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ASPERGILLUS NIGER AS THE RAPID PRESS MUD COMPOSTING AGENT

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ABSTRACT

Composting with efficient microbes is considered a sustainable strategy for effective conversion of agricultural discards into a productive biofertilizer. The present study was designed to isolate fungal strain efficient in releasing hydrolytic enzymes for accelerated decomposition and value addition of press mud (PM). In order to tolerate higher temperatures and pH variations during composting, the analysis specifies the different enzymatic behavior at varied temperature and pH range and the strain was also tested for aflatoxins production to produce biofertilizer safe for crop application. The physicochemical characteristics of PM such as total organic carbon (TOC), C/N ratio, electrical conductivity (EC), cation exchange capacity (CEC), total Kjeldahl nitrogen (TKN), total phosphorus (TP), and total potassium (TK) during composting were also studied as compared to control. The results indicated that among seven fungal isolates, *Aspergillus niger* (*A. niger)* PM-4 was selected as the best enzyme producers by producing carboxymethyl cellulase (CMCase) α-Amylase, pectinase, and xylanase at a broader temperature and pH spectrum. Composting of PM with *A. niger* PM-4 leads to significant reduction in TOC (21.2%), C/N ratio (21.3%), increase in EC (25%), CEC (98%), TKN (10.5%), TP (9.2%), and TK (41%) compared to control with one month maturity duration. The *A. niger* PM-4 also inhibiting mycotoxin growth (aflatoxins) and its inoculation into PM generated mature biofertilizer with germination index (GI) 96.5% after a month representing a cheaper, effective and sustainable approach for waste utilization.

Key words: Biofertilizer; Bio-inoculant; *Aspergillus niger;* Composting; Hydrolytic enzymes

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INTRODUCTION

Large amounts of sugar cane waste are generated from sugar mills. After the clarification of the sugar cane juice, a solid waste sludge called press mud is obtained. Press mud contains 2.8–4.5 % of sugar (Velarde *et al*. 2004), less than 85% of organic matter that is insoluble (Sánchez *et al*. 1996) and a lignocellulosic content (cellulose: 22.3%, hemicellulose: 21.67% (Nimbalkaret *et al*. 2017). Organic waste desires to be taken care through different ways. One of them is composting, which, owing to its high organic matter content, it may be used for this reason and its productive aspect, and the existence of macro- and micronutrients critical to plant health in press mud (Sánchez *et al*. 1996). In general, for the biodegradation of press mud, a period of six months is required to convert it into efficient compost with harmful effects of contamination of surface and ground water bodies, which pose a major threat not only to animals but also to human health (Sangwan *et al*. 2010).

By secreting hydrolytic enzymes, microorganisms metabolized the insoluble fractions of organic waste as a result, the biodegradation cycle accelerates and the degradation rate can be increased (Castaldi *et al*. 2008; Singh *et al*. 2016; Kong *et al*. 2018). Aspergillus sp. are capable of hydrolyzing complex plant polysaccharides by means of the synthesis of a variety of enzymes, including ligninase, cellulase, α-amylase, pectinase and xylanase, etc. *A. niger* is a well-known composting agent for the rapid degradation of the substrate to shorten the time needed for composting and increase its added value (Grujić *et al*. 2015). Additionally, *A. niger* is well-known for its significant industrial fermentation processes, which are used to produce organic acids and essential enzymes (Schuster *et al*. 2002).

Lignocellulose, which required a lot of time and energy to degrade, is a recalcitrant material among sugar products. Polysaccharide dry weight contains 70% lignin (Manna *et al*. 2003). Cellulase enzyme promotes cellulose hydrolysis. The synergistic action of three enzymes causes cellulose degradation (Zhang *et al*., 2006). Xylan is a structural part of the plant cell wall that is composed of 1,4-linked b-D-xylopyranose residues. The variety of xylanase hydrolytic enzymes supports in the complete hydrolysis of xylan. These enzymes catalysed the hydrolysis of the lignin-carbohydrate complex (LCC) generated during the kraft process (Polizeli *et al*. 2005; Dobrev *et al*. 2007). Numerous studies indicate that Aspergillus spp. are significant xylanase producers. -amylase is another enzyme that digests starch, glycogen, and associated polysaccharides.

Pectin is made up of D-galacturonic acid that is naturally esterified by methoxy groups and contains natural sugars on the side chains. Microbes enhance pectin's pectinolytic function (Aguilar and Huitron 1993).

The aim of this study was to stabilize press mud by transforming it into an efficient and productive biofertilizer through bio-augmentation with thermostable multi enzyme producing fungal strain by adding value (NPK) and accelerating the biodegradation process.

MATERIALS AND METHODS

Isolation and purification of fungal strain: Thermostable hydrolytic enzymes producing fungal strains were isolated from press mud composting at various phases, already occurred in the vicinity of the Auriga group of companies in Lahore (31° 20' 12.9" N, 74° 6' 56"E) Pakistan. Samples were collected in sterilized polyethylene bags, labeled and stored at 4 °C for further analytical work. Press mud used as substrate was collected from Macca sugar mills, Manga Road, Raiwind (31°15'34.12 **N,** 74°9'19.72" E) Lahore, Pakistan. The initial physicochemical characteristics of press mud are depicted in table 1

Table 1: Initial characteristics of raw Press mud.

Parameters	Concentration
pH	7.14 ± 0.1
EC (mS/cm)	1.32 ± 0.02
CEC (meq/kg)	217.4 ± 32
TOC %	60.9 ± 2.1
TKN %	1.5 ± 0.9
C/N	40.6 ± 3.3
P2O5%	1.1 ± 0.08
/K2O%	0.52 ± 0.01

* The mean and standard deviation of three replicates was used for all values.

