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Antibacterial activity, biological quality assessment and growth promoting effects of liquid manure

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Abstract
Growth promising formulations of liquid manure was assayed for presence of human pathogens, antibacterial activity against phytopathogens i.e. *Pseudomonas syringe, Xanthomonas citri subsp.malvacearium* & *Xanthomonas campestris* and growth promoting effect on wheat. The study showed that it does not facilitate the growth of human pathogens. It exhibited antibacterial activity against *Xanthomonas campestris* pathogen of soyabean plant. Presence of Psedomonas flurocence, a growth promoting bacteria, in liquid manure was confirmed during assay for Psedomonas aeruginosa which is a human pathogen. The pot culture experiment with wheat var. Lok 1 showed the significant positive effect of liquid manure on growth of plants.

Keywords: liquid manure, antibacterial activity

Introduction
Cow dung has an important role in preserving environment and in India it is accepted as a purifier. It is used as cooking fuel, as a disinfectant as well as mosquito repellant since long back in the history. From ancient time it is used as manure for boosting crop growth as it contains humic compounds and fertilizing bioelements (39). The low C:N ratio in cow dung manure underlines that it is a good source of protein for the microbes involved in the decomposition of organic matter[1]. Panchagavya; the term used in Ayurveda is a decoction of cow urine, cow dung, cow milk, cow ghee and cow curd. Panchagavya finds its application either alone or in combination with the drugs of herbal, animal or mineral origin [35]. Cow dung was observed to suppress mycelia growth of plant pathogenic fungi like *Fusarium solani, F. oxysporum* and *Sclerotinia sclerotiorum* [6]. Spraying of cow dung extract was also effective for the controlling bacterial blight disease of rice and was equally effective as pausthamycin, penicillin and streptomycin [21]. Antagonistic properties of bacterial genera identified (*Bacillus and Pseudomonas*) from cow dung are well known. These research findings support the use of cow dung as a purifier in religious practices and for disease suppression in organic farming over the years [15].

Many diseases in crops are caused by microorganisms consequently leading to major yield losses. The problem is much intense in developing countries. Major before and after harvest losses are caused by pathogens such as species of *Fusarium, Alternaria Sclerotium, Phytophthora, Curvularia Ralstonia, Xanthomonas* etc. In modern practices of agriculture chemical pesticides are usually used to control the plant diseases caused by microorganisms. However, the use of synthetic compounds to control plant pathogens had two main drawbacks viz., potential development of resistance in pathogens and the risk of toxicity. This has intensified the research focused on botanaical pesticides i.e. plant extracts and their probable application in agriculture. Such products of natural origin, including plant extracts, have been reported to have inhibitory effect against a variety of plant pathogens [7, 8, 9, 10, 13, 26, 30, 42].

Cow urine has found its application in several medicinal purposes in India several classical Ayurveda texts like Charaka samhita and Shushruta samhita had quoted it's medicinal use. Cow is worshiped as sacred animal in India. Cow urine is known to cure several human diseases. In Veda, cow urine is received equal recognition as nectar [16, 19]. It has been proved that cow urine can control *Meloidogyne incognita* in *Lycopersicon esculentus* [2] and watermelon diseases specifically aphids and pickleworms [11]. Inhibitory effect of cow dung was observed against several plant pathogens such as Sclerotini sclerotiorum, Fusarium solani F.sp. cucurbitae [12], Bipolaris sorokiniana [3] and Xanthomonas oryzae pv. oryzae [22]. It has been proved that cow urine extracts of certain plants as well as cow urine alone possess marked inhibitory effect on human pathogens as well as plant pathogens [3, 31, 41].

In India, soyabean is mainly cultivated in Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharashtra, Karnataka and Gujarath. [23] Bacterial postule is caused by *Xanthomonas campestris pv glycine* [33]. This is one of the most important disease in India and causes up to 18 % losses to the soya*bean* crop [36]. Bacterial blight of cotton is a disease affecting the cotton plant caused by infection by bacteria *Xanthomonas citri pathovar malvacearum* [32] which is the major bacterial disease on cotton which infects all aerial parts of the host. The estimated loss due to this disease was about 10 to 30% on different cultivars.

It has been found that the soil microbial population and soil enzyme activities have been increased with application of poultry litter [5]. There are many reports suggesting that the manure application had enhanced effect on the biological

parameters [28; 34] and availability of nutrients in the dissolved form [17]. It also improves the level of soil organic matter, soil pH and EC which are the major factors influencing activities of soil enzymes [38].

