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Effect of Oilcakes as Substrates for Multiplication of Biocontrol Agents and Biofertilizers

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Abstract

A laboratory experiment was conducted to study the effect of locally available material like oilcakes; groundnut, neem and pongamia cake to know the survivability of biocontrol agents and biofertilizers at Research Institute on Organic Farming, University of Agricultural Sciences, GKVK, Bangalore. The effectiveness of these bio-control agents and biofertilizers on different oilcake extracts showed significantly higher compatibility and their multiplication. Shelf life at different intervals was studied upto 60 days of incubation. All the oilcake extracts were able to support higher population till 60 days of incubation *viz*., with glucose (1%) and without glucose which recorded $1.00X10^8$ CFU/ ml of *Pseudomonas fluorescens* and $6.00X10^8$ CFU/ ml of *Trichoderma viride* in pongamia and neem cake extract, respectively. The biofertiliser PSB showed maximum of $7.67X10^8$ CFU/ ml in pongamia extract (with glucose) and *Rhizobium* of $1.00X10^8$ CFU/ ml in groundnut extract (without glucose) was recorded. *Azotobacter* recorded $1. 33X10^8$ CFU/ ml in neem extract (with 1% glucose) during 60th day of incubation. These findings indicate that the effective utilization of locally available oilcakes is possible for mass production of biofertilizers and biocontrol agents which is an eco friendly approach in organic agriculture.

Keywords : Oilcakes, biofertilizers, biocontrol agents, multiplication and survival

Management of pests and diseases is one of the most promising and challenging task in the sustainable agriculture. Due to plant diseases every year nearly 10-20 per cent of the total world food production was decreased and leads to loss of billions of dollars (Babu and Pallavi, 2013). However for many years, pest and diseases were controlled by the use of synthetic pesticides. Use of chemical fertilizers are non-specific in their effect, killing beneficial organisms and have undesirable health and environmental pollution risks. It is well established that the addition of organic additives increased the organic matter contents of the soil and availability of other plant nutrients. For sustaining the desired crop productivity under integrated nutrition system, the nutrient supply through oil-seed cakes, botanicals, and other organic manures not only reduce the dependence on chemical fertilizers but also improve the soil structure, encourage the growth and activity of beneficial organisms in the soil (Rizvi *et al*., 2013). Fungi that protect plants from pathogenic agents include *Trichoderma* sp. (Jin and Custis, 2011) and bacteria that protect plants from pathogenic agents include *Pseudomonas* sp., *Bacillus* sp. (Saharan and Nehra, 2011).

Bio-fertilizers are being essential component of organic farming are the preparations containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic micro-organisms and play a very significant role in improving soil fertility (Mahdi *et al.,* 2010). They are the naturally available, biological system of nutrient mobilization (Venkatashwarlu, 2008). Biofertilizer contains microorganisms which promote the adequate supply of nutrients to the host plants and ensure their proper development of growth and regulation in their physiology. Effective living microorganisms are used in the preparation of biofertilizers. (Bahadur *et al*., 2014).

Long term objective in organic farming is the use of plant growth promoting microorganisms as a viable, sustainable, economic and eco-friendly alternative to synthetic and hazardous chemicals (Rizvi *et al*., 2012). The compatible way of application is necessary for these biofertiliser and biocontrol agents in organic farming to get satisfactory results and also explore the alternate sources of substrate that could be used as a carrier material. With this objective, a laboratory study was conducted to study the effect of locally available material like oilcakes on survivability of biofertilizers and biocontrol agents. There is a need to study the role of oilcake extracts on multiplication and enhancing the activity of *Pseudomonas fluorescens* and *Trichoderma viride* as effective biocontrol agents and PSB, *Azotobacter* and *Rhizobium* as effective biofertilizers. Keeping this in view, an experiment was conducted to study the efficacy of oilseed extracts of groundnut, neem and pongamia for the survival of biocontrol agents and biofertilizers.