Qualitative screening of different fungal enzymes

Cellulase: Fungal isolates were inoculated on a minimal salt medium supplemented with 1% CMC and 2% agar. After inoculation, the strains were incubated for 48 hours at 28±2°C. After incubation, the plates were flooded with 0.1 percent Congo red solution for 60 minutes before being destain with 1M NaCl solution (Mandels & Weber 1969). A pale yellow or orange zone determines the efficiency of strain.

Xylanase: The xylanase screening medium consists of yeast extract 1.0 g/l, xylan 5.0 g/l, $(NH4)_2SO_4$ 0.2 g/l, $MgSO_4.7H_2O$ 0.5 g/l, $CaCl_2.2H_2O$ 0.25 g/l, KH_2PO_4 0.6 g/l, and agar 20 g/l. After inoculation, the plates were incubated at 28 ±2 C^0 for 48 hours. Plates were stained for 15 minutes with Congo red solution (0.5 percent w/v in 50% ethanol) before being de-stained with 1M NaCl (Mandels and Sternburg's 1976). Zone formation indicates the efficiency of strain.

Amylase: A medium containing 1.0 g/l glucose, 2.5 g/l yeast extract, 10.0 g/l starch, and 20.0 g/l agar was used to test isolates amylolytic potential. Inoculated plates were incubated for 48 hours at 28±2 C^0. After that, the plates were flooded with a 2% iodine solution (for 15 min). A starch hydrolysis zone accompanying fungal growth indicates strain proficiency.

Pectinase: The pectinase screening medium contained yeast extract 1 g/l, $(NH_4)2SO_4$ 1.4 g/l, KH_2PO_4 2.0 g/l, $MgSO_4.2H_2O$ 0.2 g/l, pectin 5.0 g/l, and agar 20 g/l. Following incubation, the plates were flooded with potassium iodide solution (0.3 percent iodine and 0.6 percent KI) for 5 minutes (Rhoban *et al*. 2008). The creation of clear zones determines the activity of the strain.

Quantitative assay for different enzymes production: Inoculated fungal isolates were put in synthetic medium containing substrates Carboxymethyl cellulose (CMC), beech wood xylan, starch, and pectin from apple cellulase, xylanase, -amylase, and pectinase action. As substrates for CMCase, xylanase, -amylase, and pectinase, 1 ml carboxymethyl cellulose (1 percent w/v), 1 ml beech wood xylan (1 percent w/v), 1 ml starch (1 percent w/v), and 1 ml apple pectin (1 percent w/v) were added to the reaction mixture. After adding 0.1 ml of culture filtered supernatant and 1 ml of 0.05 molar sodium acetate buffer at 5 pH in substrate length, the mixture was incubated at 40 °C for one hour. A quenched reaction mixture (QRM) was developed by immersing the tubes in a boiling water bath for 5 minutes. 0.5 ml of QRM was topped off with 1 ml of DNS reagent, which was then increased to 2 ml with distilled water and boiled for 10 minutes. The reaction mixture was then cooled, and the difference in absorbance (A°) at 550 nm was measured with a spectrophotometer (Siddiqui *et al*. 2000).

To calculate the standard glucose factor, the standard glucose curve was used (Figure 1). At 40 °C and pH 5, one unit enzyme activity was described as the amount of enzyme needed to release 1mol of reducing groups (calculated as glucose equivalents) min^{-1}. The formula was used to calculate the operation units:

$$\text{Units/ml/min} = \frac{\Delta A° \times \text{Glucose Standard factor (G. S)} \times \text{Dilution factor} \times \text{Total reaction mixture}}{\text{Enzyme} \times \text{Time} \times \text{Quenched Reaction Mixture for DNS assay (QRM)}}$$

Where,

ΔA° = Change in absorbance (OD) at 550 nm = Experimental 'OD' – Blank 'OD'.
Glucose Standard Factor =G.S. factor = 1.0 OD = 1/slope = µmole glucose
QRM = Quenched reaction mixture

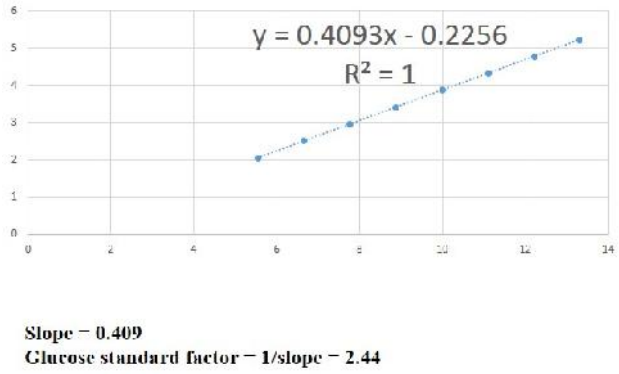

Fig 1: Glucose standard Curve

Optimization of pH and temperature for different enzyme activities: Temperature was optimized for different enzyme production, and culture medium was prepared in separate flasks. Temperatures ranging from 20º to 50º C were used to incubate the inoculated medium. A standard assay protocol was used to measure enzyme activity (Siddiqui *et al*. 2000). The pH of the production medium was optimised by adjusting the (medium) pH in separate Erlenmeyer flasks using 1M NaOH and 1M HCl solutions at different pH values (from 2-9). The samples were drawn after 48 hours of incubation, and enzyme activity was determined using the standard assay protocol (Siddiqui *et al*. 2000).