Innovative farmers in Maharashtra had evolved and using three alternative methods of liquid manures prepared using locally available materials viz., *Sanjeevak, Amarutpani* and *Panchagavva*. These liquid manures are mostly fermented products. *Jaggary* and honey in the admixture acts as a carbon source, cattle dung offers microbial cultures and other ingredients act as nitrogen (protein) source.

Earlier research was carried out on seven different promising formulations of liquid manures. These formulations were tested for nutrient level, microbial content and amount of extracellular enzymes secreted by them. The observations stated that the level of nutrients was quite low in these formulation but the population of beneficial microbes was high. The presence of high level of enzymes corresponds to mineralization of some plant nutrients which in turn was noted with the healthy growth of plants. Sanjeevak 5 and Panchagavya were the most promising formulations of liquid manure exhibiting comparatively highest microbial count and presence of high amount of extracellular enzymes. The seed germination trial showed that Sanjeevak 5 treatment was noted to have pronounced effect on root growth in case of cotton, pigeon pea and wheat seeds. Panchagavya treatment was seen to facilitate the growth of Actinomycetes and Phosphate Solubilizing Microorganisms in the soil, while Amritpani treatment was seen to accelerate the growth of microbes like Azotobacter and Pseudomonas closely followed by Sanjeevak 5 treatment [37].

The objective of the present study is to assay the biological quality of most effective formulation i.e. Sanjeevak 5 and its antibacterial activity against few phytopathogens as no such work had been carried out as revealed from available literature. The pot culture trials on wheat were undertaken in continuation with the findings of growth promoting effect of liquid manure on seed germination. The use of liquid manure for controlling the pest as well as for promotion of crop growth will reduced the risk of potential development of resistance in the pathogen, risk of toxicity and reduces the input cost of farming.

Materials and methods

Collection of raw material

For preparation of liquid manure cow dung and cow urine was collected from indigenous cow breed called Gir. Jaggery was purchased from a farmer who runs an enterprise of organic jaggary. The phytopathogenic cultures were obtained from Microbial Culture Collection (MCC), Pune

Preparation of liquid manure

10 kg cow dung, 10 lit cow urine, 1 kg jaggary, 2 kg flour of pulses, 1 kg ant hill soil and 200 lit water were mixed and allowed to ferment in a non-corrosive plastic tank for 5 days when bubble formation was stopped. Every day the mixture was stirred for 10 minutes. This quantity is sufficient to apply for an acre of land.

Antibacterial Activity

For assessing antibacterial activity of liquid manure against plant pathogen, Agar well diffusion method (Kekuda *et al*., 2012) [29] was adopted. The pathogen culture was developed in sterile nutrient broth tubes at required incubation temperature of 37ºC for 24 hours. The broth culture was swabbed on sterile King's B Agar *Pseudomonas syringe* and Sucrose Pepton agar plates for *Xanthomonas citri subsp.malvacearium* and *Xanthomonas campestris* using sterile cotton swabs. Wells of 0.6cm diameter were punched in the inoculated plates with the help of a sterile cork borer and liquid manure was transferred into wells. After incubation of plates at 37ºC and observed for formation of zone of inhibition. The experiment was repeated thrice and the average value was recorded.

Biological Quality Assessment of Liquid Manure

The presence of human pathogens i.e. *E. coli, Shigella, Salmonela and Pseudomonas aeruginosa* were determined in the formulation. For determination of *E. Coli* Mac-Conkey agar and EMB agar were used while for *Shigella* and *Salmonella* XLD agar and *Salmonella-Shigella* agar were used respectively. For *Psedomonas aeruginosa, Psedomonas* isolation agar was used.

Growth promoting effect with Wheat

Randomized Complete Block Design (RCBD) was laid out for pot culture experiment having three replications. 90 plants for each of the treatment were considered for observations.

