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Comparative Studies of the Physicochemical Properties of 3-Mercaptopyruvate Sulfurtransferase (3-MST) from *Pennisetum glaucum* (Millet) and *Sorghum bicolor* (Guinea corn)

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ABSTRACT

3-Mercaptopyruvate sulfurtransferase (3-MST) is a cyanide detoxifying enzyme that helps in the detoxification of cyanide to a less toxic thiocyanate. 3-MST was purified from the seeds of *Sorghum bicolor* and *Pennisetum glaucum*. The enzyme was purified from the seeds by 80% ammonium sulphate precipitation and ion exchange chromatography on CM-Sephadex C-25. The Enzyme from *Sorghum bicolor* had a specific activity of 0.056 U/mg with a yield of 23.86 % while *Pennisetum gluacum* had specific activity and percentage yield of 0.0445 U/mg and 27.90% respectively. The Optimum pH of the enzyme from *Sorghum bicolor* and *Pennisetum glaucum* was observed at 6.0 and 8.0 respectively, while the optimum temperature was observed at 50 ^{o}C. The results of the inhibition studies revealed that there was no significant effect of the metal ions on the enzyme activity. The study confirmed the presence of 3-MST, a powerful cyanide detoxification mechanism in the seeds of *Sorghum bicolor* and *Pennisetum glaucum.*

Keywords: 3-Mercaptopyruvate sulfurtransferase, Physicochemical Properties, *Pennisetum glaucum, Sorghum bicolor***,** ion exchange chromatography.

INTRODUCTION

Cyanide (compound which contain the –C≡N group) is found in a wide range in the various life forms including photosynthetic bacteria, algae, fungi, plants such as cassava, tapioca, almonds, corn, lima beans etc (Kuyucak and Akcil, 2013, Dubey and Holmes, 1995) and even in the animal kingdom such as beetles, butterflies and grasshoppers (Ogunlabi and Agboola, 2007). Humans are in close contact with cyanide in their daily life through food, drink, and medicines. Cyanides are found in substantial amounts in certain seeds and fruit stones, e.g., those of bitter almonds, apricots, apples, and peaches (Bhandari *et al.*, 2014). Cyanide can exist as a gas (hydrogen cyanide) or as a salt (potassium cyanide) (Culnan *et al.*, 2018). Chemical compounds that can release cyanide are known as cyanogenic compounds. Some substances in foods can also release cyanide, examples are lima beans, almonds etc. In plants, cyanides are usually bound to sugar molecules in the form of cyanogenic glycosides and defend the plant against herbivores (Borron and Bebarta, 2015).

Cyanogenic glycosides are glycosides of α-hydroxy nitriles composed of aglycone and

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sugar parts. The most commonly occurred sugar moiety is D-glucose and is followed by gentiobiose. Most of the cyanogenic glycosides, such as dhurrin, prunasin, linamarin, and lotaustralin have a β-glycosidic linkage between the aglycone and D-glucose, but some other compounds, including amygdalin and linustatin, possess the second sugar moiety attached by β-1, 6 linkages (Hartanti and Cahyani, 2020). 3-mercaptopyruvate sulfurtransferase (3-MST) (EC 2.8.1.2) is an enzyme that catalyzes the chemical reactions of 3-mercaptopyruvate. This enzyme belongs to the family of transferases, specifically the sulfurtransferases (Yadav *et al.*, 2013). The enzyme is of interest because it provides a pathway for detoxification of cyanide, especially since it occurs widely in the cytosol and distributed broadly (Patterson *et al.*, 2016).

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal that ranks fifth after rice, wheat, maize, and barley. In sub-Saharan Africa (SSA), it ranks second in importance after maize (Prajapati *et al.*, 2018). Sorghum comprises the main food source from which over half a billion people in developing countries derive their energy requirements from it (Adebo, 2020).

Millets (*Pennisetum glaucum)* are a group of highly variable small-seeded grasses, widely grown around the world as cereal crops or grains for fodder and human food. Millets are important crops in the semiarid tropics of Asia and Africa. Compared to other widely grown cereal crops like wheat, rice, and sorghum, millet offers higher protein, fat, fiber, and ash content. It is rich in essential amino acids, calcium, iron, zinc, unsaturated fatty acids, and linoleic acid, making it highly beneficial for human consumption (Chove and Mamiro, 2010).

