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Evaluation of antifungal effects and seed growth using bio-formulation

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Abstract
Fungal infection in the crop disease is major problem in world wide. Many plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. Plants have been known to relieve various diseases in Ayurveda. Today are emphasizing on evaluation and characterization of various plants and plant constituents against several diseases based on their traditional claims of the plants given in Ayurveda. Till now many plant materials are been used to form bio formulation which cure fungal infection in variety of plants. The plants selected for study were *Nicotiana tabacum*, *Azadirachta indica* and *Datura metel* of leaves and cow dung, cow urine, buttermilk and distilled water used for bio formulation. Total 15 Bio-formulations were prepared and investigated for their antifungal activity against *Aspergillus niger*, *Sclerotium rolfsii* and *Penicillium sp*. It was observed that formulation F13 and F15 showed good antifungal activity compared to other prepared formulations. Bio formulations were performed on *Pearl millet* (Bajra), *Vigna radiata* (Mung bean), *Cicer arietinum* (Chana), *Zea mays* (Makai) and morphological parameters were analyzed, results obtained showed that F13 and F15 formulation shows remarkable plants growth of Bajra, Mung, Chickpea and Maize in 15 days. All samples had performed better than the control. It is possible to establish a small working business in pilot scale primarily for providing effective and improved eco-friendly variety of seeds for better agriculture product that may also be useful for further research.

Keywords: plants, antifungal activity, formulation, seed germination, agriculture crops

1. Introduction
Fungal diseases are a major cause of morbidity mortality worldwide. Fungi are the fifth most common pathogen after *Enterobacteriaceae and Staphylococcus aureus* (Marty and Jarlier, 1998) [1]. The number of multidrug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad spectrum antibiotics and immunosuppressive agents (Dean and Buchard, 1996) [2]. Synthetic fungicides are currently used as control of plant diseases. However, the alternative control methods are needed because the negative public perceptions about use of synthetic chemical, resistance to fungicides among fungal pathogen, and high development cost of new chemicals. In agricultural, the crops loss due to plant pathogen has become major concern. Increased usage of different chemicals in target pathogens and environmental pollution. Some well-known medicinal plants are used to control some of the pathogens. This situation provided the drive to the search for new antifungal substance from various sources like medicinal plants. Traditional medicines have made use of many different plant extracts for treatment of fungal infections and some of these have been tested for antifungal activity (Ishnava *et al*., 2012) [3]. The uses of plant-derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance.

Plants generally produce many secondary metabolites which constitute an important source of antifungal agents. Plants products remain the principal source of pharmaceutical agents used in traditional medicine (Ogundipe *et al.,* 1998) [4]. The effects of plant extracts on fungus have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethno-medicinal plants in India. Interest in many rational natural products has increased (Taylor *et al.,* 1996) [5]. Plants are the sources of natural pesticides that make excellent leads for new pesticides development (Swarna and Neelakanta, 2009) [6].

It is now believed that nature has given the cure of every disease in one way or another. The most important bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds. Natural products of higher plants may possess a new source of antifungal agents with possibly novel mechanism of action (Ahmed and Aqil, 2007) [7]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu *et al.*, 1999) [8]. Therefore, it is of great interest to carry out a screening of these plants to validate their use in folk medicine and reveal the active principle by isolation and characterization of their constituents (Tomoko *et al.,* 2002) [9]. Till now many plant materials are been used to form bio formulation which cure fungal infection in variety of plants (Reddy *et al.,* 2013) [10]. Many plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide (Satish *et al*., 2007) [11]. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant

constituents against several diseases based on their traditional claims of the plants given in Ayurveda (Ishnava *et al*., 2012) [3].

The aim of the present study was to investigate the antifungal effects of Neem, Datura, Tobacco leaves and Cow dung extracts against the pathogenic fungus including *Aspergillus niger*, *Penicillium*, *Scalorotium rolfsii* based on activity the bioformulation preparation and their activity check on the different agriculture crops seed germination.

