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Aerobic composting of pig excreta as a model for inoculated deep litter system in sty using Indigenous Microorganisms (IMOs)

SEEMA YADAV[1], P K BHARTI[2✉], CHANDRAHAS[3], G K GAUR[1], ABHISHEK[1], MUKESH SINGH[1] and ARUN SOMAGOND[1]

ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh 243 122 India

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ABSTRACT

The present experiment was conducted at Swine Production Farm, IVRI, Izatnagar, Bareilly (Uttar Pradesh) during month of December and March for the cultivation of Indigenous Microorganisms (IMOs) and its application in the composting of piggery excreta as a model for inoculated deep litter system of pig sty respectively in India. The IMOs were cultivated in four steps by using half-cooked rice, rice bran, soil and different energy sources, viz. brown sugar, jaggery, molasses in treatments C (Control), T_1 and T_2 groups, respectively. As a model of deep litter sty, aerobic composting of pig faeces, with different IMOs was conducted as treatments C_C (brown Sugar), C_{T1} (jaggery), C_{T2} (molasses) and C_0 (control) without any IMOs. During the experiment morning, evening, peak, mesophilic and thermophilic temperature distribution were recorded. The chemical composition and C: N ratio of pig faeces and compost were analysed at the end of experiment. The final compost temperature in all the treatment groups was in between 55 to 65°C but in control, it was higher than 65°C. The temperature range in treatment groups suggests that IMOs layers might have helped in decomposition process at faster rate which resultantly lowered the temperature. The carbon: nitrogen ratio (C: N) was significantly lower in C_{T1} and C_{T2} (19:1) than control indicative of good quality compost in treatment groups. It was concluded that IMOs from jaggary and molasses might be used as farmer friendly inocula/ inoculums to compost pig excreta under inoculated deep litter pig production system.

Keywords: Aerobic composting, Indigenous microorganisms, Inoculated deep litter, Pig excreta

Indigenous microorganisms (IMOs) refer to a group of beneficial microbes that are native to a particular area where they exist locally (Sadi *et al.* 2006). Beneficial microbes of IMOs include bacteria, filamentous fungi and yeast which live together in harmony with the rest of nature and are mainly collected from non-cultivated soil. The IMOs have wide range of applications such as bio-degradation, bio-leaching, bio-composting, nitrogen fixation, improving soil fertility and enriching deep litter in animal husbandry. Indigenous microorganisms are inhabited by aquatic as well as oil bearing deep sub-surface environments (Magot *et al.* 2000). One of the investigations (Cai *et al.* 2013) showed that aerobic indigenous microorganisms play a role in degrading petroleum oil. Indigenous microorganisms play an important role by protecting the normal host from invasion of pathogenic microorganisms causing disease. IMOs produce bacteriocins and other inhibitory substances which prevent colonization of pathogens and also compete with the pathogens for essential nutrients and receptors on host cells. The IMO based technology also helps in composting process, where organic waste materials are degraded by activities of microorganisms into much simpler nutrients and produce organic fertilizer or soil conditioner at the end of the process. It is a decomposition process which involves succession of different microbial population to breakdown organic matter and used as their energy supply. In order to produce mature compost in a shorter time, the presence of the correct microorganisms is needed. Now-a-days, cleaning of piggery excreta has become a major problem in terms of disposal because most farmers dispose them into environment without any treatment. The IMOs can be used for aerobic decomposition of excreta for making valuable compost. Its cultivation can be done for piggery where traditional deep litter may be inoculated to make the sties odour-free and to convert the excreta in to better quality compost (Kumar and Gopal 2015) for sustainable animal husbandry and integrated agriculture. IMO replace the pathogenic organisms in the litter which produces bad smell in the excreta (Kumar and Gopal 2015). Despite the fact that IMOs have lots of scope for animal husbandry, very few reports are available on its use in animal housing for inoculated deep litter in pigs. The cultivation and application of IMOs for inoculated deep litter sties have not been

Present address: [1]ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh. [2]ICAR-Mahatma Gandhi Integrated Farming Research Institute, Motihari, Bihar. [3]ICAR-Central Avian Research Institute, Izatnagar, Uttar Pradesh. ✉Corresponding author email: pkish.1002@gmail.com

attempted in India. Therefore, the present study has been conducted to cultivate Indigenous Microorganisms (IMOs) using different substrates and its *in vitro* application for aerobic composting of pig faeces as a model of inoculated deep litter system (IDLS) in piggery.