Material and Methods

Two biocontrol agents; *Trichoderma viride*, *Pseudomonas fluorescens* and biofertilisers; phosphorus solubilising bacteria (PSB), *Azotobacter* and *Rhizobium* were collected from the organic plots of Research Institute on Organic Farming (RIOF), GKVK, Bengaluru. *Trichoderma viride* was sub-cultured in Potato Dextrose agar medium, *Pseudomonas fluorescens* in Kings B agar medium, *Azotobacter* in Waksman-77 agar medium, PSB in Sperbers agar medium and *Rhizobium* in Yeast extract mannitol agar medium (YEMA) with Congo red in the laboratory. Aqueous extracts of 10 per cent concentration of pongamia, groundnut and neem cakes were prepared by soaking appropriate amounts of finely powdered oilcakes in sterile tap water for twelve hours and filtering through a muslin cloth (Rao *et al*., 1998). 15 ml of each extract was taken in a 30 ml test tube and similarly an another set containing 15 ml of extract with 1 per cent glucose was autoclaved at 15 lb pressure for 30 minutes. Each treatment was replicated thrice. Sterilized test tubes were inoculated with *Trichoderma viride* ($1x10^9$ CFU/ ml), *Azotobacter* ($1x10^9$ CFU/ml), PSB ($1x10^9$ CFU/ml) and *Rhizobium* ($1x10^9$ CFU/ml) and *Pseudomonas fluorescens* ($1x10^9$ CFU/ml). These tubes were incubated at 28 ± 2^0 C at different intervals up to 60 days. The treatments included were T_1: Pongamia cake; T_2: Neem cake; T_3: Groundnut cake; T_4: Pongamia cake + 1 per cent glucose Cake; T_5: Neem cake + 11 per cent glucose; T_6: Groundnut cake + 11 per cent glucose.

The multiplication and survival of *Trichoderma viride*, *Pseudomonas fluorescens*, *Azotobacter,* PSB and *Rhizobium* in different oilcake extracts at different intervals was studied. Survival of these biofertiliser and biocontrol agents in different oilcake extracts with and without glucose (1%) at different intervals of 3, 6, 9, 12, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 days of incubation was determined. Survivability of the biocontrol agents were enumerated by standard plate count technique. All the microorganisms were enumerated in respective agar medium and the observations were recorded after their incubation period (28 ± 2º C and 24 - 48 hours of incubation for bacteria and 48 - 76 hours of incubation for fungi).

Results and discussion

Pseudomonas fluorescens

The population of *Pseudomonas fluorescens* varied significantly due to different treatment effects. Population of *Pseudomonas fluorescens* in groundnut, pongamia and neem extracts was quite good in all the treatments till 45 days of storage period (Fig. 1). *Pseudomonas* population was found better in all the oilcake extracts supplemented with 1 per cent of glucose and distinguished fluorescence was found around the colonies. But the population of *Pseudomonas fluorescens* in the pongamia extract supplemented with 1 per cent of glucose was found

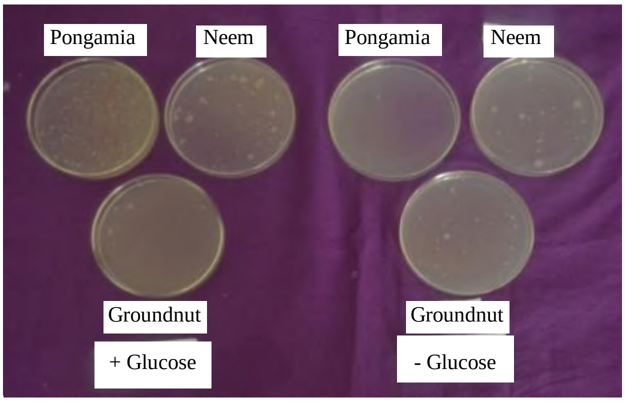

Fig. 1: Growth of *Pseudomonas fluorescens* as influenced by different oilcake extracts

superior over other extracts during storage. A gradual decrease was noticed in all the oilcake extracts over a period. The pongamia extract with 1 per cent of glucose supported significantly higher *Pseudomonas* population till 55th day of storage which recorded $1.00x10^8$ CFU/ml of sample followed by groundnut cake extract supplemented with 1 per cent of glucose which recorded $3x10^8$ CFU/ml of sample at 50th day of storage (Table I). Neem cake extract with glucose 1 per cent showed lesser viability of *Pseudomonas fluorescens* during the storage intervals which recorded

41.67×10^{8} CFU/ml of *Pseudomonas* initially and the *Pseudomonas* population was zero after 40 days of storage period. Similarly, groundnut cake supplemented with 1 per cent glucose recorded maximum of 29.33×10^{8} CFU/g of *Pseudomonas* at 20 days of storage period and gradually decreased to zero at 55th day of storage (Table I). The combination of Pongamia with 1 per cent glucose served better when compared to other oilcake extracts.