2. Material and Method

2.1 Plant materials

Based on the ethnomedicinal data the plants species selected for study were *Nicotiana tabacum, Azadirachta* indica, *Datura stramonium* leaves and cow-urine and butter milk. These plants were collected from Charotar region, Kapadavnj, Gujarat, India between March to May 2015. The leaves of all the healthy and disease-free plants were used during throughout the study of formulation preparation, antifungal screening, seeds growth (Germination time, Height of plants, Shoot-Root length, No. of leaves) were performed. The plant specimens were identified by Dr. Kalpesh Ishnava (Plant taxonomy) at Ashok and Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidhyanagar, Gujarat, India. Media used for screening of antifungal activity: potatoes dextrose agar, potatoes dextrose broth (HiMedia).

Table 1: Plant used in bioformulation

Sr. No.	Plant Name	Family	Local Name	Parts Use
1	*Azadirachta* indica	*Meliaceae*	Neem	Leaves
2	*Nicotiana tabacum*	Solanaceae	Tobacco	Leaves
3	*Datura stramonium*	Solanaceae	Datura	Leaves

2.2. Preparation of plant leaves extracts

First, the required leaves of selected plants were thoroughly washed with running tape water, blotted and dried under sunlight. To making powders it was grinded in grinder. The extraction procedure was done using the maceration method (Tiwari *et al*., 2011) [12]. From these, separate each formulation of 50 grams of powdered material was soaked in 500 mL of cow-urine and butter milk in both flask for 24 hours at room temperature under shaking condition (130-140rpm) respectively. The extract was filtered with the help of whatman filter paper number-1. The filtrate was collected in petridish and dried at room temperature. The dried extract from petridish was scrapped and transferred to eppendorf tube. This material used for the antifungal activity. The different concentrations of leaves extracts (Neem, Dhatura, and Tobacco) and cow dung extract were suspended with different concentrations of solvents (cow urine and buttermilk) used for the preparation of the bioformulation for antifungal activity and seed germination study.

Table 2: Composition of different formulations

Formulation	Neem*	Dhatura*	Tobacco*	Cow dung*	Butter milk	Cow-urine
F1	10	1	2	20	82.5	82.5
F2	20	2	3	30	137.5	137.5
F3	20	3	4	30	142.5	142.5
F4	20	4	5	30	147.5	147.5
F5	20	5	6	30	152.5	152.5
F6	20	5	7	30	155	155
F7	20	5	8	30	157.5	157.5
F8	20	5	9	30	160	160
F9	20	5	10	30	162.5	162.5
F10	30	5	10	30	187.5	187.5
F11	40	5	10	30	212.5	212.5
F12	50	5	10	30	237.5	237.5
F13	25	3	5	30	157.5	157.5
F14	15	6	7	30	145	145
F15	10	4	8	30	130	130

*All quantities are in Grams

2.3 Determination of antifungal activity

2.3.1 Preparation of sample

A sample for antifungal activity was prepared by dissolving 100 mg of different formulation (F1 to F15) 1 ml of DMSO (Qualigens Fine Chemicals, Mumbai).

2.3.2 Preparation of fungal spore suspension

Different fungal strains were obtained from different places. The *Aspergillus* niger, *Sclerotium rolfsii and Penicillium sp.* were kind gift from the Department of Biosciences, Sardar Patel University, Vallabh Vidynagar, Gujarat, India. Different strains were transferred to fresh slants in sterile conditions and were incubated at 28^0C till sporulation. These spore suspensions were preserved in 250-ml sterile flasks. This sterile spore suspension can be used for antifungal activity.