In view of the above, this study was aimed at comparative study of the Physicochemical Properties of 3-Mercaptopyruvate Sulfurtransferase (3-MST) from *Pennisetum glaucum* (Millet) and *Sorghum bicolor* (Guinea corn).

MATERIALS AND METHODS

Materials and Sample Collection

Disodium hydrogen phosphate, ammonium sulfate, potassium cyanide, magnesium chloride, sodium thiosulfate were purchased from British Drug House (BDH) Chemicals Limited, Poole, England. Guinea corn (*Sorghum bicolor*) and millet grains (*Pennisetum glaucum*) were purchased at Sabo market in Ile-Ife, Osun State, Nigeria and were authenticated at the Botany Department of the Obafemi Awolowo University, Ile-Ife, Nigeria.

Homogenization and Enzyme Extraction

50 g of the blended *Sorghum bicolor* and *Pennisetum glaucum* grains was homogenized with 200 ml of 0.1 M phosphate buffer (pH 7.4). It was then sieved with a cheese cloth and refrigerated overnight. The homogenate was centrifuged at 3000 rpm for 10 minutes. The sediment was discarded and the supernatant was collected and stored in a sample bottle in the refrigerator at 4 ^{o}C. This served as the crude enzyme (Agboola and Okonji, 2004).

Ammonium Sulphate Precipitation

The crude enzyme obtained from homogenization was brought to 80 % Ammonium saturation by the gradual addition of ammonium Sulphate with continuous stirring with a magnetic stirrer, after which it was kept in the refrigerator at 4 ^{o}C overnight. The precipitated enzyme was dissolved in 0.1 M Phosphate Buffer (pH 7.2) and the enzyme was dialyzed against the same buffer (Agboola and Okonji, 2004).

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Enzyme Assay

3-MST activity was measured according to the method of Lee *et al.* (1995) as described by Agboola and Okonji (2004), on the principle of the colorimetric determination of thiocyanate formation. The reaction mixture consisted of 0.25 ml of 0.1 M Tris buffer, 0.1 ml of 0.5 M KCN, 0.1 ml of 0.3 M mercaptoethanol, and 1 ml of the enzyme solution. The mixture was then incubated for 15 minutes at room temperature and the reaction was terminated by adding 0.25 ml of 15% formaldehyde, followed by the addition of 0.75 ml of Sorbo reagent. The absorbance was then read at 460 nm. One unit of 3-MST is defined as the amount of enzyme that will convert one micromole of cyanide to thiocynate in one minute under specified conditions.

Protein concentration determination

Protein concentration was determined by the method of Bradford (1976), using Bovine Serum Albumin (BSA) as standard. The reaction mixture consisted of 0.1 ml of the enzyme solution and 1 ml of Bradford reagent. The absorbance was then read at 595 nm.

Ion-Exchange Chromatography on CM-Sephadex C-25

CM-Sephadex C-25 cation exchanger was pretreated by boiling 11 g of the resin in 200 ml of distilled water for 1 hour. This was followed by the addition of 100 ml of 0.1 M HCl for 30 mins, after which the acid was decanted and the resin was washed with distilled water several times to ensure that the acid is totally removed. Thereafter, 100 ml of 0.1 M NaOH was added to the resin, which was decanted after 30 mins, followed by thorough rinsing of the resin with distilled water to remove all traces of the base (Chukwuejim et al., 2019). The resin was then equilibrated with 0.1 M phosphate buffer (pH 7.2) before it was packed into a 2.5 x 40 cm column. Afterwards, it was equilibrated with several changes of 0.1 M Tris-HCl buffer, pH 7.2. 10 ml of the enzyme solution from the preceding step was then applied on the column. Fractions of 1 ml were collected from the column at the rate of 25 ml per hour. The active fractions from the column were assayed for 3-MST activity and protein concentration.

Determination of Kinetic Parameters

The kinetic parameters (K_m and V_{max}) of the enzyme were determined using the Lineweaver-Burk plot by varying the concentration of KCN between 10 µl to 100 µl at a fixed concentration of 100 mM mercaptoethanol. Also, the concentration of mercaptoethanol was varied between 10 mM and 100 mM at a fixed concentration of 100 mM KCN. The kinetic parameters were determined from the plots of the reciprocal of initial reaction velocity (1/V) versus reciprocal of the varied substrates (1/[S]) at each fixed concentrations of the other substrate.

