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Original Research

Use of Biogas Slurry for Enhancing Control of Phytopathogens

Fang-Bo Yu[1a], Xiao-Dan Li[2a], Shinawar Waseem Ali[3a], Cheng-Fang Song[1], Sheng-Dao Shan[1]*, Lin-Ping Luo[1]

[1]Department of Environmental Sciences, School of Environment and Resource Sciences, Zhejiang Agricultural and Forestry University, Linan 311300, China
[2]Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing 210014, China
Institute of Agricultural Sciences, University of the Punjab, Lahore-54590, Pakistan

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Abstract

We mixed biogas slurry with 6 commercial fungicides and screened them against 11 phytopathogens. Results showed that the inhibition effect of biogas slurry was different for different pathogens, and no significant difference between treatments of *Didymella bryoniae*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Aspergillus niger*, *Rhizoctonia cerealis*, *F. graminearum*, and *Septoria tritici* was observed. However, significant differences were found among *Penicillium* sp., *Botrytis cinerea*, *Alternaria sonali*, *F. oxysporum* f. sp. *melonis*, and *Sclerotinia sclerotiorum*. The approach described here presents a promising alternative to current manipulation, although some issues still need further examination. Information obtained from this study could contribute to better utilization of biogas slurry and sustainable agriculture.

Keywords: anaerobic digestion, bacteriostastic activity, biogas slurry, phytopathogen

Introduction

Anaerobic digestion has been used for many years for recycling biological wastes. China has been promoting underground and individual anaerobic digesters to process rural organic materials. Since 1975, the "Biogas for every household" program led to the construction of approximately 1.6 million digesters per year in China, mainly by way of concrete fixed-dome digesters [1]. These household anaerobic digestion systems mainly utilize human and animal manures, along with agricultural by-products such as grain stalks (primarily rice), sweet potato vines, and weeds for producing biogas as an energy source. However, in recent years the number of digesters built each year has fallen dramatically because of the reduction in subsidies, with a consequent switching from biogas to coal as a fuel [2].

Biogas slurry is a by-product of biogas production and a good source of plant nutrients, which can improve crop and fruit qualities and yields [2-4]. Its use as a soil amendment could offer a win-win opportunity and, at the same time, prevent adverse environmental impacts of waste disposal [5]. Due to the propagations of family-sized anaerobic digesters and biogas plants in many Asian countries, including China, the amount of biogas slurry has drastically increased [1, 6]. Furthermore, since the announcement of China's 2003-10 National Rural Biogas Construction Plan in 2003, a total of 20 million household digesters had been built by 2010, and the output of slurry has soared to 600 million tons annually.

A number of studies recorded the inhibition effects of biogas slurry on phytopathogens [2, 7, 8]. The inhibition was probably due to:

*e-mail: shanshd@vip.sina.com
[a]Fang-Bo Yu, Xiao-Dan Li, and Shinawar Waseem Ali contributed equally to this work.

1) the high concentration of ammonia nitrogen
2) the existence of low-molecular-mass substances (e.g., enzymes, humic acids, and vitamins), and micro-anaerobic environment
3) the antagonism of microbes in the slurry
4) the enhancement of resistance ability of the host plant (indirect one) [8].

However, Yu et al. [2] indicated that the efficacy of biogas slurry on phytopathogen is rather weak and transient when compared with chemical counterparts due to the low content of active ingredients, with the majority being water (> 99%), and the ease of inactivation. Therefore, studies on enhancing the efficacy should be carried out.

Nowadays, with the rapid development of large-scale intensified farms, with a growth rate of 3-5% in China [9], the associated environmental problems have attracted considerable public attention, leading to the substantial manpower and financial resources invested for the prevention and control of pollution. Nevertheless, with the output of livestock and poultry manure of 4.5 billion tons per year, the situation is still severe [10]. In addition, there are more than 700 million tons of crop stalks produced simultaneously. How to utilize these resources is key to the sustainable development of modern agriculture. Although the use of small household biogas digesters has been fairly sophisticated and widespread throughout China, especially southern China, more and more attention has been paid to the construction of large and medium-sized biogas projects due to their advantages, such as power generation and land saving [10]. According to the data provided by Wang et al. [11], more than 2,761 large- and 12,864 medium-sized biogas projects had been constructed in China by the end of 2008. However, there is a severe lack of information concerning the utilization of biogas slurry generated from these projects.

The objectives of this research were to:

1) investigate the feasibility of mixing commercial fungicides with biogas slurry to control test phytopathogens,
2) select efficacy-enhanced formulations.