2.3.3 Antifungal assay

A drop of fungal spore suspension was placed in the center of PDA (Hi Media, Mumbai) plates and spread all over with a sterile glass spreader. Wells were bored with a sterile cup borer and filled with 100 µl of formulation extract. Plates were placed in the refrigerator for 10 min and then transferred to the incubator held at 28^0C and were incubated for 72 hours, and then the plates were observed for zone of inhibition. Antifungal activity was calculated by measuring the diameter of the zone. The experiment was carried out in duplicate and mean of diameter of inhibition zone was calculated. 100% DMSO (Qualigens Fine Chemicals, Mumbai) used as a

control.

2.4 Seed germination

The experiments were conducted to the study of enhancement in the germination growth and yield of *Pearl millet* (Bajra), *Vigna radiata* (Mung bean), *Cicer arietinum* (Chana), *Zea mays* (Makai) using pre-sowing bioformulation treatment of seeds. Experiments were conducted at Chemistry laboratory of ARIBAS, New V.V.Nagar, Anand, Gujarat. Genetically uniform seeds of Bajra, Mung bean, Chana and Makai were collected from Kapadwanj, Gujarat. Before the formulation treatment, seeds were given primary treatment. Bajra, Mung bean, Chana and Makai seeds were socked in tap water for overnight to make its skin softer. The selection of the formation based on the best antifungal activity performance those only selected 05 (F3, F6, F10, F10, F13 and F15) out 15 formulations. Seeds were divided in to 6 group (05 - F3, F6, F10, F10, F13, F15 and 01- Control) treatment is given to the seeds. For this purpose, seeds were socked in different five formulations for 24 hrs and one group consider as control, which without giving seed treatment it's socked only in tap water for 24 hrs.

2.5 Data analysis

After giving different five formulation treatment to seed following observations were taken. The numbers of normal seedling were count and percentage of germination count. 1. Germination was counted till 3rd day of experiment. 2. Four normal seedlings were selected from each treatment and shoot length was measured from the base of mesocotyl and mean shoot length was expressed in cm.3. Four normal seedlings were selected from each treatment, the root length was measured from the tip of primary root to base of mesocotyl and mean shoot length was expressed in cm.4. The height (in cm) of plants grown on the pots of seed treatment with formulations. Observations were taken 15 days growth with 5 days intervals. The height was measured from the soil surface within pots.5. The normal plant was put in dry tube and put it in hot air oven at 65 to 70^0 C for 48 hrs, after drying plant were cooled the weight of dried plant were recorded (gm/plant).6. The number of plant leaves produce observations were taken 15 days growth with 5 days intervals.

3. Result and Discussion

3.1 Antifungal activity of different formulation

Different 15 bioformulations were tested on antifungal activity by agar well diffusion method (Figure 1). 12 out of 15 bioformulation give show the zone of inhibition against *Aspergillus niger*. The maximum zone of inhibition is 2.7 mm in formulation no.15 against *Aspergillus niger*. 11and minimum 11 out of 15 bioformulation give show the zone of inhibition against *Sclerotium rolfsii*. The maximum zone of inhibition is 4.5 mm in formulation no.06 against *Sclerotium rolfsii*. 14 out of 15 bioformulation give show the zone of inhibition against *Penicillium sp.* The maximum zone of inhibition is 4.1 mm in formulation no.10 against *Penicillium sp.* 05 (F3, F6, F10, F13 and f15) out of 15 bioformulation give show maximum zone of inhibition against *Aspergillus niger*, *Sclerotium rolfsii and Penicillium sp.* Muhammad *et al.*, (2010) [13] reported the *in-vitro* evaluation of *Nicotiana tabacum* and *Azadirachta* indica, leaves plant extracts on mycelial growth of *sclerotium rolfsii* using the different organic solvent. In our study both the plant using in bioformulation better result against *sclerotium rolfsii*. Suleiman (2012) [14] reported the antifungal properties of leaf extract of neem and tobacco on three fungal pathogens (*Aspergillus niger*, *Sclerotium rolfsii and Penicillium sp.).* This result compared to our result using the bioformulation after checking the antifungal activity is increasing. This result shows the bioformulation use the cow-dug, butter milk and water use in the extraction give better response. 05 out of 15 bioformulation which showed maximum zone of inhibition were selected for seeds treatment experiment as shown in Figure 1.