Mycotoxin's determination: An inexpensive qualitative method ammonia vapour test was performed to assess the aflatoxigenic effect of a selected fungal strain (Saito and Machida 1999). Many research findings demonstrated the test's compatibility with thin layer chromatography and the enzyme-linked immune sorbent assay, which were used to determine the aflatoxigenic effects of the isolates (Shekhar *et al*. 2017). The colour production of the culture was documented after it was exposed to ammonia vapour.

Identification: Fungal isolates were identified and confirmed on the basis of morphological and cultural characteristics (Domsch *et al.* 2008; Samson *et al*. 2010).

Preparation of fungal inoculum and processing of biofertilizer: Using aerobic composting technology, raw press mud was bio-augmented with an efficient fungal strain and transformed into biofertilizer. T1 (PM) press mud heap alone was used as a control treatment, and T2 (PM + inoculant) press mud heap with fungal inoculum was also used. Both treatments had four replicates. Each heap is made up of a five-ton load of press mud. An efficient fungal culture was first grown on potato dextrose agar for 7 days at 28+2 °C (PDA). Following incubation, the biomass was suspended for two days in five liters of sterile molasses at 28+2 °C. Molasses containing a fungal strain (10^8 CFU/ml) was distributed over the press mud heaps to ensure the best possible spread of the inoculum. To evaluate the decomposition rate and value addition parameters, TOC, Total nitrogen (TN), C/N ratio, total phosphorous (TP), and total potassium (TK) were measured at the beginning and end of a one-month composting period. To keep composting in an aerobic environment going, turning was done weekly with the aid of a loading shovel, and moisture content was kept at 60%.

Physicochemical analysis: TOC (Tandon, 2005) was determined using the dry combustion method, TN (Tandon, 2005) was determined using the Kjeldahl method, TP (PSQCA 1996) was determined using a spectrophotometer (Spectroscan, 80D), Zn (AOAC 2005), and K (Richards 1954) were determined using an atomic absorption spectrophotometer (Thermo fischer, ICE 3300). Other important parameter for compost maturity and stability are CEC and GI (Rashad *et al*. 2010) CEC was determined by the method of Yasud and Akio (1980).

It is important to evaluate the phytotoxicity of biofertilizers to avoid any harmful effects on crop (Luo *et al*. 2018). Phyto-toxicity assay was performed by measuring seed GI of maize (Jiang *et al*. 2015) using eq. (1)

$$GI\% = \frac{\text{(Germinated seeds in treatment \% × length of root in treatment)}}{\text{(Germinated seeds in control\% × length of root in control)}} \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

Statistical analysis: Data were represented as mean and standard deviation (SD) of four replicates.

RESULTS AND DISCUSSION

Screening and characterization of fungal strain: Among seven fungal isolates PM-1 to PM-7, only one fungal strain has been shown to be effective for all desired enzymatic activities. The efficient strain was morphologically studied for its identification. Fresh culture appears like white cotton, which finally turns black. The microscopic examination revealed that the strain was filamentous fungi, a smooth walled surface with black molds, a glubose shape and a conidia surface that is very rough and irregular. The strain was described as *A. niger* based on morphological characteristics. The selected strain was used for rapid and value-added press mud composting based on its enzymatic efficiencies.

A. niger PM-4 showed higher enzymatic capacity by generating a zone for cellulase, xylanase, α-α-amylase, and pectinase on the substrate CMC, xylan from beech wood, starch and pectin from apple respectively, fig 2. The higher degrading ability of *A. niger* PM-4 makes it more imperative to use a composting agent. The dinitrosalisyl acid (DNS) method was used to determine quantitatively the activities of various enzymes across a broader temperature and pH spectrum. Under standard assay conditions, one International Unit (IU) of enzyme activity is characterised as the amount of enzyme that releases one umol of reducing sugar per minute. A glucose standard curve was developed to measure the reducing sugar from different enzymes. *A. niger* PM-4 optimization was performed to explore the optimum temperature and pH for the efficient activity of *A. niger* as temperature and pH varies during composting.