MATERIALS AND METHODS

Location of experiment/study: The experiment for the cultivation of Indigenous Microorganisms (IMOs) was conducted at Swine Production Farm, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh (India). The farm is located at latitude of 28° 22' North, longitude of 79° 24' East and altitude of 169.2 meter above the mean sea level. The location comes under upper gangetic plain region and has semi tropical climatic condition with high humidity, especially during the monsoon season. The farm comes under upper gangetic plain region and has semi tropical climatic condition with high humidity, especially during the monsoon season. Winter stretches from November to February whereas summer ranges from May to August months annually. The extreme temperature ranges between 4°C to 44°C. The annual mean temperature is 25°C (77°F) and monthly mean temperatures range from 14°C to 33°C (58°F to 92°F). Annual rainfall ranges from 90 to 120 cm and mostly received during the months of July and August. The nearby open area of the farm was selected for cultivation of IMO.

Materials used for IMOs cultivation: The IMOs in the present study were cultivated by using three protocols (based on energy source) with four steps (step I, II, III and IV) in each, i.e. brown sugar, jaggery and molasses in control (C), treatment-1 (T_1) and treatment-2 (T_2), respectively. The other materials like half cooked rice, rice bran, distilled water, native soil etc. used from step I to step IV were common for various protocols. A wooden box of size 12″×12″×4″ (volume of 576 cubic inches), wire mesh, rubber band and white paper were also used. The IMOs cultivated at step I, II, III and IV were named as IMO-1, IMO-2, IMO-3 and IMO-4, respectively, based on the end product of the cultivation. The IMOs cultivated at step I, II, III and IV were named as IMO-1, IMO-2, IMO-3 and IMO-4, respectively, based on the end product of the cultivation.

Cultivation of IMOs in control (C)

Step-I (*Cultivation of IMO-1*): Here, 2.5 kg raw rice was half cooked and placed in a wooden box up to 2/3rd part and labelled properly. The remaining 1/3rd part of the box was kept empty to facilitate the aerobic environment to the microbes and, thereafter, the box was covered with a sheet of white (tissue) paper. A wire mesh equal to the wooden box top was placed over and, thereafter, a thin plastic sheet was also covered to protect the materials from rodents or stray animals in view of avoiding any damage or interference to the contents. The box containing the half cooked rice was buried 5 cm deep in the soil under the trees for 7 days. The top surface of the box was again covered with bamboo leaves collected from the nearby area to maintain optimum moisture for microbial growth. All possible measures were taken to protect the materials from direct sunlight and rainwater while placing them under the native soil near to swine production farm.

Step II (*Cultivation of IMO-2*): It involved the cultivation of IMO-2 by mixing IMO-1 (0.5 kg) with brown sugar (0.5 kg) in the ratio of 1:1. The mixture of IMO-1 with brown sugar (IMO-2) was placed in a cool environment for the next 7 days. The enrichment of IMO-1 with energy source further helped in growth of microbes and fermentation of the substrate.

Step III (*Cultivation of IMO-3*): In this step, 5g of IMO-2 was mixed with 500 ml of distilled water until the colour of the solution turned brownish (IMO-3). The completely mixed solution was sprinkled over 4 kg of rice bran spread on a gunny bag. The materials were covered with rice straw to allow the growth of microorganisms for the next 5 days.

Step IV (*Cultivation of IMO-4*)*:* In the last step, 4 kg of IMO-3 was mixed with 4 kg of native soil in the ratio of 1:1. This mixture was allowed to propagate for next 7 days to get the final product. The step wise complete procedure for cultivation of IMOs in different groups has been summarized below and presented in Table 1.

Cultivation of IMOs in treatment groups: The procedure for cultivation of IMOs in T_1 and T_2 was similar to control group except at step-II, where jaggery and molassess were used as readily available source of energy for microbes.