Treatment variations and Doses of fertilizers used

Eight variations of treatments were selected for experimentation which were as follows;

1. Control (T1)
2. Organic manure (T2)
3. Chemical fertilizer (T3)
4. Liquid manure (T4)
5. Organic manure + Chemical fertilizer (T5)
6. Liquid manure + Organic manure (T6)
7. Liquid manure + Chemical Fertilizer (T7)
8. Liquid manure+ Organic manure + Chemical fertilizer (T8)

Following doses of fertilizers were given as per recommendations of Dairy of Panjabrao Krushividyapit, Akola, 2013

Table 1

Treatments	Organic manure	Liquid manure	Chemical fertilizer		
			Urea	***SSP***	***MOP***
T1	-	-	-		
T2	0.035 kg / 35 gm	-	-		
T3	-	-	0.91 gm (0.42 gm N)	1.31 gm (0.21 gm P)	0.35 gm (0.21 gm K)
T4	-	1.73 ml	-	-	-
T5	0.035 kg / 35 gm	-	0.91 gm (0.42 gm N)	1.31 gm (0.21 gm P)	0.35 gm (0.21 gm K)
T6	0.035 kg / 35 gm	1.73 ml	-	-	-
T7	-	1.73 ml	0.91 gm (0.42 gm N)	1.31 gm (0.21 gm P)	0.35 gm (0.21 gm K)
T8	0.035 kg / 35 gm	1.73 ml	0.91 gm (0.42 gm N)	1.31 gm (0.21 gm P)	0.35 gm (0.21 gm K)

Note - Recommended dose of liquid manure - 200 lit / acre

Doses of potassium and phosphorus were applied at the time of sowing and nitrogen fertilizer was applied in splits i.e., half at the time of sowing and remaining at 1st irrigation schedule. The crop was irrigated at continuous intervals considering the need of crop and the weeds emerged during crop growth were manually pulled out from whole of the experimental pots. The observations were recorded after formation of tillers before infecting the plants with pathogen.

Results and discussions

Antibacterial Activity

The results showed that liquid manure does not have antibacterial activity against *Pseudomonas syringe and Xanthomonas citri subsp.malvacearium*. Antibacterial activity was observed against *Xanthomonas campestris* with a zone of inhibition of 1.5 cm on third day of incubation which had enlarged up to 2.1 cm on fourth day.

There are many reports which supports that cow urine based extracts of plants exhibit antibacterial and antifungal activity. IT was reported that extract of *Calotropis procera*, in combination with cow urine inhibits conidial germination *of Bipolaris sorokiniana*, causative agent of leaf blight of wheat up to 91% [3]. Similarly there are findings that some medicinal plant extracts prepared in cow urine inhibited the growth of *Rhizoctonia solani*, causative agent of sheath blight of rice. Murugan *et al*. (2012) [22] showed the efficacy of cow urine and cow urine with *Pongamia pinnata* seed against bacterial leaf blight of paddy caused by *Xanthomonas oryzae pv. Oryzae* [22].

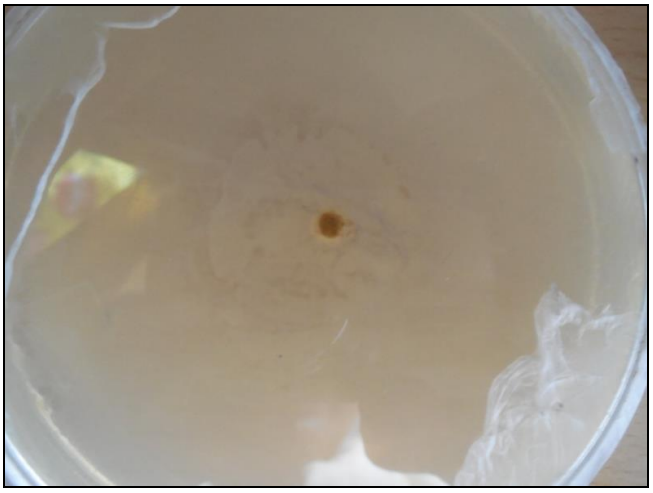

Fig 1: Antibacterial activity against *Xanthomonas campestris*

Study of antibacterial activity of cow urine extract of 9 plants against *P. aphanidermatum* and *F. oxysporum* showed that the *F. oxysporum* was highly susceptible to cow urine extracts of plants. [29] Similar kind of report was raised quoting that plant extracts were also effective against *R. solanacearum.* [38] For controlling onion blight the farmers of Uttarkhand traditionally using cow dung and cow urine by making their solution with water. [27] Earlier reports also reveals biocontrol activity of *Bacillus subtilis* obtained from fresh cow dung against plant pathogenic fungi *F. oxysporum* and *Botryodioplodia theobromae* [26]. All these findings are in support with the result of present study.

Biological Quality Assessment of Liquid Manure

There was absence of all pathogens i.e. *E. coli, Shigella*, *Salmonela and Pseudomonas aeruginosa* tested under laboratory conditions in the formulation of liquid manure. In the plate of *Psedomonas* isolation agar presence of *Psedomonas fluroscence* was observed. The observation was confirmed with UV Transh Illuminator.