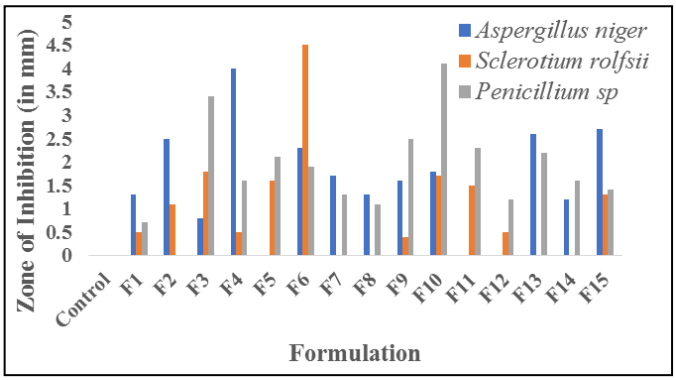

Fig 1: Antifungal effects of different bioformulations.

3.2 Effect of bioformulation in seed germination and growth development

3.2.1 Seed germination of different crops seeds

The selected 05 formulation further study of seed germination. The seed germination study in the *Pearl millet* (Bajra), *Vigna radiata* (Mung bean), *Cicer arietinum* (Chana) and *Zea mays* (Makai) using pre-sowing bioformulation treatment. The formulation accelerates seeds germination, seedling growth and root formations study. The various experiments performed for seed germination in petri plates and pots. The use of formulation pre-sowing treatment was found to enhance growth of Bajra, Mung bean, Chana and Makai plays in early stage of growth compare to the control. The seed germination percentage after 48 hrs show in figure 2. In all measurements, plant grown from treated seeds performs better growth than control plants.

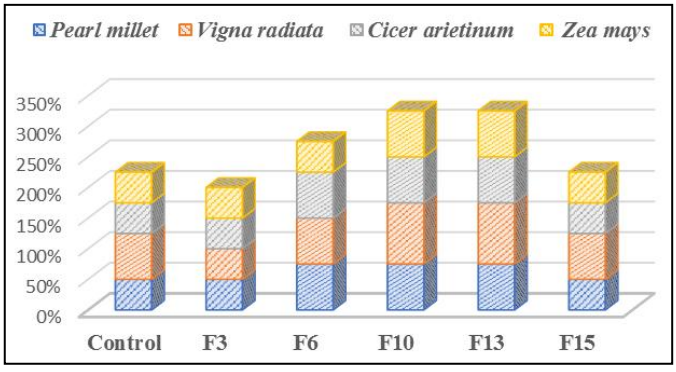

Fig 2: Seeds germination percentages after 48hrs

Figure 2 shows the germination rate of Bajra, Mung bean, Chana and Makai and control seeds treated with five different formulations. Treatment after 24 hrs it was observed the effect of bioformulation in different seed germination. In Bajra, F6, F10 and F13 germination rate were increased compared to control and F3 and F15 were same as control. In Mung bean, F10 and F13 germination rate were increased compared to control and in F6 and F15 were as remain same. In Chana, F6 and F13 germination rate were increased compared to control and F3, F10 and F15 were same as control. In Makai, F13 germination rate were increased compared to control and F3, F6, F10 and F15 were same as control.

3.2.2 Growth development in different crops

3.2.2.1 Different time interval study of growth (Height in cm)

The selected 05 formulation further study of effect on the plant growth. The growth study in the *Pearl millet* (Bajra), *Vigna radiata* (Mung bean), *Cicer arietinum* (Chana) and *Zea mays* (Makai) using bioformulation treatment. The formulation accelerates growth, plant growth study in form of height length after different time interval (5 Days, 10 Days and 15 Days), The various experiments performed for growth in pots. The use of different formulation treatment was found to enhance growth of Bajra, Mung bean, Chana and Makai plays in early stage of growth compare to the control. The growth observed in after 5 Days, 10 Days and 15 Days show in figure 3. In all measurements, plant grown from treated plants performs better growth than control plants.