Table 1. Procedure for cultivation of IMOs in different groups

Stages of IMOs	Control	Treatment 1 (T1)	Treatment 2 (T2)
IMO-1	2.5 kg half-cooked rice in wooden box under tree was kept for 7 days	2.5 kg half-cooked rice in wooden box under tree was kept for 7 days	2.5 kg half-cooked rice in wooden box under tree was kept for 7 days
IMO-2	IMO-1 and Brown sugar was mixed (1:1) and kept for 7 days	IMO-1 and Jaggery was mixed (1:1) and kept for 7 days	IMO-1 and Molasses was mixed (1:1) and kept for 7 days
IMO-3	IMO-2 (5 g) +DW (500 ml) + 4 kg Rice Bran were mixed and kept for 5 days	IMO-2 (5 g) + DW (500 ml) + 4 kg Rice Bran were mixed and kept for 5 days	IMO-2 (5 g) + DW (500 ml) + 4 kg Rice Bran were mixed and kept for 5 days
IMO-4	IMO-3 (4 kg) + Soil (4 kg) mixed (1:1) and kept for 7 days	IMO-3 (4 kg) + Soil (4 kg) mixed (1:1) and kept for 7 days	IMO-3 (4 kg) + Soil (4 kg) mixed (1:1) and kept for 7 days

Aerobic composting of pig faeces using IMOs as a model of inoculated deep litter piggery

Mini composter: A total of four specially designed iron meshed compost bins of equal dimension ($1.2 \times 1.2 \times 1.2$ m^3) were taken for the composting and fixed on impervious floor made up of concrete material. The sidewall of the bins was fixed from three sides and one hinged door was made from the front side for easy filling of ingredients.

Ingredients used for composting: Fresh pig manure was collected from sheds of crossbreed (Landrace × Desi) pigs at Swine Production Farm. Composting recipe was prepared by using paddy straw as carbonaceous material for C : N ratio of 25 : 1 and moisture 45–55%. The paddy straw was chaffed to a size of 2 to 3 inches for easy filling of ingredients in the compost bins.

Composting procedure: The experimental trial on composting was conducted during February to March, 2018 for an initial period of 37 days. Four different composting groups were used. The first group was named as compost-control (C_0), with pig excreta and paddy straw but without IMO. The other groups were compost-C_C (Cc = C_0 + IMO obtained from control group of cultivation), compost-T_1 ($C_{T1} = C_0$ + IMO obtained from T_1) and compost-T_2 ($C_{T2} = C_0$ + IMO of T_2 group). Composting bins were filled by layering of 100 kg chaffed paddy straw and 250 kg piggery excreta equally divided in 10 layers with C : N ratio of 25 : 1 and 45–55%, moisture. For each treatment group, 4 Kg of IMOs inoculums were used for their respective groups. The temperature of composting bins was recorded from centre of contents using a digital composting thermometer (Mextech® multi-thermometer). After the decomposition of materials, the compost samples were collected from different groups and analysed for proximate principles (AOAC 1995).

Parameters recorded: The cultivated IMOs in different groups were physically evaluated based on development of colour and texture of product at various steps. During the aerobic composting of pig faeces, morning, evening temperature was recorded daily to assess average, peak, mesophilic and thermophilic temperature distribution. The samples (pig faeces and compost from different treatment groups) were analysed for DM, ash, OM, CP, CF and EE as per AOAC (1995). The total carbon was calculated from total organic matter value using the conventional "Van Bemelem Factor" of 1.724. The weight loss on ignition was divided by 1.724 to give the percentage of total carbon (Navarro *et al.* 1993). Carbon: nitrogen ratio (C: N) for samples were calculated based on the total carbon and total Kjeldahl nitrogen concentration (Zhu, 2006)

Statistical analysis: The data generated from the experiment, were subjected to statistical analysis as per the standard statistical method (Snedecor and Cochran 1994) using Statistical Package for Social Sciences (SPSS, version 20.0). The one way analysis of variance (ANOVA) was done to test the difference between various treatment groups. The significant means between different treatments were compared by Tukey's b test.

RESULTS AND DISCUSSION

Cultivation and physical evaluation of IMOs: The IMOs were cultivated successfully from three groups, viz. control, T1 and T2 and in four steps, i.e. I, II, III and IV. The total duration for cultivation of IMOs (IMO-4) was 26 days. The present findings are in similar lines of Bakar and Ibrahim (2013), who cultivated IMOs by similar methodology for composting process. The environmental conditions of the present study were almost similar to the earlier reports on cultivated IMOs. However, in contrast to our findings, Hoonpark and Du Ponte (2008) and Anyanwu *et al.* (2013) reported 5 days to complete the step-I process based on the prevailing environmental temperature at the locality. The duration for cultivation varies from place to place and depending upon the climatic conditions of the experimental site. The duration for cultivation of IMO-2, IMO-3 and IMO-4 were similar to previous reports (Hoonpark and Du Ponte 2008, Bakar and Ibrahim 2013).