The results obtained from this study will provide valuable information for better utilization of biogas slurry, and contribute to the sustainable agricultural development of modern China.

Experimental Procedures

Biogas Slurry

Biogas slurry was sampled from a 950 m^3 anaerobic filter at Brother Farm, Yongning, Zhejiang Province, China. There are about 10,000 pigs on site. The properties of biogas slurry were analyzed in the National Analytical Centre, Guangzhou, China, according to the standard methods of the American Public Health Association [12], and are presented in Table 1.

Table 1. The physico-chemical properties of biogas slurry obtained from Brother Farm. The data are reported as means (n = 3).

Item	Content	Item	Content
Total N ($g \cdot l^{-1}$)	0.234	Fe ($mg \cdot kg^{-1}$)	<1
Total P ($g \cdot l^{-1}$)	0.042	Ni ($mg \cdot kg^{-1}$)	<1
Total K ($g \cdot l^{-1}$)	0.36	Cu ($mg \cdot kg^{-1}$)	<1
NH4+-N ($mg \cdot l^{-1}$)	162	Zn ($mg \cdot kg^{-1}$)	<1
EC[a] (25ºC) ($dS \cdot m^{-1}$)	6.50	As ($mg \cdot kg^{-1}$)	<1
CODCr ($mg \cdot l^{-1}$)	520	Se ($mg \cdot kg^{-1}$)	<1
Li ($mg \cdot kg^{-1}$)	<1 [b]	Rb ($mg \cdot kg^{-1}$)	<1
B ($mg \cdot kg^{-1}$)	<1	Sr ($mg \cdot kg^{-1}$)	<1
Na ($mg \cdot kg^{-1}$)	105	Mo ($mg \cdot kg^{-1}$)	<1
Mg ($mg \cdot kg^{-1}$)	36	Ag ($mg \cdot kg^{-1}$)	<1
Al ($mg \cdot kg^{-1}$)	<1	Cd ($mg \cdot kg^{-1}$)	<1
Ca ($mg \cdot kg^{-1}$)	20	Sn ($mg \cdot kg^{-1}$)	<1
Ti ($mg \cdot kg^{-1}$)	<1	Sb ($mg \cdot kg^{-1}$)	<1
Cr ($mg \cdot kg^{-1}$)	<1	Ba ($mg \cdot kg^{-1}$)	<1
Mn ($mg \cdot kg^{-1}$)	<1	Pb ($mg \cdot kg^{-1}$)	<1

[a] EC – electrical conductivity.
[b] The result was lower than the limit of detection of the inductively coupled plasma mass spectrometry (ICP-MS).

Microbial Strains Used

Highly aggressive strains of *Botrytis cinerea* Pers. exfr NJAU 01 (designated as BC, the same below), *Alternaria sonali* NJAU 02 (AS), *Fusarium oxysporum* f. sp. *melonis* NJAU 04 (FOM), *Didymella bryoniae* NJAU 08 (DB), *Sclerotinia sclerotiorum* (Lib.) de Bary NJAU 11 (SS), *Penicillium* sp. NJAU 12 (PS), *F. oxysporum* f. sp. *vasinfectum* NJAU 16 (FOV), *Aspergillus niger* V. Tiegh NJAU 17 (AN), *Rhizoctonia cerealis* Vander Hoeven NJAU 20 (RC), *Septoria tritici* NJAU 21 (ST), and *F. graminearum* Schw. NJAU 22 (FG) were used. The fungi were provided by Mrs. Yan-Ling Ji, Department of Microbiology, Nanjing Agricultural University. The cultures of the phytopathogenic organisms were maintained on potato dextrose agar (PDA) at room temperature (25ºC) with a 12 h photoperiod of 180 μE $(m^2 s)^{-1}$ per day, and were subcultured on fresh PDA every 4 weeks.

Chemicals

The commercial formulations Benzimidazole 44#® WP (carbendazim 50%), Cuixi® ME (prochloraz 45%), Hekang™ WP (polyoxin 3%), Equation Contact® WG (famoxadone 6.25% + mancozeb 62.5%), Pulec™ SL (propamocarb 72.2%), and Barleton® WP (triadimefon 15%) were used in the following tests.