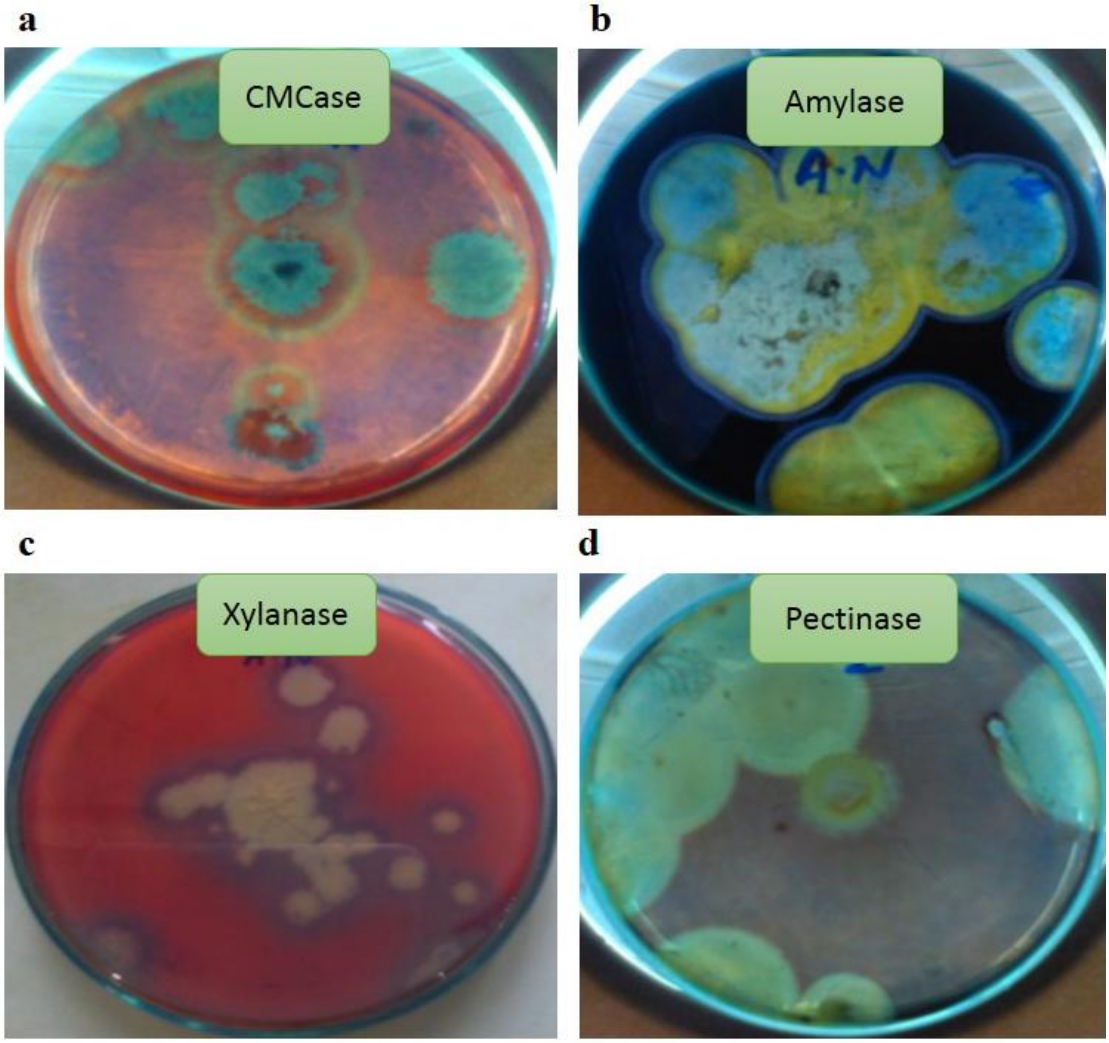

Fig 2: Qualitative screening of *A. niger* for different enzymatic activities. (a) CMCase (b) α-amylase (c) Xylanase (d) Pectinase

Quantitative estimation of enzymatic activities by *A. niger* PM-4: In the present study, *A. niger* PM-4 was selected as efficient cellulase producers due to the formation of zone. Around microbial colonies cellulose-solvent zones were made in CMC agar plate after 2 days of incubation.

The highest titer of CMCase (18.2 ± 0.01U/ml) was obtained when *A. niger* PM-4 was grown at incubation temperature 40ºC with initial pH 6 of the medium (19.0±0.09) (Fig. 3). Enzyme activity was also noticeable at initial pH 7 of the medium (18.2 ± 0.01 U/ml). The effect of temperature and pH variation on the trend of A. niger cellulase development PM-4 backs up the observations of El-Hadi *et al*. (2013) and Sohail *et al*. (2009).

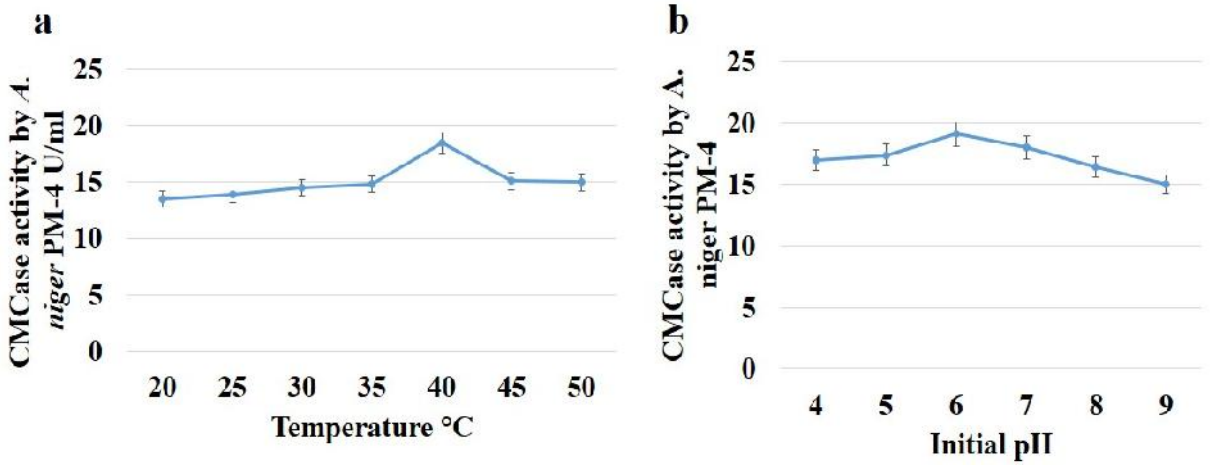

Fig 3: Effect of Incubation temperature (20-50C^0) and initial pH (4-9) for CMCase production in submerged fermentation during 48 hour. (a) CMCase activity by *A. niger* PM-4 at incubation temperature (20-50C^0); (b) CMCase activity by *A. niger* PM-4 at initial pH (4-9). Values are means and standard deviation of triplicates.