Trichoderma viride

The bioformulation of *Trichoderma viride* in different oil cake extracts showed good microbial load during the different storage period. There was no significant difference among different treatments tested with and without glucose conditions. However, neem cake extract with 1 per cent of glucose showed better and consistent population of *Trichoderma viride* till the 60 days of storage period (4.33×10^{8} CFU/ml) followed by neem cake alone (4.33×10^{8} CFU/ml) at 60th day (Table II). Even though groundnut + 1 per cent glucose and pongamia+ 1 per cent glucose extract showed higher population(1.33×10^{8} CFU/ ml) at initial stages, the population decreased to 1.33×10^{8} CFU/ ml in both the extracts at 55th day of extended storage period. Neem cake extract found better with respect to the survival of *Trichoderma viride* and also good sporulation was observed at all the storage intervals followed by groundnut and pongamia cake extract (Fig. 2).

Phosphorus Solubilising Bacteria (PSB)

Among different treatments tested at different intervals till 60 days of incubation, showed significant difference in population of PSB (Table III). All the treatments showed better population till 40 days of incubation period with maximum of 41.17×10^{8} CFU/ ml of PSB in groundnut extract + 1 per cent glucose at 3rd day of incubation and minimum of 8.67×10^{8} CFU/ ml of PSB population in neem extract alone at 40th day of incubation. In later days of incubation the population has decreased in all the treatments. The treatments supplemented with 1 per cent of glucose served better with respect to PSB counts when compared to the treatments with out glucose. Pongamia extract with 1 per cent glucose showed significantly maximum population till 60th days of storage period which recorded 34.83×10^{8} CFU/ ml of sample at initial day of incubation and 7.67×10^{8} CFU/ ml of PSB at 60th day of incubation. The next better treatment was found to be groundnut extract with 1 per cent of glucose which recorded 6.33×10^{8} CFU/ ml of sample during 60th day of incubation. The extracts without glucose retained less population during different intervals of storage period. Among the treatments without glucose, neem and groundnut extract was found better than pongamia extract which recorded 25.67×10^{8} CFU/ ml and 39.50×10^{8} CFU/ ml of PSB initially and upon incubation at 60th day the population was 3.67×10^{8} CFU/ ml in neem and groundnut extract, respectively (Table III).

Azotobacter

Azotobacter population differed significantly upto 30 days of incubation with the oilcake extracts supplemented with 1 per cent glucose when compared to with out glucose. Among oilcake extracts, higher population was present in the extract of neem with 1 per cent of glucose which recorded 6.33×10^{8} CFU/ ml of sample initially and the population was consistent and retained to 1.33×10^{8} CFU/ ml of *Azotobacter* population at 60th day of storage (Table IV). Even

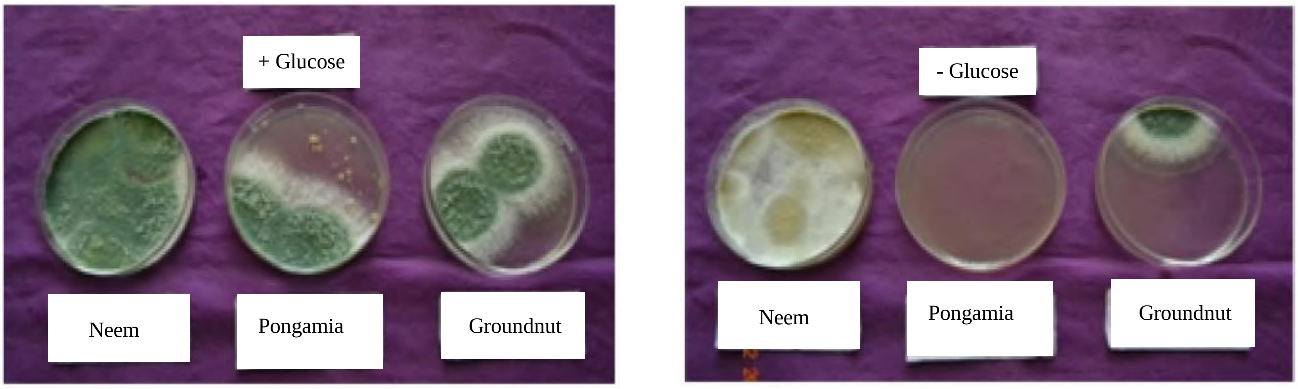

Fig. 2 : Growth of *Trichoderma viride* as influenced by different oilcake extracts