Effect of pH on enzyme activity

The effect of pH on enzyme activity was performed according to the methods described by Agboola and Okonji (2004). The enzyme was assayed using different buffers of different pH: 50 mM Citrate buffer (pH 3-5), 50 mM Phosphate buffer (pH 6-7), 50 mM Tris-HCl buffer pH 8 and 50 mM Borate buffer (pH 9-10). The 3-MST activity was assayed as described in the assay section with the assay buffer being replaced by these different buffers with different pH.

Effect of temperature on enzyme activity

The effect of temperature on the enzyme activity was studied between 30 ^{o}C and 80 ^{o}C. The assay mixture was first incubated at the indicated temperature for 15 minutes before initiating the reaction by addition of an aliquot of the enzyme which had been

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equilibrated at the same temperature. 3-MST Activity was assayed routinely as previously described.

Effect of urea and EDTA on enzyme activity

The effect of Urea and EDTA on the enzyme activity was determined at the concentrations of 0.5 mM, 1.0 mM, 2.5 mM, and 5.0 mM. Absorbance was read at 460 nm.

Effect of metal ions on enzyme activity

The effect of various metallic ions on the enzyme activity was determined following the procedure described by Lee *et* al (1995). The different metallic ions used were $HgCl_2$, NaCl, $ZnCl_2$, $SnCl_2$ and $MnCl_2$ at concentrations of 1 mM, 5 mM and 10 mM. The reaction mixture without the metals served as the control with 100% activity.

RESULTS AND DISCUSSION

In this research work, 3-mercaptopyruvate sulfurtransferase was isolated and purified from the grains of *Sorghum bicolor* and *Pennisetum glaucum* using 80 % ammonium sulphate precipitation and ion exchange chromatography on CM-Sephadex C-25. The results showed *Sorghum bicolor* had a specific activity of 0.056 U/mg with a purification fold and percentage yield of 2.45 and 23.86 % respectively. *Pennisetum glaucum* grains had a specific activity of 0.045 U/mg with a purification fold of 1.84 and percentage yield of 27.90% (Figure 1 and 2, Table 1 and 2). Specific activities of 3-MST from different sources have been reported. Agboola et al (2006) reported that the activity of 3MST from liver of chicken, duck and pigeon are 0.21 U/mg/ml, 0.09 U/mg/ml and 0.19 U/mg/ml. Fagbohunka et al. (2004) obtained a specific activity of 0.19 µ/mg/ml from the gut of Tilapia mariae fish and 0.29 µ/mg/ml from the gut of Tilapia zilli fish.

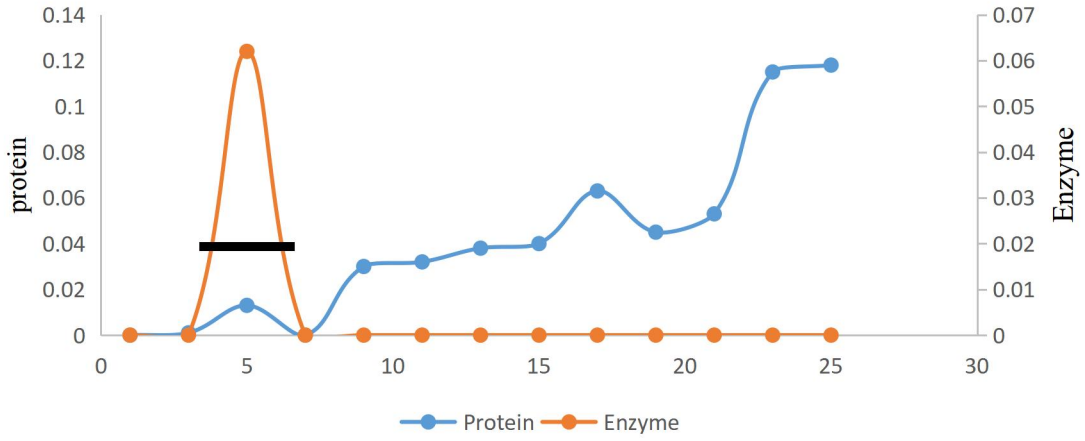

Figure 1: Elution profile of 3-MST from *Sorghum bicolor* on ion exchange chromatography CM-Sephadex C-25.