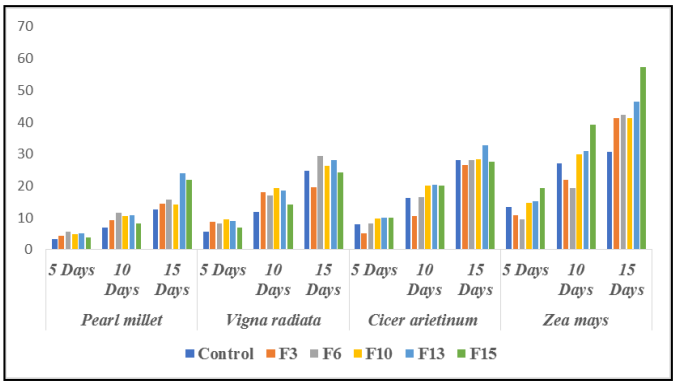

Fig 3: Different time intervals study of growth (in height in cm)

In *Pearl millet*, afterv15 days best response in the formulation no.13 observed the 24 cm height. In *Vigna radiata*, after 15 days best response in the formulation no.06 observed 29.3 cm height. In *Cicer arietinum*, 15 days best response in the formulation no.13 observed 32.8 cm height. In *Zea mays*, 15 days best response in the formulation no.15 observed 57.3 cm height. It is compare to other plants maximum height observed. It is observed that selected plants among best response in the formulation 15 in *Zea mays*. It showed that there is tremendous increase in the height of maize plant (57.3cm) grown with treatment of F15compare to control (30.6 cm) while in case of Chana and Bajra plant growth is increase in plants which was treated with F13. Besides this it was observed there was no significant growth was observed in mung.

3.2.2.2 Different time interval study of growth in shoot and root

The selected 05 formulation further study of effect on the plant growth of shoot and root length. The shoot and root study in the *Pearl millet* (Bajra), *Vigna radiata* (Mung bean), *Cicer arietinum* (Chana) and *Zea mays* (Makai) using bioformulation treatment. The formulation accelerates growth, plant growth study in form of length after different time interval (5 Days, 10 Days and 15 Days), The various experiments performed for growth in pots. The use of different formulation treatment was found to enhance the shoot and root growth of Bajra, Mung bean, Chana and Makai plays in early stage of growth compare to the control. The growth observed in after 5 Days, 10 Days and 15 Days show in figure 4. In all measurements, plant shoot and root from treated plants performs better growth than control plants.

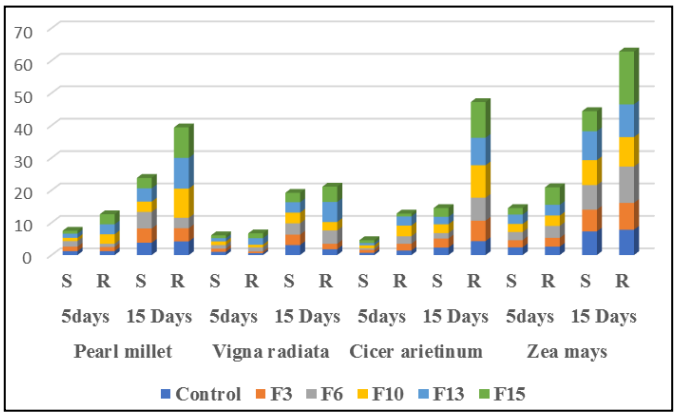

Fig 4: Different time intervals study of shoot and roots (in length in cm)

Results obtained showed that there are tremendous increases shoot and root length. In Bajra, Mung bean, Chana and Makai. Root growth measured shows significant differences among treatments of formulations. In *Pearl millet*, after15 days best response in the formulation no.10 and 15 observed the 5.1 cm and 9.5 cm shoot and root length respectively were increased compared to control. In *Vigna radiata*, after15 days best response in the formulation no.10 and 15 observed the 3.5 cm and 6.2 cm shoot and root length respectively were increased compared to control. In *Cicer arietinum*, 15 days best response in the formulation no.3 and 15 observed 2.8 and 11 cm shoot and root length respectively were increased compared to control. In *Zea mays*, 15 days best response in the formulation no.13 and 15 observed 8.9 and 16.3 cm shoot and root length respectively were increased compared to control.