TABLE I

Effect of oilcake extracts and glucose on population of Pseudomonas fluorescens *at different intervals of incubation*

Oil Cake Extracts	*Pseudomonas fluorescens* (10^8 CFU/ ml of sample)													
	Number of Days													
	3	6	9	12	15	20	25	30	35	40	45	50	55	60
Pongamia	4.33	12.33	8.33	8.33	39.33	18.00	1.33	3.33	3.00	6.33	7.33	2.33	0.00	0.00
Neem	38.67	8.00	9.33	6.67	26.33	19.33	2.33	14.33	11.33	7.00	5.00	1.33	0.00	0.00
Groundnut	59.33	39.33	31.67	31.33	14.67	20.00	14.00	10.00	8.00	6.00	3.33	1.00	1.00	0.00
Pongamia + 1% glucose	81.33	56.33	47.33	38.33	30.67	22.00	23.33	14.00	13.67	10.33	9.00	3.00	1.00	0.00
Neem + 1% glucose	41.67	3.33	2.00	2.00	3.00	5.00	2.00	1.33	1.33	0.00	0.00	0.00	0.00	0.00
Groundnut + 1% glucose	21.33	10.33	20.00	27.67	25.00	29.33	20.67	19.67	11.00	13.67	4.33	3.00	0.00	0.00
S.Em ±	2.39	1.54	0.95	1.02	1.23	0.93	0.68	0.83	1.00	0.62	0.47	0.38	0.33	0.00
CD at 1%	10.33	6.65	4.11	4.40	5.32	4.03	2.94	3.58	4.32	2.69	2.04	1.66	NS	0.00

TABLE II

Effect of oilcake extracts and glucose on population of Trichoderma viride *at different intervals of incubation*

Oil Cake Extracts	*Trichoderma viride* (10^8 CFU/ ml of sample)													
	Number of Days													
	3	6	9	12	15	20	25	30	35	40	45	50	55	60
Pongamia	4.00	2.33	2.33	2.00	4.33	3.00	2.00	2.33	1.33	0.33	0.00	0.00	0.00	0.00
Neem	4.33	2.33	2.33	4.00	7.00	6.33	3.33	3.33	6.00	4.00	3.00	4.00	4.33	4.33
Groundnut	3.00	8.33	4.00	2.33	7.33	5.00	4.33	3.33	1.33	2.33	1.00	1.33	0.00	0.00
Pongamia + 1% glucose	1.33	1.33	2.33	2.33	7.33	5.33	3.00	2.00	6.00	2.00	2.33	2.00	1.33	0.00
Neem + 1% glucose	4.00	3.33	3.33	3.00	6.00	6.00	3.33	4.67	7.33	4.33	4.33	5.33	6.00	4.33
Groundnut + 1% glucose	1.33	3.33	3.00	3.33	7.00	6.33	3.33	2.33	4.33	4.33	4.00	2.33	1.33	0.00
S.Em±	0.62	0.71	0.36	0.47	0.82	0.78	0.59	0.38	0.59	0.49	0.38	0.33	0.33	0.19
CD at 1%	2.69	3.05	1.56	NS	NS	NS	NS	1.66	2.56	2.12	1.66	1.44	1.44	0.83

TABLE III

Effect of oilcake extracts and glucose on population of PSB at different intervals of incubation