The cultivated IMOs at various stages were evaluated based on their physical texture and colour. The development of white mycelium (Fig. 1) at IMO-1 stage indicates the positive direction towards the cultivation process of IMOs (Hoonpark and Du Ponte 2008). The physical evaluation at IMO-1 was very crucial as any deviation from the white coloured moulds over the substrate may become undesirable for further cultivation of IMOs. The finding in the present study was in agreement with earlier reports (Hoonpark and Du Ponte 2008, Anyanwu *et al.* 2013, Bakar and Ibrahim 2013).

At the end of step-II, the IMO-2 was physically evaluated in all groups. The texture of IMO-2 in control and T_1 was semi-moist but not wet consistency due to less water contents in brown sugar and jaggery, respectively. However, the consistency of IMOs in T_2 was semi-moist with wet emergence. The colour of cultivated IMO-2 in control, T_1 and T_2 was dark brown, light brown and light black, respectively which might have imposed due to colour of added ingredients like brown sugar, jaggery and molasses at this step. The colour of IMO-3 appeared as white patches developed over the rice bran in all the groups. The development of white patches in IMO-3 seems to be due to proliferation of diverse microbes upon enrichment of

Fig. 1. White colored fungal hyphae developed at IMO-1 after 7 days.

nutrients. The colour of IMO-4 was light brown, irrespective of the groups. The fungal mycelium developed on IMO-4 was not prominent as compared to IMO-1 stage. However, the mycelium of developed moulds over the substrates was observed as white threads interwoven within the soil. The texture of IMO-4 was similar to granular soil in all the groups. The colour and texture developed at IMO-4 in the present study were positively correlated with previous reports on IMO cultivation under various farming systems (Hoonpark and Du Ponte 2008, Anyanwu *et al.* 2013). Based on the development of colour, mainly the presence of white coloured fungal mycelium, the IMO-1 and IMO-4 stage were considered as a positive indicator for successful cultivation of IMOs.

Aerobic composting of pig faeces using IMOs as a model of Inoculated deep litter system (*IDLS*): The morning, evening and average temperature of composting bins during study period was significantly (P<0.01) higher in C_0 than other three treatment groups with non-significant differences among them, i.e. C_C, C_{T1} and C_{T2} (Table 2). Higher (P<0.01) morning, evening and average temperature of composting bins during study period in C_0 than other three treatment groups indicated uncontrolled microbial decomposition than other treatment groups where composting process might be under control due to various kind of IMOs in different composting bins.

In pig farming, especially under tropical conditions, managing pigs under deep litter system will require lower temperature and present findings under treatment groups indicate the positivity in this direction. This finding may further be confirmed by trends of thermophilic temperature (>45°C) which was higher in C_0 than C_C, C_{T1} and C_{T2} groups. Peak temperature during morning as compared evening hours was lower in C_{T1} (16.97%), C_{T2} (11.57%) and Cc (9.26%) than C_0 group (Table 3). Although mesophilic temperature (<45°C), was non-significant among all four groups (Table 4), the thermophilic temperature was significantly higher in C_0 than C_C, C_{T1} and C_{T2} groups. Similarly, thermophilic temperature (>45°C) was significantly (P<0.01) higher in Cc than C_{T1} and value of C_{T2} was in-between C_C and C_{T1} groups. The temperature range of >75°C was absent in C_{T1} and C_{T2}, therefore, T_1 (IMO from jaggery) and T_2 (IMO from molasses) may be recommended for inoculums of in deep litter system of pig production. Since, T_1 and T_2 were cultivated using jaggery and molasses, its cost of production will be certainly lower than control which was cultivated using brown sugar. So, T_1 and T_2 may act as farmer friendly inoculum for model of Inoculated deep litter system (IDLS) due to its lower cost of production and may further reduce the cost of pig production due to lesser labour required in daily cleaning and washing of sty. The temperature below 55°C was, non-significant among all four groups (Table 5).