A total volume of 10 ml of the dialysate was applied to the column. The column was washed with 0.1 M phosphate buffer pH 7.2. Fractions of 2 ml were collected at a flow rate of 42 ml/hr from the column.

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Pooled fraction
Protein concentration at 595 nm
Enzyme activity at 460 nm

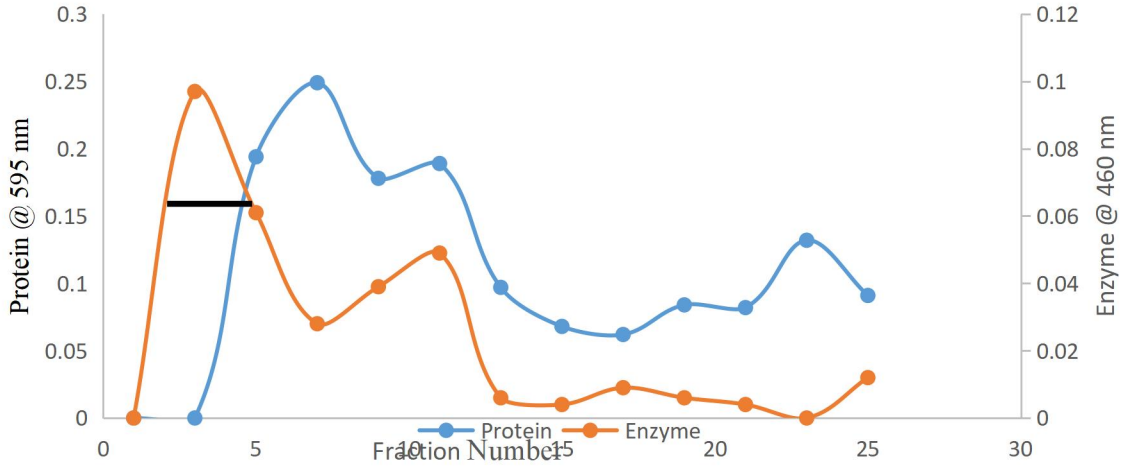

Figure 2: Elution profile of 3-MST from *Pennisetum glaucum* on Ion exchange chromatography on CM-Sephadex C-25**.**

A total of 10 ml of the dialysate sample was applied to the column. The column was washed with 0.1 M Phosphate buffer pH 7.4. Fractions of 2 ml were collected at a flow rate of 42 ml/hr from the column.

Pooled fraction
Protein concentration at 595 nm
Enzyme activity at 460nm

Table 1: Summary of the purification of 3-mercaptopyruvate sulphurtransferase from *Sorghum bicolor*

	Total protein (mg)	Total Activity (U)	Specific activity (U/mg)	Purification fold	Percentage yield (%)
Crude Extract	219.96	5.059	0.023	1	100
Ammonium sulphate precipitation	43.4	1.389	0.032	1.39	27.45
Ion exchange chromatography	21.5	1.207	0.056	2.45	23.86

Table 2: Summary of the purification of 3-mercaptopyruvate sulphurtransferase from *Pennisetum glaucum*

	Total protein (mg)	Total Activity (U)	Specific activity (U/mg)	Purification fold	Percentage yield (%)
Crude Extract	184.8	4.48	0.02424	1	100
Ammonium sulphate precipitation	44.8	1.57	0.03504	1.45	35.04
Ion exchange chromatography	28.09	1.25	0.0445	1.84	27.9

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K_m is equivalent to the substrate concentration at which the reaction rate is half maximal and is often used as an indicator of the affinity of an enzyme for its substrate (Raybuck, 1992). A high K_m indicates weak binding that is low affinity of the enzyme for the substrate while a low Km indicates strong binding that is high affinity of the enzyme for the substrate (Nelson and Cox, 2004). The Km and Vmax values of 3-MST from *Pennisetum glaucum* as determined by the lineaweaver-buck plot for KCN and mercaptoethanol were 0.143 mM and 0.059 µmol/ml/min and 0.25 mM and 0.138 µmol/ml/min respectively. The Km and Vmax values for *Sorghum bicolor* as determined by the lineweaver-burk plot for mercaptoethanol and KCN were estimated to be 0.053 mM and 0.066 µmol/ml/min and 0.25 mM and 0.0385 µmol/ml/min respectively. The K_m and V_{max} of 3-MST in some plant tubers for sodium thiosulphate and potassium cyanide were 16.67 mM and 20 mM and 0.2 µmol/ml/min and 0.24 µmol/ml/min respectively (Ehigie *et al*., 2013).(Table 3 and 4, Figures 3-6).