Oil Cake Extracts	PSB (10^8 CFU/ ml of sample) Number of Days													
	3	6	9	12	15	20	25	30	35	40	45	50	55	60
Pongamia	36.00	20.50	26.50	18.67	19.67	15.83	30.67	22.33	7.67	12.00	4.00	3.33	5.67	1.67
Neem	25.67	21.67	22.00	17.83	22.50	22.17	21.67	25.33	14.67	8.67	7.67	3.67	3.67	3.67
Groundnut	39.50	11.00	31.50	22.33	24.00	26.83	13.33	15.33	12.67	9.67	8.33	9.67	3.67	3.67
Pongamia + 1% glucose	34.83	29.67	34.50	29.00	28.50	18.50	25.00	17.33	12.33	13.00	10.67	9.67	9.00	7.67
Neem + 1% glucose	33.67	24.67	21.50	19.83	17.50	22.17	23.00	13.67	15.33	11.00	7.33	6.33	7.67	5.33
Groundnut + 1% glucose	41.17	28.50	66.00	25.00	24.83	26.83	26.00	13.00	18.33	18.00	15.00	4.67	6.67	6.33
S.Em±	1.94	1.17	1.94	1.44	1.62	1.40	1.80	1.26	1.00	1.33	0.72	0.67	0.73	0.33
CD at 1%	8.39	5.04	8.37	6.24	7.01	6.06	7.75	5.45	4.32	5.73	3.11	2.88	3.17	1.44

though a significantly higher population of *Azotobacter* (24.33X 10^8 CFU/ ml) was noticed in extracts of groundnut with 1 per cent glucose at initial levels, the survival of PSB was reduced to 4.67X 10^8 CFU/ ml at 40th day of incubation and there was no population observed in later days of incubation. Similar trend was observed with Pongamia + 1 per cent of glucose which recorded 8.83X 10^8 CFU/ ml at 3rd day and 0.67X 10^8 CFU/ ml of *Azotobacter* at 55th day of incubation. Pongamia and neem extract without glucose also supported significant number of *Azotobacter* population till 60 days of incubation which recorded 21.50X 10^8 CFU/ ml and 8.50X 10^8 CFU/ ml of *Azotobacter* initially and 1X 10^8 CFU/ ml of *Azotobacter* population at final day of incubation, respectively.

TABLE IV

Effect of oilcake extracts and glucose on population of Azotobacter *at different intervals of incubation*

Oil Cake Extracts	*Azotobacter* (10^8 CFU/ ml of sample) Number of Days													
	3	6	9	12	15	20	25	30	35	40	45	50	55	60
Pongamia	21.50	5.50	4.17	10.33	8.17	5.17	5.00	4.67	4.00	6.33	5.67	1.67	1.00	1.00
Neem	8.50	21.67	5.50	4.83	4.33	5.00	3.00	4.00	3.33	5.33	6.67	2.67	1.33	1.00
Groundnut	13.17	7.00	6.00	14.33	5.50	7.17	4.33	3.67	3.00	4.67	3.33	1.67	1.00	0.00
Pongamia + 1% glucose	8.83	9.17	8.00	5.83	6.67	14.50	9.00	6.67	4.00	5.67	1.00	1.33	0.67	0.00
Neem + 1% glucose	6.33	7.17	10.17	6.67	5.83	8.83	4.33	6.33	4.33	6.00	5.00	5.00	1.33	1.33
Groundnut + 1% glucose	24.33	7.67	12.50	10.67	19.00	10.67	5.33	4.33	5.00	4.67	0.00	0.00	0.00	0.00
S.Em±	0.95	0.60	0.59	0.75	0.74	0.80	0.41	0.38	0.56	0.45	0.41	0.43	0.33	0.27
CD at 1%	4.08	2.61	2.53	3.26	3.19	3.47	1.76	1.66	NS	NS	1.76	1.86	NS	1.18

Rhizobium

Rhizobium population varied significantly due to oil cake extracts and glucose and presented in Table V. Significantly higher population of (1.00×10^8 CFU/ ml) *Rhizobium* at final days was recorded in the treatment of groundnut cake with out 1 per cent of glucose followed by neem cake extract which recorded 0. 33×10^8 CFU/ ml of *Rhizobium* at final days of incubation. Treatments supplemented with glucose showed significantly higher population till 50 days of storage period when compared to the treatments without 1 per cent of glucose as an additive (Table V). Groundnut extract without 1 per cent glucose recorded maximum of 16.17×10^8 CFU/ ml and minimum of 1.00×10^8 CFU/ml of *Rhizobium* counts at 3rd and 60th day of incubation, respectively.

