick to surfaces and each other through an unpredictable grid involving an assortment of “extracellular polymeric substances” (EPS) consider exo-polysaccharides, macromolecule and “DNA”. Bacterial adherence to seeds is a procedure that firmly impacts rhizosphere colonization. Providers regularly intentionally cover their seed stocks with microbial bio-films to immunize the creating rhizosphere.

Maize (Zea mays L.) is the third significant grain crop close to rice and wheat in the World. It is considered as the "Sovereign of Cereals". It's developed over a territory of around 159000000 ha with a creation of around 817 million tons and efficiency of 5 tons of grain ha-1 of every 2009 (en.wikipedia.org/wiki/maize). By 2020 AD, the prerequisite of maize in different segments will associate with 100 million tons, of which the poultry area request alone will be 31 million tonnes. It is an exceptionally troublesome errand

for our agriculturists to build the maize creation from the current degree of 34 to 100 million tons (Seshaiah, 2000).

In this study, maize seeds were treated with nitrogen Bio-fertilizers like Azotobacter and phosphorus bio-fertilizer like *Pseudomonas putida, Bacillus megaterium, Bacillus polymyxa, Pseudomonas fluroscence* and biocontrol *Trichoderma viride* in hydroponic culture.

MATERIALS AND METHODOLOGY

MATERIALS

Microbes-

Pseudomonas putida, Bacillus megaterium, Bacillus polymyxa, Pseudomonas fluroscence Trichoderma viride

Media:

Fungal agar, Nutrient agar, Actinomyces agar, Rhizobium agar, Pikovaskaya's agar, Pseudomonas agar, Bacillus agar, Jensens medium

Reagents:

70% ethanol, HgCl2

METHODOLOGY

Pure culture

Pure culture was obtained from the microbiology department of BAIF CRS Urulikanchan. Commercial product of bacterial inoculants of BAIF was used as primary culture.

Identification of Microbial inoculants

By using gram staining method, bacterial inoculants of *Pseudomonas putida, Bacillus megaterium, Bacillus polymyxa, Pseudomonas fluroscence*, *Trichoderma viride* were identified.

Production of primary bacterial Culture

Pikovskaya's broth pseudomonas and PDB broth of 2.5 lit were prepared and autoclaved at 15 psi at 121 c for 15 mins. When broth was cooled, loopful culture from the pure culture of *Bacillus megaterium, Bacillus polymyxa, Pseudomonas putida* was inoculated in pikovskaya's broth, *Pseudomonas fluroscence* was inoculated in Pseudomonas broth and *Trichoderma viride* was inoculated in potato dextrose broth. After immunization, the flasks were kept on the revolving shaker for four days at RT at 250. TVC was performed to check bacterial growth.

STUDY OF BACTERIAL ADHERANCE

Plant Materials:

The plant material used for this study included seeds of African tall variety of maize collected from BAIF development research foundation, CRS, Uralikanchan, Pune.

Seed analysis

Before seed treatment, seeds were analyzed for infectious pathogens and testing of naturally adhered micro-flora. Some seeds were manually taken from sorted stock of seeds. Nutrient agar & potato Dextrose agar was prepared and poured in petriplates allowed to solidify. After media solidification seeds were inoculated on both media. Entire seed was kept on both media. Some seeds were teased into the saline. Crushed seeds were inoculated on both media. All these plates kept for incubation. After 48hrs, the results were observed.

Seed treatment

After seed analysis, seeds were soaked for 5min for individual treatment; seeds were soaked in *bacillus megaterium , Bacillus polymyxa, Psuedomonas fluorescens, Psuedomonas putida* and *Trichoderma viride*. Naturally adhered microbial counting of maize seeds before washing. The TVC was performed to check cell count i.e. viable cells present on maize seeds before washing the maize seeds. Above procedure was carried out for checking naturally adhered microbial count on maize seeds rinsed with tap water and distilled water.

Same procedure is carried out for counting of naturally adhered and interiorly colonized microbes of maize seeds after washing with distilled water and TVC was performed.

Seed treatment with bacterial inoculants (old culture)-

Arranged old bacterial inoculants were utilized for seed treatment. The maize seeds were very much washed with refined water and they submerged in different bacterial inoculants for 15-20 mins. After 20mins the seeds were expelled from bacterial inoculants and dried in hot air stove at 40oc for 20mins. The TVC was performed to check cells present on maize seeds after bacterial inoculants treatment to the maize seeds.

Seed treatment with bacterial inoculants (fresh culture)-

2days newly arranged bacterial inoculants were utilized for seed treatment of maize seeds. The seeds were all around washed with refined water and afterward submerged in different bacterial inoculants for 15-20 minutes. After 20mins the seeds were expelled from bacterial inoculants and dried in hot air stove at 40oc for 20mins.TVC performed to check cell tally for example reasonable cells present on maize seeds after bacterial inoculants treatment to the maize seeds.

Sterility testing of seeds with various concentration of HgCl2-

Maize seeds collected from agriculture department of BAIF, CRS uralikanchan. The seeds were manually sorted disease free seeds were selected. Maize seeds were treated with various concentrations of hgcl2 (0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.5, 2, and 2.5). The seeds were immersed in hgcl2 solution for 5 min under aseptic condition later seeds was rinsed with4-5 times with sterile distilled water. For sterility testing nutrient media and PDA was prepared and poured in petriplates after solidifying the media the treated seeds was placed on media under LAF. The plates were kept in incubator for further analysis.