Table 3: Kinetic parameters of 3-MST from *Sorghum bicolor*

	KCN	Mercaptoethanol
K_m (mM)	0.025	0.053
V_{max} (µmol/ml/min)	0.0385	0.066

Table 4: Kinetic parameters of 3-MST from *Pennisetum glaucum*

	KCN	Mercaptoethanol
K_m (mM)	0.143	0.25
V_{max} (µmol/ml/min)	0.059	0.138

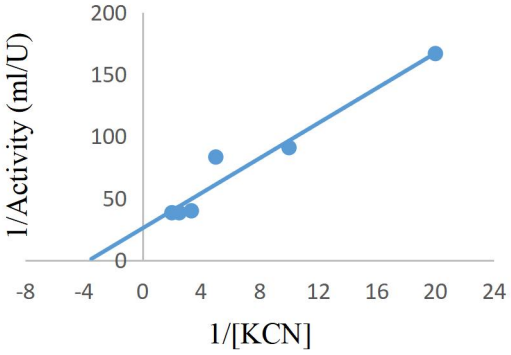

Figure 3: Lineweaver-Burk plot of 3-MST from *Sorghum bicolor* at varying concentrations of KCN (Lineweaver and Burk, 1934).

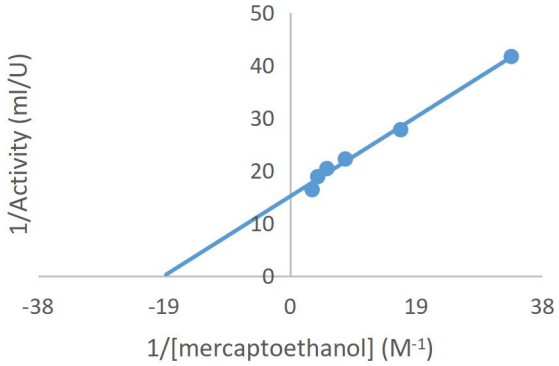

Figure 4: Lineweaver-Burk Plot of 3-MST from Sorghum bicolor at varying concentrations of mercaptoethanol (Lineweaver and Burk, 1934).

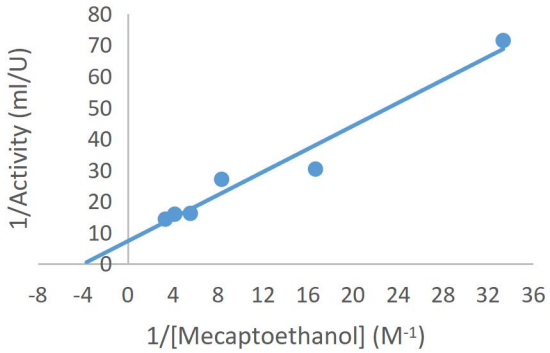

Figure 5: Lineweaver-Burk Plot of 3-MST from *Pennisetum glaucum* at varying concentration of mercaptoethanol.

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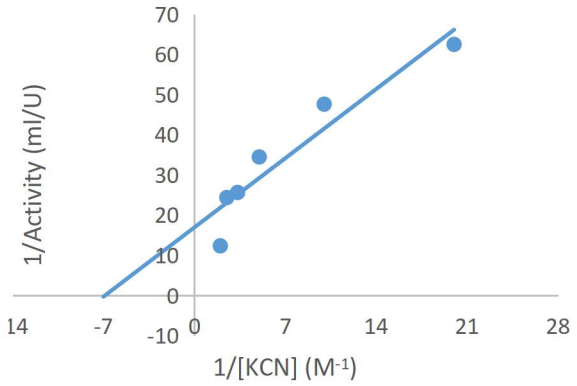

Figure 6: Lineweaver-Burk plot of 3-MST from *Pennisetum glaucum* at varying concentration of KCN

The activity of 3-MST from the seeds of *Sorghum bicolor* was found to be optimum at pH 6.0, while that of 3-MST from *Pennisetum glaucaum* was found to be optimum at pH 8.0. The result is shown in Figure 7 and 8. There was however a decrease in enzyme function with increasing alkalinity. Different pH values have been reported for the enzyme from different species of organism. Williams et al (2003) reported pH values of 6.9-7.6 for *L. major* and *L.* Human 3-MST has optimum pH of 8.2 (Yadav *et al*, 2013).