3.2.2.3 Fresh and Dry weight and number of leave

The fresh weight and dry weight of plants treated by bio formulations and control plants show in the figure 5. In *Pearl millet*, maximum fresh and dry weight observed in the formulation no.6 fresh and dry weight 26.2gm and 0.045 gm respectively and number of leaves four produce. In *Vigna radiata*, maximum fresh and dry weight observed in the formulation no.6 fresh and dry weight 26.2gm and 0.045 gm respectively and number of leaves five produce. In *Cicer arietinum*, maximum fresh and dry weight observed in the

formulation no.13 fresh and dry weight 1.112 gm and 0.078 gm respectively and number of leaves five produce. In *Zea mays*, maximum fresh and dry weight observed in the formulation no.15 fresh and dry weight 4.16 gm and 1.098 gm respectively and number of leaves five produce. In formulation no.3 decreased fresh weight and dry weight compared with control and formulation no.10,13 and 15 were increasing fresh weight and dry weight compared with control.

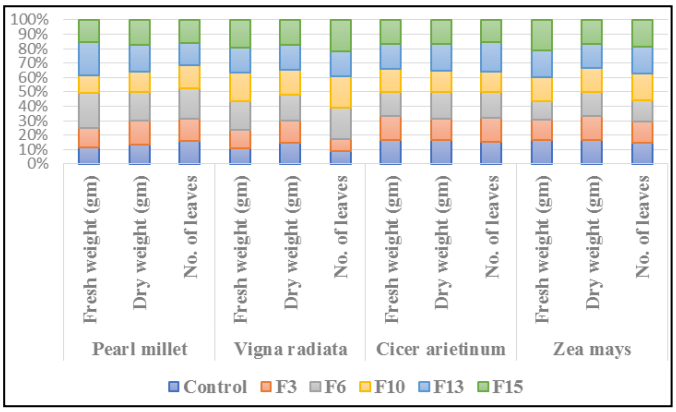

Fig 5: Fresh and Dry weight in gm) and number of leaves

4. Conclusion

Bio-formulation using the different plant extracts of *Nicotiana tabacum*, *Azadirachta* indica, *Datura stramonium* leaves with cow dung, cow urine and buttermilk were prepared. Total 15 Bio-formulations were prepared and investigated for their antifungal activity. It was observed that 05 (F3, F6, F10, F13 and F15) out of 15 formulation showed good antifungal activity compared to other prepared formulations. Based on the good antifungal activity formulation selected for study of seed germination and growth of different agriculture crops of *Pearl millet* (Bajra), *Vigna radiata* (Mung bean), *Cicer arietinum* (Chana), *Zea mays* (Makai). The results showed that F15 formulation shows remarkable plants growth of *Pearl millet* (Bajra), *Vigna radiata* (Mung bean), *Cicer arietinum* (Chana), *Zea mays* (Makai) after15 days. The result revealed that the effects of formulations well as duration of exposure influence growth of plant. The significant improvements were seen in growth and yield of formulation no.13 and 15 treated plant as compared to their control. Pre-sowing formulation treatment significantly increased the wet and dry weight and height of plant in compare to control. Formulation treatment plant positively affected both the shoot and root length and number of leaves.

It is possible to establish a small working business in pilot scale primarily for providing effective and improved eco-friendly variety of seeds for better agriculture product that may also be useful for commercial agriculture.

5. Acknowledgements

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