Furthermore, the temperature trend in decomposition of pig faeces remained higher in C_0 which did not reduce due to microbial activities of mesophilic bacteria as reported by De Bertoldi *et al.* (1983) who suggested that the optimum temperature for the composting process was between 40 to 65 °C. While comparing the temperature range of 55 to 65°C, there was a significant difference (P<0.01) among the treatment groups with higher and lower values in C_{T1} and C_C, respectively, and values of C_0 and C_{T2} were in-between both the groups. The final temperature in all the treatment groups were in between 55 to 65°C but in control, it was higher than 65°C. The temperature range in treatment groups suggests that IMO layers might have helped in decomposition process at faster rate which ultimately lowered the temperature. Bakar and Ibrahim (2013) also reported similar findings by inoculating the IMO layer in

Table 2. Temperature (°C) of bins during composting

Parameter	C_0	C_C	C_{T1}	C_{T2}	SEM
Temp. (Morning) in °C	73.79^b	66.75^a	62.40^a	64.73^a	0.92
Temp. (Evening) in °C	74.28^b	66.73^a	63.10^a	64.75^a	0.86
Av. temp. in °C	73.36^b	66.43^a	61.94^a	63.56^a	0.99

Mean with different superscripts in a row differs significantly at 1% level (P<0.01).

Table 3. Peak temperature (°C) of composting bins during study period

Parameter	C_0	C_C	C_{T1}	C_{T2}
Temp. (Morning) in °C	83.1	81.0	69.00	73.50
Temp. (Evening) in °C	83.5	81.0	81.30	73.40
Av. temp. in °C	83.3	81.0	73.75	73.45

Table 4. Mesophilic and thermophilic temperature distribution during composting

Temperature (°C)	C_0	C_C	C_{T1}	C_{T2}	SEM
Mesophilic (<45)	34.75	33.83	32.85	35.26	1.20
Days	3	3	3	3	
Thermophilic (>45)	76.76^c	69.31^b	64.51^a	66.13^{ab}	0.65
Days	34	34	34	34	
SEM	2.00	2.19	1.53	1.68	

[abc]Mean with different superscripts in a row differs significantly at 1% level (P<0.01)

Table 5. Temperature (°C) distribution (days) during composting

Temperature (°C)	C_0	C_C	C_{T1}	C_{T2}	SEM
<55 (days)	34.75 (3)	44.04 (6)	32.85 (3)	35.27 (3)	2.35
>55–65 (days)	60.78^{ab} (1)	57.11^a (3)	63.22^b (20)	60.71^{ab} (14)	3.09
>65–75 (days)	73.36^d (9)	63.96^a (14)	66.36^b (14)	70.13^c (19)	4.17
>75 (days)	78.09 (24)	79.09 (14)	NA	NA	0.33

Figures in parenthesis indicates day. [abc]Mean with different superscripts in a row differs significantly at 1% level (P<0.01).

organic material. The difference in the temperature between treatment groups might be due to the presence of different IMO layers obtained from control, T_1 and T_2. The increased temperature during the initial phase indicates a positive effect on decomposition process (Anyanwu *et al.* 2013). The temperature range of 65 to 75°C was significant (P<0.01) in all other groups with increasing order of C_C, C_{T1}, C_{T2} and C_0. However, in the temperature range of > 75°C, the values not differed significantly among various groups.

After primary phase trial, proximate analysis of prepared compost in different groups has been presented in Table 6. The dry matter percentage was significantly (P<0.01) lower in pig faeces than $C_{0,}$ C_C, C_{T2} and C_{T1} (Fig. 2). Furthermore, DM% of C_{T1} was significantly (P<0.01) higher than $C_{0,}$ Cc and C_{T2} groups. This might be due to addition of paddy straw in all treatment groups including the compost control group as compared with pig faeces. Among treatment groups, ash content and organic matter were higher in C_{T1} and C_{T2} which were indicative of better biodegradation due to addition of paddy straw and IMO layers in mentioned groups. That was further confirmed by lower crude fibre in C_{T1} group. Higher nitrogen and crude protein in pig faeces was their inherent property. However, it was lowered in all treatment groups probably due to the evaporation of ammonical nitrogen.