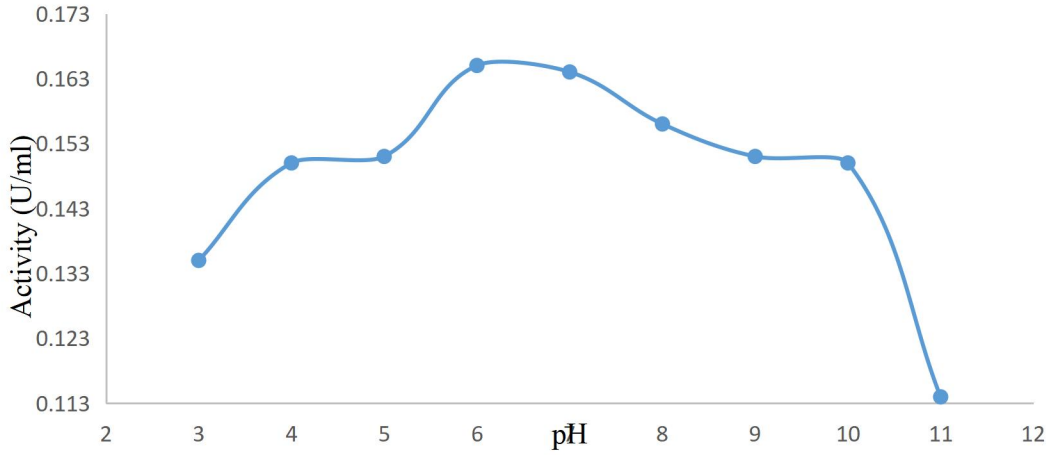

Figure 7: Effect of pH on 3-MST from *Sorghum bicolor*

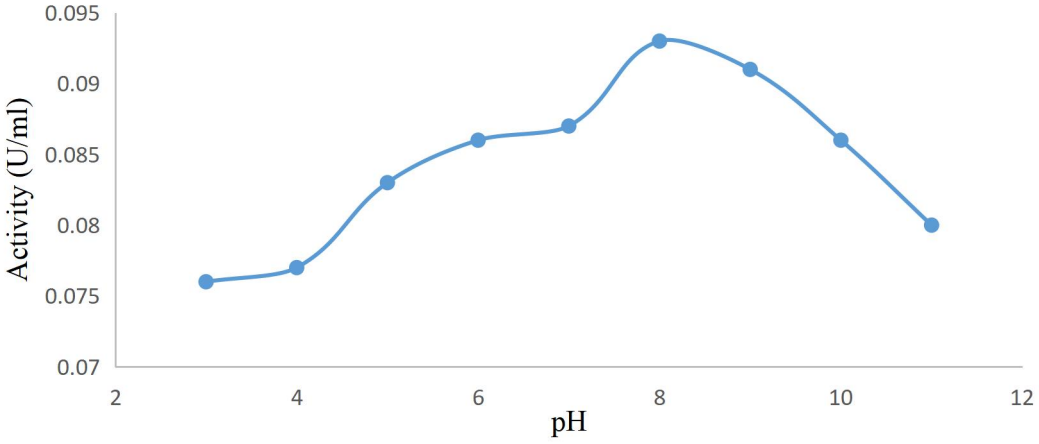

Figure 8: Effect of pH on 3-MST from *Pennisetum glaucum*

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An optimum temperature of 50ºC was obtained for 3-MST from *Sorghum bicolor* and *Pennisetum glaucum*. (Figures 9 and 10). This result is in agreement with optimum temperature obtained from different sources (Yadav et al., 2013). MST from *Limicolaria flammea* have an optimum temperature of 60 ºC (Ehigie *et al.,* 2020). The optimum temperature reported for 3-MST from intestine of cane rat (*T. swinderianus*) was 40°C (Sanni *et al.,* 2020).