Study of seed germination of seeds treated with microbial inoculants both fresh and old cultures-

Along with study of adherence the study on

effect of microbes on seed germination and seedling formation at room temperature was also done. Germination rate of maize seeds was checked with help of filter paper. One set of 10 seeds was place on sterile filter paper in petriplate in aseptic condition. 3replication was done for avoiding error. These petriplates was kept in incubator for constant temperature (32 ^{0c}) daily observation was done up to seeding development. Reading was taken on every 2nd and 4thday. The seeds selection was done from the stock of seeds kept for study of adherence.

RESULTS AND CONCLUSION

1. Identification of microorganisms-

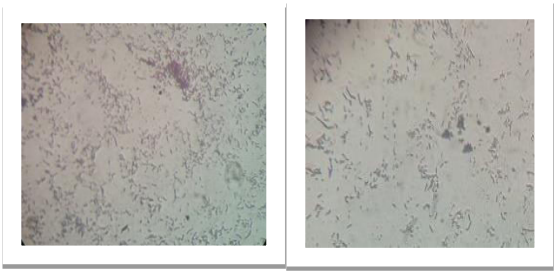

Fig no.1.1 *Bacillus megaterium* and *Bacillus polymixa*

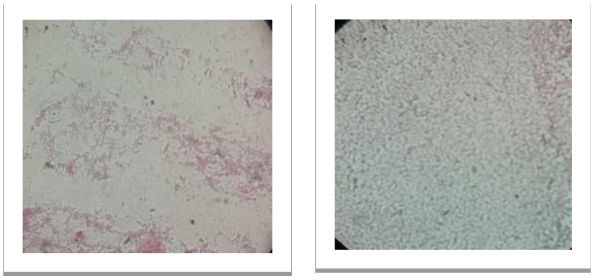

Fig no.1.2 *Psuedomonas putida* and *Pseudomonas fluorescens*

2. Study of microbial adherence Microbial adherence on maize seed naturally-

Microbial growth was observed up to $1*10^{12}$dilutions

Fig 2.1Microbial adherence on maize seed naturally

Microbial adherence on maize seed after rinsing with distilled water-

Microbial growth was observed up to $1*10^{10}$dilutions.

Fig 2.2 Microbial adherence on maize seed after rinsing with distilled water

Microbial adherence on maize seed grinded after rinsing with distilled water

The microbial growth was observed up to $1*10^{12}$ dilutions.

Fig 2.3 Naturally adhered micro flora on water rinsed and grinded seeds

Microbial adherence on maize seed after treated with 0.5% hgcl2 and rinsed with distilled water-

The microbial growth was observed up to $1*10^{4}$dilutions

Microbial adherence in maize seed grinded after treated with 0.5% hgcl2 and rinsed with distilled water-

The microbial growth was observed up to

$1*10^{4}$dilutions

3. Study of sterility

Positive after effect of Sterility and germination was seen at seeds rewarded with 0.5% HgCl2. Negative impact on sterility was seen on seeds rewarded with 0.1% and 0.2%. Negative impact on germination was seen on seeds rewarded with 2% HgCl2**.**

Fig 3.1- Positive result of sterility on seeds treated with 0.5% HgCl2

4. Study of germination

Fig 5.1 Germination of seeds treated with HgCl2 and fresh bacterial content- 2 nd day and 4 th day

Fig no.5.2- germination of seeds treated with old bacterial culture 2nd day and 6th day.

CONCLUSIONS

To satisfy the future interest of a developing populace for food, solid and high yielding plants are the prerequisite. Utilization of microorganisms as Bio-fertilizer for plant development advancement and bio-control action is a desire for the food security issue. Along these lines in this examination we had rewarded maize seeds with bacterial inoculants to improve their germination and seedling advancement for this we considered the microbial adherence on seed so we can appraise the adherence limit of organism on seed to create standard SOP for ranchers for pre-planting treatment. Vaccination of micro-organism in maize crops prompts a high germination prospective. Specifically, P-solubilizing demonstrated incredible potential for use as bio-inoculants. The utilization of these bacterial strains effectively affected development and improvement of seedlings of maize.

Seeds treated with 0.5% hgcl2 for 5 mins have shown positive result in sterility testing as well as in rate of germination. Seeds treated with 0.1% and 0.2% hgcl2 for 5 mins had shown negative impact on seed sterility. Fungal growth was observed on sterile media plates. Seeds treated with 1.5%, 2%and 2.5% hgcl2 for 5 mins had shown negative impact on germination. 20-30% germination rate was observed.

Naturally adhere microflora adhere on seed surface was up to 10^{-12} dilutions. Naturally interiorly colonized microflora in seed surface was up to 10^{-12} dilutions. Microbial count adhered on seed surface after washing with water was up to 10^{-10} dilutions. Microbes of fresh culture sustain for longer duration on seed surface as compare to old culture.

In semisolid pikovaskaya's media mat growth of PSB inoculants was observed. Old bacterial culture can sustain up to a month on seed surface. Seeds washed with water and treated with *Trichoderma viride* had shown minimal fungal growth where as other seeds was infected with fungal flora. Seeds treated with 0.5% hgcl2 and inoculated in bacterial culture had shown no fungal growth. Greenish and black color fungal

flora was observed on seeds which show fast seedling development. At constant room temperature in incubator the rate of germination was fast as compare to natural environment. In incubator seeds get germinated within 48 hrs.

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