



Comparative Study of Germination Rate and Plant Growth by Secondary Metabolites and *In Vitro* LDPE Biodegraded Fragments by Microbes

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Accepted on: 10-05-2013; Finalized on: 31-07-2013.

ABSTRACT

The biodegradable polymers are the best alternatives than the conventional plastic materials. Though polymers are biodegraded by living cells, these monomers and the metabolites produced by those microbial cells may cause toxic effects to the environment. In this study, low density polyethylene fragments were degraded by different bacterial and fungal samples. After the biodegradation the monomeric fragments along with bacterial and fungal metabolites were used for germination of seed and seedling growth of *Cicer arietinum* and *Vigna radiata*. It showed efficient results in almost all the cases for both plants which implies that the extracts are disposing safe.

Keywords: LDPE fragments, Secondary metabolites, Seed germination, Seedling growth.

INTRODUCTION

Soils are contaminated by undesirable compounds, these compounds may be biochemical or chemical in nature. These toxic materials cause several adverse effect on seed germination, plant growth etc. Because these compounds can alter the various physicochemical characters of particular soils, such as, temperature, pH, total carbon content, texture of soils. Plastics are man-made long chain polymeric molecules¹. In recently, plastics are the biggest threatening for all types of living systems. Thus, there is a kin quest for some alternatives which can reduce this problem. Biodegradable polymer is the apt solution for this in which degradation of polyethylene is done by microorganisms. Biodegradation resulting from the utilization of polyethylene as nutrient may be more efficient if the degrading microbes form a biofilm on the polypeptide surface². Microbial degradation of plastic caused by oxidation or hydrolysis using microbial enzymes that lead to chain cleavage of the high molecular weight polymer to low molecular weight oligomer and monomer by aerobic or anaerobic metabolism³. There is a wide range of microbes which can degrade the plastics. The microbial species are associated with the degrading materials were identified as bacteria (*Pseudomonas*, *Streptococcus*, *Staphylococcus*, *Micrococcus* and *Moraxella*), fungi (*Aspergillus niger*, *Aspergillus glaucus*), *Actinomyces* sp. and *Saccharomonospora* genus⁴.

In case of degradable plastics, polymeric compounds depolymerized to monomers, which may or may not cause adverse effect to the plants or other living organisms in case of disposing those without any treatment. Though the polymers degrade to monomers, these materials may be toxic to the environment. Unofficial methods of recycling particularly in developing countries can cause the release of toxic chemicals to the environment. After degradation of plastic the long chain

of polymers changed to small monomers termed as micro plastics ie., not visible to naked eye and is toxic to living organism if ingested⁵. Some of the major plastics such as PVC and polystyrene found to produce monomers which is linked to cause cancer and reproductive problems and usually remains in the environment as bioaccumulated form^{6,7}. Apart from the products of depolymerization, the secondary metabolites produced by the degrading microbes also impact on all types of biological systems.

The chickpea, *Cicer arietinum* L. (Fabales: Fabaceae), is one of the most important grain-legume crops in the world, and the Asia region comprising Turkey contributes 89% of the world chickpea production⁸.

Vigna radiata (L.) Wilczek is a native of India and Central Asia and has been grown in these regions since prehistoric times⁹. It is an important source of easily digestible high quality protein for vegetarians and sick persons and contains 24 per cent protein, 0.326 per cent phosphorus, 0.0073 per cent iron, 0.00039 per cent carotene, 0.0021 per cent of niacin and energy 334 cal/100 g of green gram¹⁰.

Thus, the present study was undertaken to investigate the change in percentage of seed germination, the level of growth in terms of root and shoot length of seedlings of *Cicer arietinum* and *Vigna radiata* using the depolymerized fragments and secondary metabolites produced by LDPE degrading microorganisms.

MATERIALS AND METHODS

Plant materials and chemicals

Mature and healthy seeds of *Cicer arietinum* and *Vigna radiata* were purchased from a traditional medicine market in Tamil Nadu, India. The synthetic media used for culturing LDPE (low density polyethylene) degrading bacteria and fungi isolates were purchased from Hi Media (Mumbai). All the chemicals were of higher grade. The

LDPE film was obtained from B.N.Polymers, Bangalore, India.