Many research studies reported activity of *Aspergillus spp.* to produce xylanase (Shah and Madamwa 2005). Optimum enzyme activity for *A. niger* PM-4 was achieved at 45C^0 (25.0±1.1) (Fig. 4a). The minimum amount of enzyme production (8.9±0.1) was noted at 20C^0. Enzyme production increases with increase in temperature and after getting to optimum temperature, gradual decline observe. However, enzyme activity remains active at higher temperature 50C^0. Optimum pH for *A. niger* PM-4 xylanolytic strains exhibited its peak activity at pH 7.0 and becomes very low at higher pH 8 and 9(Fig. 4b). The optimum temperature and pH range was in accordance with results of Pal and Khanum (2010) and Uday *et al*. (2017). Numerous other research results indicated that *A. niger* xylanase enzyme activity fluctuated between pH 3.0 and 9.0 (Laxmi *et al*. 2008; Bajaj *et al*. 2010).

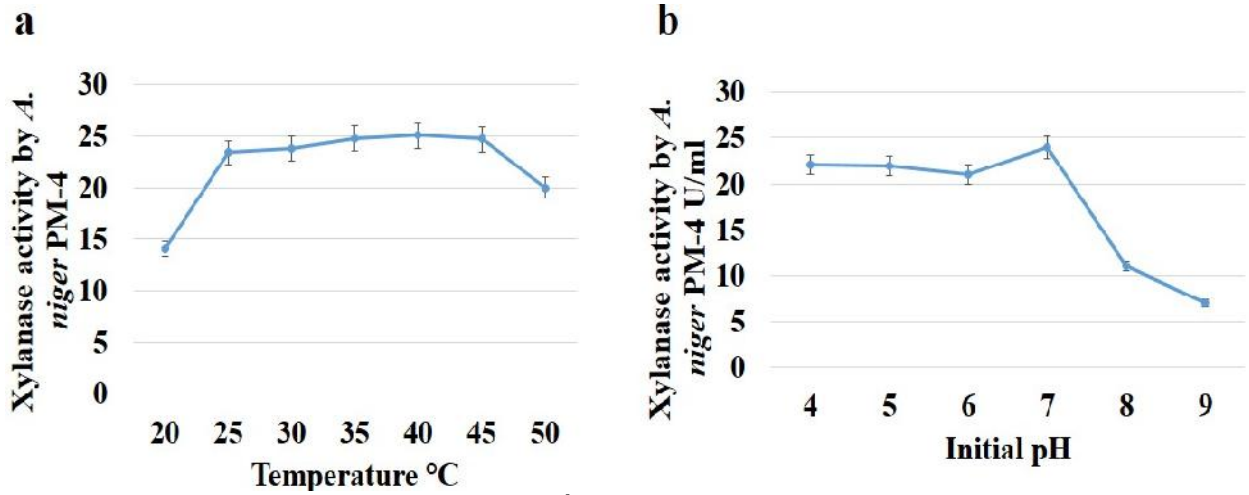

Fig 4: Effect of Incubation temperature (20-50C^0) and initial pH (4-9) for Xylanase production in submerged fermentation during 48 hour. (a) Xylanase activity by *A. niger* PM-4 at incubation temperature (20-50C^0); (b) Xylanase activity by *A. niger* PM-4 at initial pH (4-9). Values are means and standard deviation of triplicates.

Higher α-amylase production by *A. niger* PM-4 was noticed at 30 °C (25±1.1 U/ml) and at pH 7 (24.0±1.1 U/ml) respectively (Fig. 5). α-amylase production started to increase from pH 4 to 7, however, the mean α-amylase production was lower at pH 8. The findings for optimum temperature (Abdullah *et al*. 2014) and pH by *A. niger* PM-4 were in accordance with the results of (Rose´s and Guerra, 2009) and the trend in variation was according to Wang *et al*. (2016).

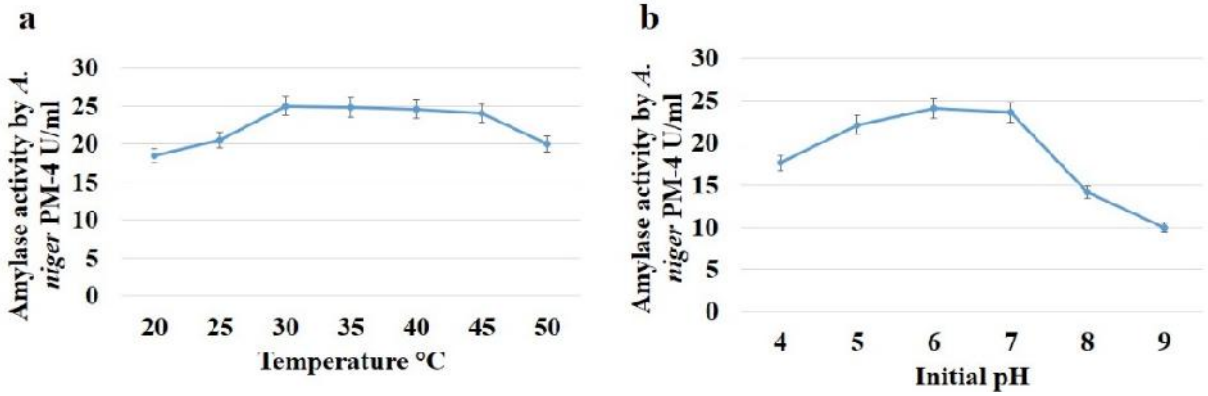

Fig 5: Effect of Incubation temperature (20-50C^0) and initial pH (4-9) for α-amylase production in submerged fermentation during 48 hour. (a) α-amylase activity by *A. niger* PM-4 at incubation temperature (20-50C^0); (b) α-amylase activity by *A. niger* PM-4 at initial pH (4-9). Values are means and standard deviation of triplicates.

A. niger PM-4 showed pectinolytic enzyme activity by hydrolyzing pectin from apple. Maximum pectinase activity by *A. niger* PM-4 was found (25.0±1.9 U/ml) at 30°C and remained nearly stable up to 40C^0 and after that enzyme activity gradually decline (Fig. 6b).