Spore Germination Assay

The fungicides were dissolved either in biogas slurry or distilled water and diluted to various concentrations chosen

based on their performance in a preliminary study. The solutions in the broad inhibition range were further diluted for testing. Spores were collected from 3 to 10-day-old cultures using aseptic procedures and made into suspensions that were added to the test compound solution to give a final concentration of 1.0×10^6 spores per milliliter. The spore suspensions were applied onto a micro-slide placed in a closed Petri dish with high humidity. The slides containing the cultures were incubated at 25ºC in the dark for 12 to 22 h, which show maximum growth in the preliminary test. Each concentration of test fungicide was investigated in triplicate and all the experiments were repeated at least three times. The germination was stopped and germ tubes were fixed and stained by adding one drop of 1% cotton blue to each slide. The spore number was then determined, and the percentage of germination inhibition was calculated by the following formula.

$$\text{Percent germination inhibition} = \frac{g_c - g_t}{g_c}\times 100\% \qquad (1)$$

...where g_c = average germination (%) of control and g_t = average germination (%) of treatment.

EC_{50} values (the effective concentration for 50% inhibition) were calculated by regression analysis of the percentage of germination inhibition with log values of concentrations.

Calculation of Germination Percentage

The germination was observed and photographed with an Olympus BH2 microscope. Six views of each slide were photographed, and every view contained about 50 spores. A total of 300 spores were observed per concentration treatment per compound. Every object on the photos was measured for length and area. If the length or area of objects was less than average length or area of the spore, the objects were defined as a spot, and the remainder was defined as spores. Germination was defined as when the germ tube was longer than the average half-length of spores; i.e., if the length of the long axis of the object was longer than 1.5 times the average half-length of spores, it was defined as a germinating spore. The average percentage of germination was obtained by counting the number of total spores and germinating ones. Statistical analysis was performed by Tukey's Studentized range test from analysis of variance (ANOVA) with P value < 0.05 considered significant.

Mycelial Growth Assay

The antifungal activity was also tested against 11 pathogens by the plate diffusion method. PDA (18 ml) was poured into sterilized Petri dishes (90 mm diameter) and 100 µL spore suspensions (1.0×10^5 spores per milliliter) were spread onto the agar plates. Filter paper disks (6 mm diameter) immersed in biogas slurry, biogas slurry + fungicides (B+F), and distilled water + fungicides (D+F) were placed in the middle of each dish, and distilled water treated disks were used for the control sets. The test fungi were incubated at 28ºC. On day 7, inhibition zone diameters for fungicides at different concentrations were recorded and compared. All the experiments were carried out in triplicate. Toxicity was expressed in terms of percentage of mycelial growth inhibition and calculated following equation (2):

$$\text{Percentage of mycelial growth inhibition} = \frac{d_t - d_c}{90}\times 100\% \qquad (2)$$

...where d_c = average diameter (mm) of clear inhibition zone in control and d_t = average diameter (mm) of inhibition zone in treatment.

The method used for EC_{50} was the same as in the germination assay except that the percentage of mycelial growth inhibition was used instead of percent germination inhibition.

Results

In the present study, 11 pathogens were investigated and results showed that the inhibition of biogas slurry was different for different pathogens. No significant difference between treatments B+F and D+F on the test *D. bryoniae*, *F. oxysporum* f. sp. *vasinfectum*, *A. niger*, *R. cerealis*, *F. graminearum*, and *S. tritici* was observed (data not shown).

Among the 11 test pathogens, significant differences between treatments B+F and D+F both in spore germination and mycelial growth were only observed in *Penicillium* sp. (Tables 2 and 3). Biogas slurry alone could inhibit conidial germination and mycelial growth, but the effects were rather weak (only 8.47 and 23.20%, respectively). B+F was effective at lower concentrations (EC_{50} for B+F was smaller than for D+F) (Table 3). The EC_{50} for biogas slurry incorporated with Cuixi (conidial germination), Hekang, and Pulec (mycelial growth) were 272.1, 198.7, and 20.6 $mg\cdot l^{-1}$, respectively, but the corresponding values for D+F were 454.4, 322.9, and 464.4 $mg\cdot l^{-1}$.