Preparation of extract by LDPE degrading microbes

The synthetic medium was supplemented with 1.5% of LDPE powder was prepared for degradation of plastic by two bacterial samples (two *Bacillus* sp., denoted as BSM-1 & 2) and eight fungal samples (six *Aspergillus* sp., denoted as FSM- 1,3,4,5,6,8 and two *Fusarium* sp., denoted as FSM- 9,10). The medium composition as follows: (g/l: K_2HPO_4 1, KH_2PO_4 0.2, $(NH_4)_2SO_4$ 0.001, $ZnSO_4 \cdot 7H_2O$ 0.001) with pH 7.0. The broth cultures for bacterial and fungal samples were incubated for 60 d in shaker incubator at 200 rpm at 37°C. After the degradation period, the individual culture broth was centrifuged at 10,000 rpm for 15 min, to remove cellular debris and clear the supernatant for each ten cultures used for germination and plant growth study.

Germination study

Previous to germination test, both types of seeds were surface sterilized by immersion in 1% sodium hypochlorite for 5 min, and washed afterwards with distilled water¹¹. Twenty two petriplates were taken, out of two plates were marked as control plate for individual plant. The rest 20 plates separated for both types of plant. Each 10 plates marked for ten specific culture supernatant. Ten seeds of each plant were placed on filter paper (initially sterilized) in petridishes (90 mm in diameter). To moisture the filter paper, 5ml of specific culture supernatant per day was added in all 20 plates, but in case of individual control plate, distilled water was added. The petridishes were set in an incubator with constant temperature at 25°C and light and dark periods were controlled for 12 h each. This germination trials were performed for 10 d and the germination rate were calculated. A seed was considered germinated when the radicle break through the seed coat¹².

Pot experiment

Before starting this experiment, the collected garden soil was tested (Hi Media kit, 1K054). 5 kg of soil were taken for each treatment. Two different types of seeds were used. Ten seeds were sowed in each soil pot. An extra set of pots in which distilled water was added instead of culture supernatant, served as control for each plant. 20ml of extract per day was added in each pot. After 10 d, the seedlings were harvested and root and shoot lengths were recorded for both plants.

RESULTS AND DISCUSSION

The present work demonstrates the toxic effects of LDPE biodegradable fragments and metabolites produced by LDPE degrading microorganisms on two plants, namely *Cicer arietinum* and *Vigna radiata*. To investigate this, two parameters were observed such as germination rate and growth of seedlings in terms of root and shoot length.

Comparison of seed germination of *Cicer arietinum* and *Vigna radiata*

The effect of extract of LDPE degrading microbes on *Cicer arietinum* and *Vigna radiata* on seed germination were presented in Figure 1. In case *Cicer arietinum*, bacterial sample extract BSM-2 and fungal sample extract FSM-4,6,10 showed 80% seed germination and rest all isolates 60% including control. The microbial metabolites and monomers of LDPE exhibited better results compared to control. For *Vigna radiata*, bacterial sample BSM-2 and fungal sample FSM- 1,3 along with control produced 80% results, FSM-10 70% and rest all showed 50% seed germination. Among all the extracts, BSM-2 showed efficient results for both plants. Thus the monomeric fragments of polyethylene and metabolites did not reduce seed germination significantly; in case of *Cicer arietinum*, they exhibited better results than the control.

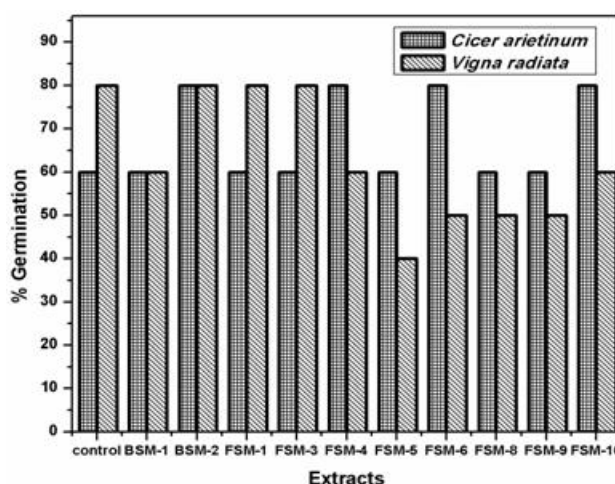


Figure 1: Percentage of seed germination of *Cicer arietinum* and *Vigna radiata*

Comparison of seedling growth of *Cicer arietinum* and *Vigna radiata*

Seedling growth of any plant include the combination of growth of root and shoot length. Thus separately root and shoot growths of those two plants were measured. The soil used for the seedling growth was tested before the experiment and Table 1. shows the different parameters of soils, such as, temperature, pH and the total organic carbon content.