The strains had a good thermal stability (30–50 °C) and more pectinase activity at acidic to neutral pH range of 4.0-7.0. Maximum pectinase activity by *A. niger* PM-4 was found at initial pH 6 (Fig. 6a) The decay in pectinolytic enzyme activity was noted as the pH level increased from 7. Ahmed *et al*. (2016) reported highest enzyme production (117.1 ± 3.4 U/ml) at 30 C^0 with pH 5.5. El Enshasy *et al*. (2018) described that controlling 5.5 pH during cultivation generated a pectinase production of 109.63 U/mL, which was about 10% greater than the uncontrolled culture pH.

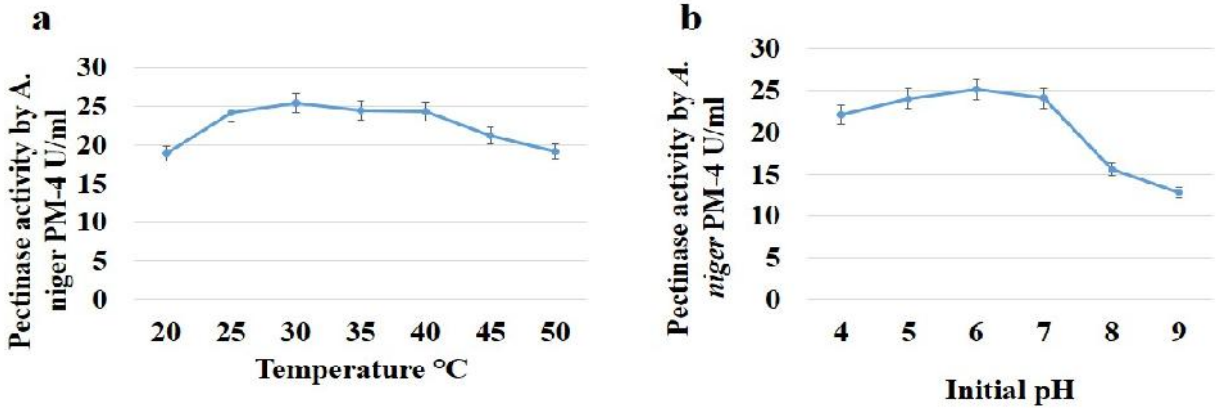

Fig 6: Effect of Incubation temperature (20-50C^0) and initial pH (4-9) for Pectinas production in submerged fermentation during 48 hour. (a) Pectinase activity by *A. niger* PM-4 at incubation temperature (20-50C^0); (b) Pectinase activity by *A. niger* PM-4 at initial pH (4-9). Values are means and standard deviation of triplicates.

The isolated microbial strains for hydrolytic enzymatic activities are moderate thermophile with minimum, optimum and maximum temperature of growth at 20, 30-40 and 50°C, respectively. All the selected isolates work best at acidic to neutral environment with minimum, optimum and maximum pH 8-9, 5-6 and 7. The temperature above 55C^0 abolish all enzyme activity by *A. niger* PM-4.

Changes in physico-chemical characteristics of PM during composting

Effect on temperature: The composting mass temperature indicates the rate of organic degradation and the appropriate time for the formation of suitable conditions supporting degradation by microbes.

The temperature of the heaps from treatments T2 (PM+*A. niger)* increased rapidly, entered in thermophilic stage at day 5, However, it peaked at day 10 and remained relatively stable until day 20. At the end of thermophilic stage, temperature gradually declined leading to cooling

and maturation stage and becomes stable. Higher temperature was observed in heap containing *A. niger* T2 (PM+A. *niger)* due to the activity of microbes and quicker breakdown of substrate compared to $T1_{Control.}$ The temperature of the heap from control treatment gradually increased and thermophilic stage starts at day15 and failed to obtain maximum temperature within a month as achieved by the heap T2 (PM+*A. niger*). The optimum temperature range for effective decomposition has been determined to be 50–60 °C (Awasthi *et al*. 2014), which is reached on day 25 in the control heap. The slower temperature rises and shorter thermophilic stages in the control heap suggested that the absence of an effective starter bio-inoculant slowed the decomposition of this substrate. The addition of a bio-inoculant had an impact on the rate of warming and subsequent heat generation of the composted material (Meng *et al*. 2018).

Effect on pH: Due to the fermentative metabolism of all treatments, the pH of the solution initially decreases, resulting in the creation of large amounts of organic acids. Following that, it increased in accordance with Awasthi *et al* (2014). The pH of heaps T2(PM+A. niger) decreased for the first five days of composting and then increased slightly at the end, while the pH of the control heap decreased for the first 20 days and then increased slightly. The low pH was caused by microbial activity producing organic acids during the initial stage of composting and Nakasaki *et al*. (2005) explain how nitrifying bacteria convert ammonia into nitrogen dioxide, which is then converted to nitrate in the presence of sufficient oxygen. H+ ions are released during the nitrification process, lowering the pH of the atmosphere. The gradual rise in pH caused by the development of NH4+-N during the decomposition of complex proteins (Awasthi *et al*. 2014; Gou *et al*. 2017; Yang *et al*. 2017). During ammonification, the aerobic atmosphere promotes organic N mineralization into ammonia, and the heap pH rises (Wong *et al*. 2001). The control heap's comparatively slow level of low pH may be attributed to a lack of microbial activity, resulting in slow biodegradation of the substrate.