Biogas slurry alone showed a suppressive effect on mycelial growth of the 4 phytopathogens *in vitro*, especially *B. cinerea* and *A. sonali* (Table 4). The percentages of inhibition were 51.10% (BC), 46.20% (AS), 17.60% (FOM), and 11.00% (SS), respectively. A remarkable improvement in biological activity was observed when some fungicides were mixed with biogas slurry, with their efficacies positively correlated with the dosage (Tables 4 and 5). For example, the EC_{50} for BC was enhanced from 2250.2 to 551.0 $mg\cdot l^{-1}$ (Pulec); for AS from 54.4 to 2.84 $mg\cdot l^{-1}$ (Equation Contact) and 1,697.0 to 934.4 $mg\cdot l^{-1}$ (Hekang), respectively; for FOM from 249.4 to 43.1 $mg\cdot l^{-1}$ (Hekang); and for SS from 421.5 to 92.6 $mg\cdot l^{-1}$ (Hekang). However, the percentage of spores germinated in all B+F treatments was similar of slightly lower than that in D+F control (data not shown).

Table 2. Inhibition effects of test formulations on *Penicillium* sp.

Item	Treatment	Concentration ($mg \cdot l^{-1}$)	Percent inhibition[a]	
			Biogas slurry	Distilled water
Spore germination	Distilled water			2.10 (0.2)
	Biogas slurry		8.47 (2.1)	
	Cuixi	1,000	78.33 (0.8)	69.25 (4.2)
		500	70.00 (2.6)	61.40 (3.6)
		333.3	60.00 (2.8)	48.68 (2.4)
		250	50.00 (3.2)	30.60 (1.8)
		200	35.00 (1.2)	15.40 (4.2)
	Equation Contact	1,250	78.50 (1.4)	68.18 (2.3)
		1,000	68.33 (2.2)	57.30 (1.6)
		833.3	55.00 (0.8)	39.70 (3.8)
		666.7	41.67 (1.2)	17.30 (3.2)
		500	21.67 (0.7)	12.90 (1.2)
	Benzimidazole 44#	2,000	83.33 (1.4)	77.40 (4.3)
		1,666.7	76.67 (2.1)	69.52 (1.1)
		1,428.6	61.67 (0.2)	54.67 (0.7)
		1,250	50.00 (2.7)	41.20 (1.7)
		1,111.1	38.33 (1.3)	24.33 (0.6)
		1,000	23.33 (1.3)	18.33 (0.5)
Mycelial growth	Distilled water			0 (0)
	Biogas slurry		23.20 (0.6)	
	Benzimidazole 44#	10,000	82.21 (0.8)	74.25 (2.6)
		4,000	62.00 (2.6)	58.40 (1.8)
		2,000	58.37 (2.8)	48.68 (1.8)
		1,000	51.07 (3.2)	30.60 (0.4)
		500	42.80 (1.2)	15.40 (2.2)
	Hekang	10,000	90.90 (1.4)	82.18 (2.1)
		5,000	86.00 (2.2)	75.30 (1.5)
		2,500	83.50 (0.8)	71.40 (2.0)
		1,666.7	83.10 (1.2)	68.75 (1.2)
		1,250	80.57 (0.7)	62.20 (1.0)
	Pulec	5,000	89.10 (0)	79.40 (1.2)
		2,500	85.50 (0.6)	71.82 (0.6)
		1,666.7	83.90 (1.2)	68.47 (0.2)
		1,250	82.20 (0.7)	63.26 (0)
		1,000	80.60 (1.1)	59.88 (1.3)

[a]The values are the average of three independent experiments, and the numbers in parentheses indicate the standard deviation (the same below).

Discussion

No significant difference between treatments on *D. bryoniae*, *F. oxysporum* f. sp. *vasinfectum*, *A. niger*, *R. cerealis*, *F. graminearum*, and *S. tritici* was observed. However, due to the fact that digested slurry contains organic nitrogen (mainly amino acids), abundant mineral elements, and low-molecular-mass bioactive substances (e.g., hormones, humic acids, vitamins, etc.) [13], which could be used as organic manure in the sowing season and as a source of water in other seasons [2], the test samples still possess values for field application. Besides, there were formulations worked better against phytopathogens as compared with the controls, and they are the emphasis of this work. What's more, although inhibition effects of biogas slurry on *F. axysporum* f. sp. *fragariae*, *Colletotrichum gloeosporioides* (Penz.) Sacc., *Verticillium dahliae*, *Phytophthora capsici* Leonian, *Pythium aphanidermatum* [8], *Alternaria alternate* [14], *Magnaporthe oryzae*, *Ceratocystis fimbriata*, *Bipolaris sorokinianum*, *Rhizoctonia solani*, *Exserohilum turcicum*, *Bipolaris maydis* [15], and *F. graminearum* [16] have been confirmed, more studies especially on vegetable pathogens should be carried out to further evaluate the comprehensive utilization potential of biogas slurry.