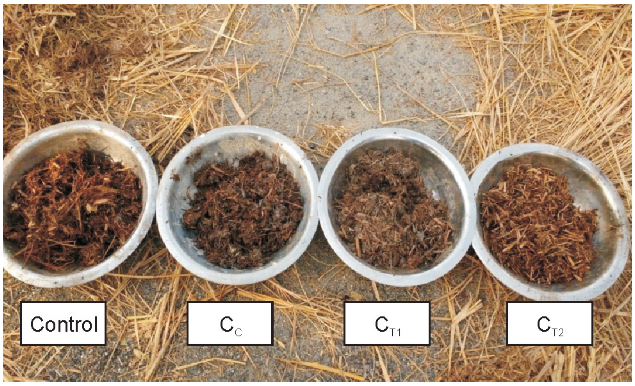

Fig 2. Compost obtained after 37 days in control and treatment groups.

The trend of carbon: nitrogen (C:N) ratio was found to be decreased in treatment groups where IMO layers were used which indicates that composting could have balanced the ratio to optimum level if complete decomposition process (2.5 months or little higher duration) were followed. The present results were supported by the findings of Tripathi (2017) who also reported lowering trend of C: N ratio after addition of organic substance (paddy straw). The addition of IMO layers in the pig faeces and paddy straw might have established certain groups of mesophilic and thermophilic bacterial population which might have increased the decomposition process (Bakar and Ibrahim, 2013). The present trends on C: N ratio indicates that microorganisms require carbon for their growth, energy for metabolism and nitrogen for protein synthesis which is in the similar line of earlier reports (Leonard 2001, Anyanwu *et al.* 2013).

The application of IMOs over the organic waste could help in degradation due to soil microorganisms which produce tremendous ranges of potentially useful enzymes that help in breaking down macromolecules (Bartha and Atlas 1977, Eze *et al.* 2011). The application of IMOs in piggery waste was successfully used for composting trials and found better results as compared with decomposition of sole faecal material of pig. It is also expected that inoculating IMO layer in the deep litter of pigs excreta might help to reduce odour and make waste disposal easier and convenient for the pig producers. The IMOs replaces the pathogenic organisms in litter that produce bad smell in the excreta (Kumar and Gopal, 2015). However, the present trial on composting process using IMOs was done only for 37 days of the primary phase, the complete duration of composting after 2.5–3.0 months may give clear and concrete recommendation for its use in traditional deep litter system of piggery.

The cultivation of Indigenous Microorganisms (IMOs) can successfully be done at pig farms under Indian conditions. The IMOs had beneficial effects on aerobic decomposition process of organic waste materials as a model in-vitro inoculated deep litter system of piggery. The Indigenous Microorganisms (IMOs) can be inoculated in deep litter materials of pig production system for improving the health, hygiene and comfort of the animals by reducing the bad smell, harmful gases and flies in the traditional sties due to improper decomposition of organic wastes. IMOs from jaggery and IMOs from molasses may act as farmer friendly inocula / inoculas for inoculated deep litter system of piggery due to its lower cost of production and may further reduce the cost of pig production due to lesser labour required in daily cleaning and washing of sty. It can, further, proved to be a boon for Indian piggery industry due to its

Table 6. Chemical composition of pig faeces and final product (compost) in different groups

Sample %	DM %	Ash %	EE %	CF %	N2 %	CP %	OM %	C %	C/N
Pig feaces	21.20^{a}	14.84^{a}	3.75^{b}	13.00^{c}	2.83^{c}	17.70^{b}	85.16^{c}	49.40^{d}	17.43^{a}
C_0	31.14^{b}	$34.88b^{c}$	1.17^{a}	9.40^{b}	1.50^{a}	9.38^{a}	65.12^{b}	$37.77b^{c}$	25.44^{b}
C_C	32.60^{b}	40.27^{d}	1.34^{a}	9.80^{b}	1.53^{a}	11.04^{a}	59.73^{a}	34.67^{a}	22.89^{ab}
C_{T1}	44.30^{c}	33.21^{b}	1.21^{a}	7.68^{a}	2.00^{b}	12.50^{a}	66.45^{b}	38.54^{c}	19.38^{a}
C_{T2}	30.80^{b}	36.86^{c}	1.91^{a}	9.40^{b}	1.93^{b}	12.08^{a}	65.47^{b}	36.62^{b}	19.02^{a}
SEM	2.03	2.39	0.26	0.48	0.13	0.79	2.37	1.38	0.91

^{abc}Mean with different superscripts in a row differs significantly at 1% level (P<0.01).

novice technique and easy applicability under Indian conditions. It was concluded from the above experiment that IMOs from jaggery and molasses might be used as farmer friendly inocula/ inoculums to compost pig excreta under deep litter pig production system.

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