The results from the study showed that the oilcakes of plant origin, served as a better carrier material for biofertiliser and biocontrol agents and also in their survival. Oil-cakes are generally rich in nutrient ingredients such as nitrogen, phosphorus, potash and other nutrients and are more effective under moist conditions. These results are in agreement with the use of number of organic additives of plant origin, including oil-seed cakes, chopped plant parts and seed dressing with plant extracts which have been used as nematode control agents (Tiyagi and Alam, 1995). Neem cake and pongamia cake supplemented with glucose (1 per cent) was found better for multiplication of *Trichoderma* and *Azotobacter*, *Pseudomonas*, respectively. It was observed that response of microorganisms varied differently with different oilcake extracts. Addition of glucose as a carbon source has been found advantageous since these biofertilizer and biocontrol agents were found to survive better for more number of days in the extract. Similar results are noticed where shelf life of inoculants can be improved by the addition of polymers, chemicals and amendments (Sivasakthivelan and Saranraj, 2013). Also these results were in conformity with Devakumar *et al*. (2011) and Devakumar *et al*. (2014) who have also recorded higher population of beneficial microorganisms in different locally available organic substrates with one per cent of glucose solution. One of the methods of production of these potential biocontrol and biofertilizer agents is production on compost and its application associated with the organic source of material. Organic matters or compost addition along with any carbon source has increased *Trichoderma* performance in the application (Panahian *et al*., 2012). Among bacteria, *Pseudomonas* species are aggressive colonizers of the rhizosphere of various crop plants and have been shown to attach to the root and efficiently colonize root surfaces (Hayat *et al.*, 2010). These bacteria use quorum sensing mechanisms

TABLE V

Effect of oilcake extracts and glucose on population of Rhizobium *at different intervals of incubation*

Oil Cake Extracts	*Rhizobium* (10^8 CFU/ ml of sample) Number of Days													
	3	6	9	12	15	20	25	30	35	40	45	50	55	60
Pongamia	4.83	13.17	6.17	8.17	9.00	3.17	2.00	4.00	2.33	5.67	3.33	2.33	1.00	0.00
Neem	7.00	8.83	8.50	13.17	10.67	2.50	1.67	8.00	7.00	9.33	3.00	4.67	1.67	0.33
Groundnut	16.17	11.00	6.17	5.50	6.67	12.00	11.00	3.67	5.67	4.00	3.00	4.33	2.00	1.00
Pongamia + 1% glucose	13.17	14.50	8.17	5.67	17.00	16.67	7.33	7.67	5.33	5.33	3.00	3.67	0.00	0.00
Neem + 1% glucose	9.33	15.00	8.67	5.17	8.83	12.17	3.00	3.67	2.33	6.67	3.00	3.33	0.00	0.00
Groundnut + 1% glucose	14.67	11.33	7.67	6.33	13.50	11.50	4.33	4.33	2.33	4.00	2.33	0.67	0.00	0.00
S.Em±	0.81	1.22	0.38	0.66	0.96	0.54	0.58	0.53	0.38	0.54	0.65	0.53	0.27	0.14
CD at 1%	3.48	5.29	1.66	2.86	4.17	2.33	2.49	2.30	1.66	2.35	NS	2.28	1.18	0.59

to coordinate and regulate gene expression according to local population density, to protect against plant pathogens (Siddiqui and Khan, 2017) and have a broad spectrum antagonistic activity against plant pathogens. PGPR like *Azotobacter*, PSB and *Rhizobium* have the potential to contribute to sustainable plant growth promotion and these PGPR microorganisms function in three different ways such as synthesizing particular compounds for the plants, facilitating the uptake of certain nutrients from the soil, and lessening or preventing the plants from diseases.

The potential biocontrol agents *Trichoderma*, *Pseudomonas* and biofertilizers *Azotobacter*, *Rhizobium* and PSB can be easily mass produced by the locally available materials like neem, ponagmia and groundnut cakes. This paved the way for production of biofertilizer and biocontrol agents by using locally available substances effectively. Utilization of oilcakes as a carrier for these biofertiliser and biocontrol agents has dual benefit to the crop production by improving the crop growth and for crop protection. Thus the aqueous oilcake extracts are found compatible, economical and ecologically safe and it can be used for crop production.

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