Penicillium sp. is among the most successful and omnivorous fungal plant pathogens, with a host range of greater than 200 plant species [17, 18], and *B. cinerea*, *A. sonali*, *F. oxysporum* f. sp. *melonis*, and *Sclerotinia sclerotiorum* are destructive pathogens to a broad range of hosts and can cause significant yield losses [19-21]. Their abilities to infect plants at different stages of growth make them very serious diseases. The present results indicated that the enhancement on mycelial inhibition was much more remarkable when compared with spore germination. The results were inconsistent with that of Ma et al. [8] which revealed a stronger inhibition of biogas slurry on spore germination of *F. axysporum* f. sp. *fragariae* rather than on mycelium growth. The reason for the above observations is still not well understood.

Currently, fungicides are the major means to control vegetable diseases. They are used alone, combined in mixtures, or applied separately in sequence. However, the growing public concern over the health and environmental hazards and the development of fungicide-resistant strains have generated interest in and encouraged research on the development of alternative control methods [17, 22]. Furthermore, there is a considerable utilization requirement of biogas slurry produced from anaerobic digestion of animal manure and slurries in China, as well as in many other parts of the world, due to the fact that associated environmental problems have attracted considerable public attention, and that anaerobic digestion could offer several agricultural, environmental, and socio-economic benefits through improved fertilizer quality of manure, considerable reduction of odors, and inactivation of pathogens and, last but not least, production of biogas production as clean, renewable fuel for multiple utilizations. The present study is the first report where the inhibition effects of biogas slurry on spore germination and mycelial growth of above 11

Table 3. Probability analysis for inhibition effects of test formulations against *Penicillium* sp.

Item	Treatment	Toxicity regression	*R*	EC_{50} (mg·l^{-1})
Spore germination	Distilled water +			
	Cuixi	y = 0.8944x - 0.4753	0.923	454.4
	Equation Contact	y = 1.9129x - 8.1444	978	965.1
	Benzimidazole 44#	y = 2.4407x - 12.69	0.983	1405.2
	Biogas slurry +			
	Cuixi	y = 0.6835x + 1.1683	0.957	272.1
	Equation Contact	y = 1.7173x - 6.42	0.998	773.0
	Benzimidazole 44#	y = 2.2084x - 10.75	0.993	1251.2
Mycelial growth	Distilled water +			
	Benzimidazole 44#	y = 0.4426x + 1.6317	0.823	2017.4
	Hekang	y = 0.2649x + 3.4695	0.981	322.9
	Pulec	y = 0.3478x + 2.864	0.996	464.4
	Biogas slurry +			
	Benzimidazole 44#	y = 0.3428x + 2.6359	0.962	988.1
	Hekang	y = 0.207x + 4.3814	0.966	19.8
	Pulec	y = 0.2234x + 4.3245	0.998	20.6

Table 4. Mycelial growth inhibition of test formulations on *B. cinerea*, *A. sonali*, *F. oxysporum* f. sp. *melonis*, and *S. sclerotiorum*.

Pathogen	Treatment	Concentration (mg·l^{-1})	Percent growth inhibition	
			Biogas slurry	Distilled water
BC	Distilled water			0 (0)
	Biogas slurry		51.10 (2.3)	
	Equation Contact	1000	84.20 (0.8)	76.25 (2.6)
		500	77.10 (1.6)	71.40 (1.8)
		333.3	70.00 (0.8)	64.06 (0.6)
		250	59.50 (2.3)	53.60 (4.2)
		200	52.10 (1.6)	41.32 (2.2)
	Benzimidazole 44#	769.2	70.70 (1.4)	65.40 (4.2)
		588.2	67.80 (2.2)	62.30 (3.2)
		200	58.50 (0.8)	55.40 (2.0)
		125	56.20 (1.2)	52.30 (1.8)
		100	52.80 (0.7)	49.20 (2.1)
	Pulec	5000	76.41 (0.4)	58.49 (0.2)
		2500	69.62 (1.8)	51.36 (1.1)
		1666.7	67.67 (0)	49.80 (0.7)
		1250	63.25 (0)	44.22 (0)
		1000	53.90 (0.1)	36.53 (2.0)

Table 4. Continued.