Effect on EC: EC value describes the total salt content of the fertilizer, representing the quality of the biofertilizer. The initial EC values of heaps $T1_{(control)}$, $T2_{(PM+\ A.\ niger)}$ were 1.55±0.01 and 1.52±0.04, $mScm^{-1}$ respectively and gradually increases as the composting proceed. The preliminary increase in EC is caused by the biotransformation of composite materials into basic compounds such as phosphate, potassium, and ammonium mineral ions (Awasthi *et al*. 2014). When microbe-inoculated treatments were compared to controls, the EC values were higher (2.5±1.1). A high EC value suggests the presence of more soluble nutrients and can have a detrimental effect on plant development, including slow germination and withering. Fertilizers with a low EC value may be applied directly to crops; however, fertilizers with a higher EC value must be combined with another substrate or soil. At the end of composting, the EC values of the heaps from all treatments were higher, but less than the toxic EC limit.

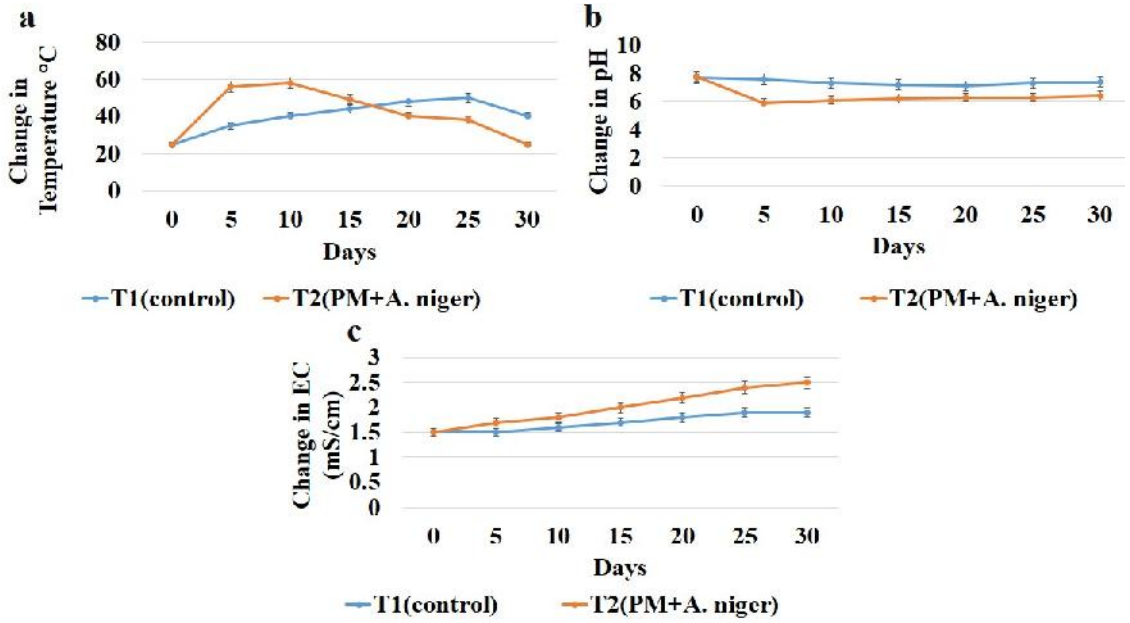

Fig 7: Change in physicochemical parameters during composting. (a) Change in temperature; (b) Change in pH; (c) Change in EC. $T1_{(control)\ Press}$ mud alone, $T2_{(PM+A.\ niger)}$- Press mud inoculated with fungal isolate. Results are the mean of three replicates, and error bars indicate standard deviation.

Change in CEC: Another important parameter for compost maturity is CEC (Harada and Inoko 1980; Karak *et al*. 2013). CEC for all treatments were measured before and after composting period. The initial and final CEC values for T1 (control) and T2 (PM+*A. niger*), are presented in Table 2. After a month, higher difference was observed in treatment T2 (PM+*A. niger*), however, control treatment showed slight increase in CEC.

Table: 2 Change in CEC during composting.

Sr No	Treatments	Initial CEC	Final CEC
1	T1 (control)	242.6±31.2	314.2±43.2
2	T2 (PM+*A. niger*)	251.4±30.1	623.4±31.1

Note: The results reflect the average of three replicates.

Greater CEC level were detected due to generation of carboxyl and phenolic functional groups. The mean CEC ratio increased with decomposition time for all treatments. The probable reason for rapid press mud mineralization are oxidation of molecules and radicals which leads to increased CEC values (Sahu *et al*. 2019). Table 4.4 shows that mean CEC value was significantly higher in T2 (PM+*A. niger*) than T1 (control). The finding support the notion that the inoculation of microbes fastens the degradation of substrate cause higher CEC value (Sahu *et al*. 2019).

Change in TOC and C/N: The various parameters investigated for composting decomposition and value addition showed a significant difference between T1 (PM) and T2 (PM + Bio-inoculant) (fig. 8). TOC is a critical parameter for monitoring the total amount of organic compounds present, and C/N has an effect on both the rate of decomposition and the amount of nitrogen recycled from a residue. The initial TOC and C/N ratios in both heaps were 60.9 percent and 40.6 percent, respectively. After a month, the ratio between control and inoculated heaps significantly decreased. In comparison to T1, T2 (PM + Bio-inoculant) significantly improved the rate of decomposition by reducing 21.2 percent TOC and 21.3 percent C/N. (PM). The highest reductions in TOC and C/N in heaps inoculated with *A. niger* T2 (PM + Bio-inoculant) after one month of composting verify Awasthi *et al*. (2014) claim that A. niger had the capacity to rapidly degrade lignocellulosic waste by increasing the temperature of the heap and its ability to function at a wide temperature range. Compost containing more than 40% TOC and 20% C/N, respectively, suggested incomplete composting (Raut *et al*. 2008). After one month, a control heap with a TOC of 36.5 percent and a C/N ratio of 21.4 generated immature and incomplete compost. A significant amount of CO_2 was released during the thermophilic process, resulting in the rapid decomposition of easily degradable organic carbon in the presence of vigorous microbial activity. Certain fungi and actinomycetes degraded complex organic molecules such as lignin and lignocelluloses, releasing CO_2 and contributing to the reduction of TOC. Gou *et al*. (2017) reported that psychrotrophic microbes decreased the TOC ratio of dairy manure from 42.9 to 29.8 percent. Similarly, after inoculation, the increased involvement of other microbes results in TOC degradation.