It has been proved that all major root diseases of wheat caused by *Gaeumannomyces graminis var tritici can be suppressed by Pseudomonas fluorescens* and its rifampin resistant derivative 2-79RN10 [20]. Its role in protecting cotton from seed colonization and pre emergence damping-off caused by *Pythium ultimum* had also been proved [18]. On the other hand it had been reported that *Pseudomonas fluorescens* produces IAA and tryptophan which has a growth promoting properties [14]. During the experimentation presence of this growth promoting bacteria was observed in the liquid manure which supports the findings of growth promoting effect and antibacterial activity of liquid manure against soyabean pathogen.

Height of plant, number of leaves and length of leaves were considered for measuring growth of the wheat plants. The observations were noted before infecting the plants with phytopathogens. Data obtained showed significant difference in the plant height.

The height of plant was significantly higher in the T7 treatment ($F(3, 1432)= 166.51$, $p < 0.001$), followed by T8 treatment and T6, in case of Lok 1 variety.

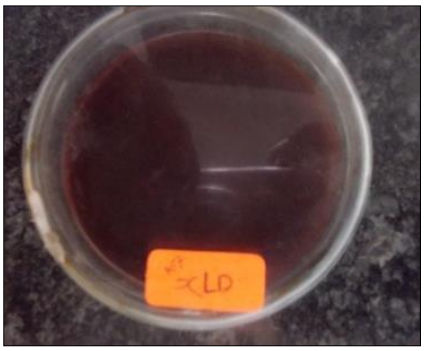

Fig 2: Absence of E.Coli in liquid manure

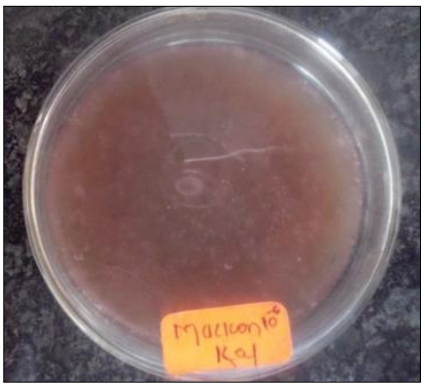

Fig 3: Absence of *Shigella in liquid manure*

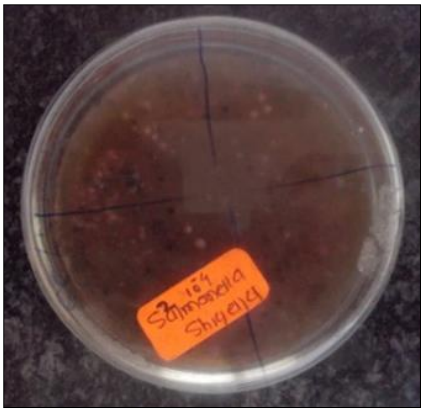

Fig 4: Absence of *Salmonella in liquid manure*

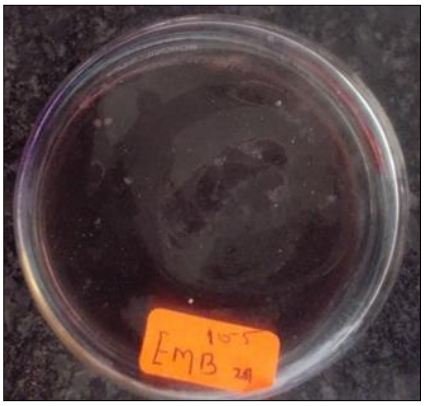

Fig 5: Absence of E.Coli in liquid manure

Fig 6: Absence of *Psedomonas aeruginosa*

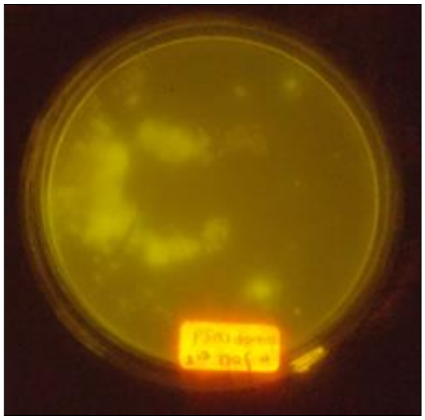

Fig 7: Evidence of presence of *Psedomonas flurocence in liquid manure*

Growth promoting effect with Wheat

Height of plant, number of leaves and length of leaves were considered for measuring growth of the wheat plants. The observations were noted before infecting the plants with phytopathogens. Data obtained showed significant difference in the plant height.