Pathogen	Treatment	Concentration ($mg \cdot l^{-1}$)	Percent growth inhibition	
			Biogas slurry	Distilled water
AS	Distilled water			0 (0)
	Biogas slurry		46.20 (1.4)	
	Equation Contact	6000	86.20 (0.3)	81.05 (0)
		5000	84.90 (0.3)	75.40 (1.0)
		4000	83.37 (1.2)	71.38 (0.5)
		3000	82.40 (1.2)	69.60 (0.3)
		2000	80.00 (0.4)	67.40 (1.1)
	Hekang	1000	79.80 (0.7)	58.18 (2.0)
		800	70.20 (0)	55.30 (0)
		600	61.80 (0.3)	51.22 (0)
		400	53.90 (0)	48.71 (1.1)
		200	52.30 (0.2)	42.20 (0.1)
	Pulec	1000	90.50 (0.0)	85.30 (0.7)
		800	89.00 (0.6)	81.62 (0.1)
		600	88.40 (1.2)	78.45 (0.3)
		400	80.20 (0.7)	73.21 (0.2)
		200	69.80 (1.1)	65.18 (0.2)
FOM	Distilled water			0 (0)
	Biogas slurry		17.60 (1.2)	
	Hekang	10000	84.90 (0)	78.15 (0)
		5000	77.90 (0.4)	75.24 (0)
		1666.7	75.60 (1.2)	68.60 (0.1)
		1250	73.42 (0.2)	63.26 (0.3)
		1000	70.80 (0.2)	60.12 (2.3)
SS	Distilled water			0 (0)
	Biogas slurry		11.00 (0.2)	
	Hekang	1000	87.30 (0)	79.08 (1.1)
		833.3	86.72 (0.2)	73.30 (0.5)
		714.3	83.33 (0)	68.46 (2.2)
		625	81.20 (0.8)	62.70 (0.2)
		500	80.00 (0.2)	58.20 (0.8)

phytopathogens were evaluated in detail. The mixing is simple, does not require a sophisticated machine, and has manifold application effects. This approach therefore presents a promising alternative to current manipulation for disease control. However, some issues need further examination before the technology can be recommended for practical use, for example the appropriate dosage for field application, economic feasibility on biogas slurry transportation, and evaluation of comprehensive application effect. In addition, as concentrated biogas slurry is now available [2], and there are reports on some inexpensive additives such as essential oil [23] and garlic extract [24] having good inhibition effects, further research should be carried out around efficacy enhancement.

Table 5. Probability analysis for mycelial growth inhibition of test formulations against *B. cinerea*, *A. sonali*, *F. oxysporum* f. sp. *melonis,* and *S. sclerotiorum*.

Pathogen	Treatment	Toxicity regression	*R*	EC_{50} (mg·l^{-1})
BC	Distilled water +			
	Equation Contact	y = 0.5489x + 2.0358	0.933	221.4
	Benzimidazole 44#	y = 0.1891x + 4.1269	0.995	101.2
	Pulec	y = 0.3111x + 2.5985	0.944	2250.2
	Biogas slurry +			
	Equation Contact	y = 0.5849x + 2.0324	0.977	159.8
	Benzimidazole 44#	y = 0.2215x + 4.0598	0.996	69.7
	Pulec	y = 0.3411x + 2.847	0.946	551.2
AS	Distilled water +			
	Equation Contact	y = 0.3882x + 3.4483	0.993	54.4
	Hekang	y = 0.2295x + 3.2931	0.936	1697.0
	Pulec	y = 0.3788x + 2.8858	0.944	265.4
	Biogas slurry +			
	Equation Contact	y = 0.2133x + 4.7797	0.974	2.84
	Hekang	y = 0.5059x + 1.5399	0.995	934.4
	Pulec	y = 0.4385x + 2.7052	0.842	184.5
FOM	Distilled water + Hekang	y = 0.2191x + 3.791	0.977	249.4
	Biogas slurry + Hekang	y = 0.1801x + 4.3219	0.955	43.1
SS	Distilled water + Hekang	y = 0.8895x - 0.3566	0.991	421.5
	Biogas slurry + Hekang	y = 0.4844x + 2.8066	0.963	92.6

Conclusions

In general, the present study showed that biogas slurry + fungicide could significantly improve the inhibition effect on 5 test pathogens (*Penicillium* sp., *B. cinerea*, *A. sonali*, *F. oxysporum* f. sp. *melonis*, and *S. sclerotiorum*) *in vitro* as compared with the control. The application of biogas slurry with appropriate fungicides is more eco-friendly, normally safe, and may provide long-term protection to crops, and in agriculture has a good promise. The information obtained from the study could contribute to better utilization of biogas slurry and sustainable agriculture.

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