Table 1: Determination of biochemical parameters of soil sample

Parameters		
Temperature	pH	Total organic carbon
30.1°C	6.8	30-50%

Effect of extracts on Root length

The root growth was presented in Figure 2. In case of *Cicer arietinum*, root growth was observed by all extracts except FSM-1. Among all, BSM- 1 & 2, FSM- 3,6,10 produced significant results compared to that control. FSM-1 was with no result and FSM-9 showed minimum

results but rest extracts acted as root growth inducing agents. For *Vigna radiata*, FSM-3,5,9 exhibited similar result like control. But other extracts specially, BSM-1 & 2, FSM- 1,4,6,10 increased root length more than the control one. Thus all the extract showed better root growth for *Cicer arietinum* than *Vigna radiata*.

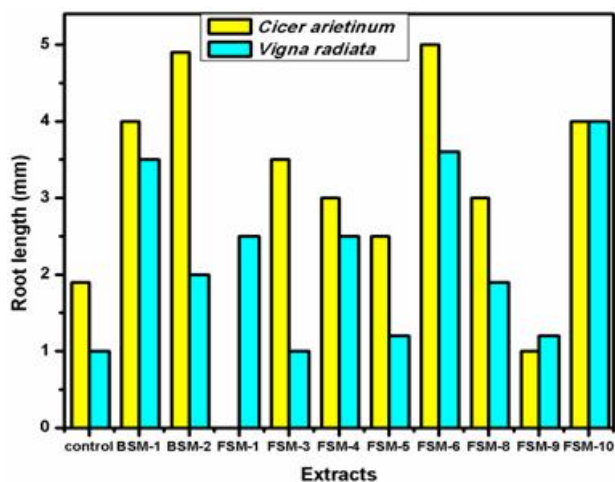


Figure 2: Comparison of root length for *Cicer arietinum* and *Vigna radiata*

Effect of extracts on Shoot length

The effects of all extracts over the shoot growth were different as compared to the effects of root growth as seen in Figure 3. For *Cicer arietinum*, except BSM-2 and FSM-9, other all inhibited the shoot growth compared to the control. FSM-1 totally stopped the shoot growth. Similarly, in case of *Vigna radiata*, only FSM- 6, 10 induced shoot growth. Though there was less increment in shoot length for both plants, still these extracts did not show any toxic effect on the plants.

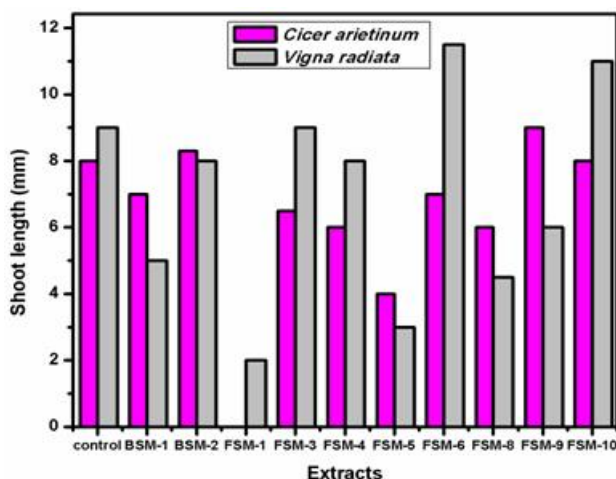


Figure 3: Comparison of shoot length for *Cicer arietinum* and *Vigna radiata*

CONCLUSION

Biodegradable plastics are quite good answer for current environmental hazardous pollution. At the same time the degraded compounds and secondary metabolites produced by LDPE degrading microbes also showed significant germination rate and seedling growth. As a comparison, *Cicer arietinum* showed better results than *Vigna radiata* towards both tests. Therefore, the discardation of culture supernatant is environmentally safe and can be used as plant growth promoting agents.

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Source of Support: Nil, Conflict of Interest: None.