The height of plant was significantly higher in the T7 treatment ($F(3, 1432)= 166.51$, $p < 0.001$), followed by T8 treatment and T6, in case of Lok 1 variety.

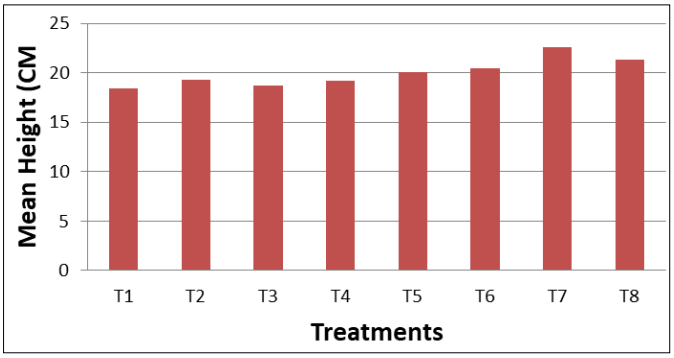

Fig 8: Mean Height of plant of the Wheat in 8 experimental conditions

Length of leaves was also significantly more in T7 ($F(3, 1432)=357.72$, $p < 0.001$), almost equally followed by T8 treatment as compared to T1. But the numbers of leaves were significantly more in the T6 and T8 treatment ($F(3, 1432) =$

40.75, $p < 0.001$), followed T2 and T7 as compared to T1 and T3 treatments.

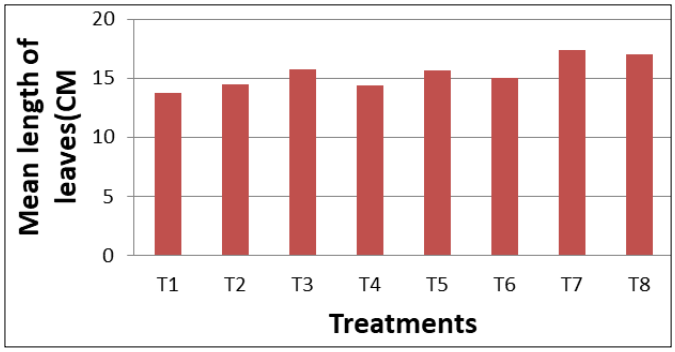

Fig 9: Mean length of leaves of plant of the Wheat in 8 experimental conditions

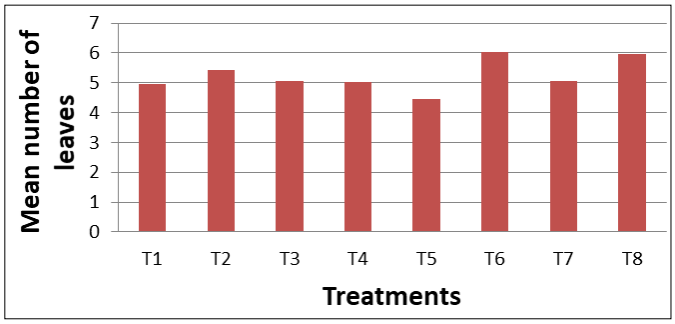

Fig 10: Mean number of leaves of plant of the Wheat in 8 experimental conditions

These research findings are in accordance with the observations of enhancement in growth of wheat reported with the application of sewage sludge [24]. It was noted that the increase in plant height could be due to the ample supply of organic matter, Nitrogen, Phosphorus and other nutrients by the sludge and improvement in soil texture and structure due to sludge application. The swine manure was reported to boost the yields of cereals, legumes, oilseeds, vegetables and pastures and in improving the level of plant nutrient in soil, especially Nitrogen, Phosphorus and potassium [25].

It has been reported that liquid cattle (Bos taurus) manure application did not affect the seed germination but improved dry biomass at two growth stages and grain production and nutrient uptake comparable to the inorganic N and P fertilization [40]. Similar study showed the enhanced corn yields with maintenance of soil fertility at desirable level without increasing soil salinity at unacceptable level [4].

The results of the present study are in accordance with all these research findings.

Conclusion

The study showed absence of common human pathogens in the formulation of liquid manure. It proved its antibacterial activity against *Xanthomonas campestris* pathogen of soyabean plant. The presence of Pseduomonas fluroscence supports the antibacterial activity as well as growth promoting property of liquid manure. It had been observed that even though height of plant and length of leaves were higher for the treatment T8, the number of leaves were higher in case of T6 treatment.

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