Nutrient Content Variation (NPK): The value addition parameters TN, TP, and TK increased in both procedures, but the increase was significantly greater in T2 (PM + Bio-inoculant), with TN (10.5 percent), TP (9.2 percent), and TK (41%) rising significantly more than in T1 (PM) (fig 8). In terms of nutrient value, the NPK quality of the compost is important. *A. niger* inoculation resulted in increased TN, TP, and TK in T2 (PM + Bio-inoculant) compared to the control heap due to the rapid decomposition and concentration effect (Wei *et al*. 2015). Awasthi *et al*. (2014) reported similar findings, stating that because the organic carbon in the compost mineralized into CO_2, the carbon lost at a faster rate than TP, TN, and TK, resulting in the concentration effect. On the other hand, nitrogen-fixing bacteria increased TN. (Nakasaki *et al*. 2005).

Germination index: The GI is used to determine a compost material's phytotoxicity (Young *et al*. 2016). Unstable and immature compost products have a detrimental effect on seed germination and the plant soil environment by releasing phytotoxic compounds. At the end of composting, the GI for T1 (PM) and T2 (PM + Bio-inoculant) was 75.5+2.1 percent and 96.5+2.2 percent, respectively. A GI value of 50% indicates phytotoxic-free compost (Zucconi *et al*. 1981), and a GI value of more than 80% indicates mature compost (Wei *et al*. 2000).

Conclusions: The present study determine the potential of indigenously isolated *A. niger* PM-4 strain for the efficient conversion of PM into biofertilizer by releasing hydrolytic enzymes cellulase, xylanase, α-amylase and pectinase at a varied range of temperature and pH. The *A. niger* PM-4 proved its efficacy by inhibiting the aflatoxins generation, shortening the maturity duration for the degradation of substrate, improved nutrient contents (TKN, TP, and TK) and GI compared to non-inoculated substrate. Adding value and reducing decomposition time, the strain is presented as accelerated composting agent with diversified metabolic capacity.

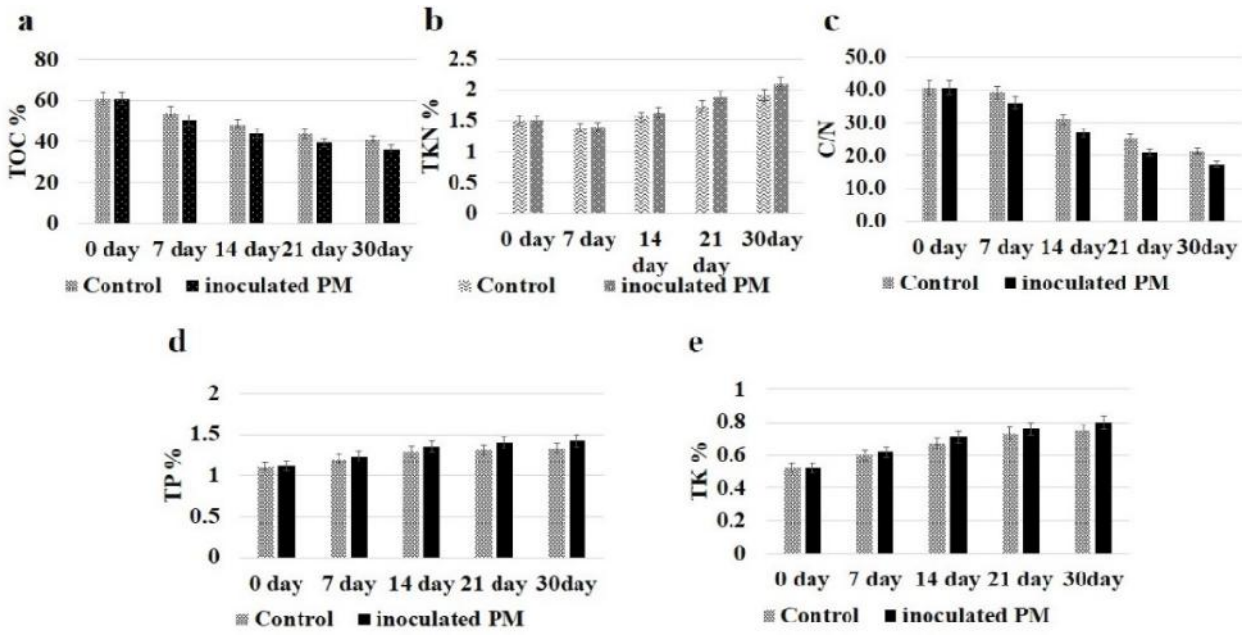

Fig 8: change in physicochemical parameters during composting (a) Change in TOC%; (b) Change in TKN%; (c) Change in C/N (d) Change in TP%; (e) Change in TK%, T1 (control) Press mud alone, T2(PM+*A. niger*)- Press mud inoculated with *A. niger*. Results are the mean of three replicates, and error bars indicate standard deviation.

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