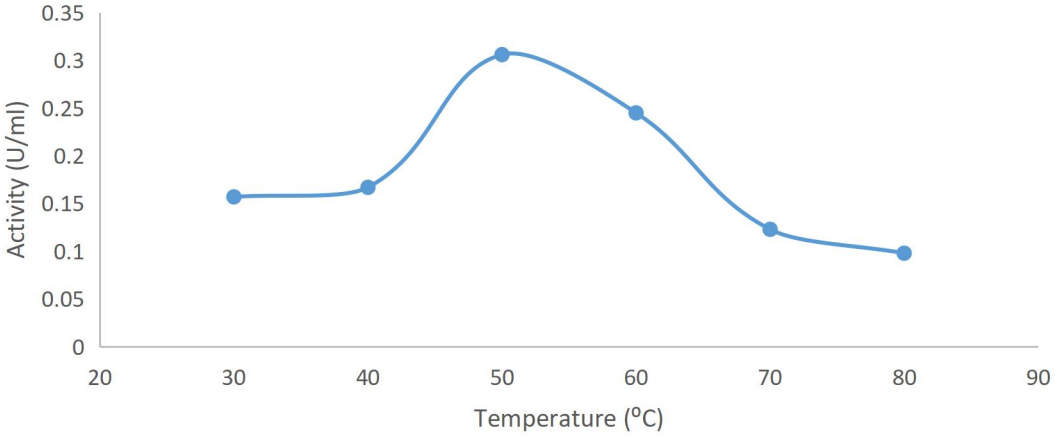

Figure 9: Effect of temperature on 3-MST from *Sorghum bicolor*

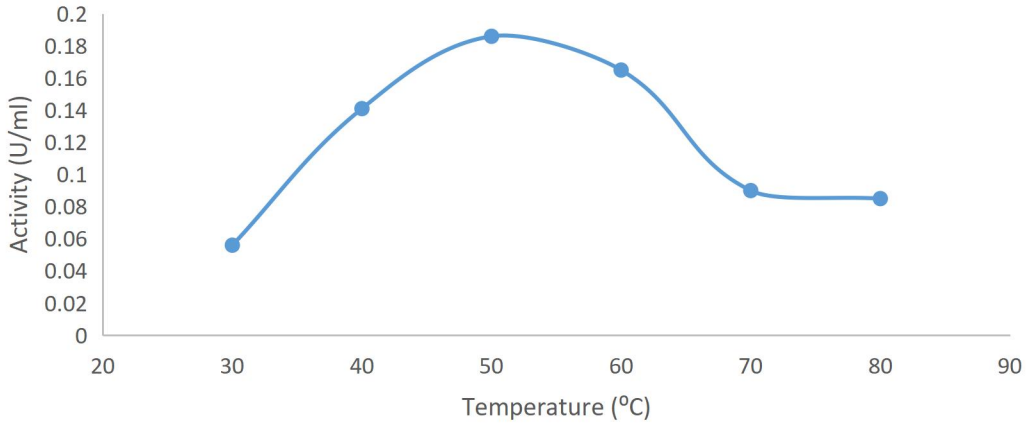

Figure 10: Effect of temperature on 3-MST from *Pennisetum glaucum*

The effect of the divalent metal ions revealed that most of the ions had no significant effect on the enzyme activity. Metals salts such as NaCl, HgCl, $MnCl_2$, and ZnCl showed no significant effect at 1 mM, 5 mM, and 10 mM concentration (Table 5 and 6). The exposure of these plants to the metal ions could be the reason why the enzyme is effective in the presence of these metal ions. Urea and EDTA showed inhibitory properties against the enzyme which may be blocking the active site.

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Table 5: Effect of Metal ions on 3-MST *Sorghum bicolor*

	NaCl	$MnCl_2$	$HgCl_2$	ZnCl	$SnCl_5$
1 Mm	85.45	84.45	71.82	58.18	76.64
5 mM	92.72	87.27	57.27	70.00	80.00
10 mM	90.91	93.64	77.27	72.73	80.00

Table 6: Effect of Metal ions on 3-MST *Pennisetum glaucum*

	NaCl	$MnCl_2$	$HgCl_2$	ZnCl	$SnCl_5$
1 Mm	66.13	66.94	54.03	72.18	64.52
5 mM	71.37	72.98	56.45	63.71	93.15
10 mM	66.53	71.77	62.90	64.92	68.55

Table 7: Effect of other compound on 3-MST *Sorghum bicolor*

	0.5 mM	1.0 mM	2.5 mM	5.0 mM
Urea	62.90	58.06	63.71	70.97
EDTA	64.11	58.06	54.44	61.29

Table 8: Effect of other compound on 3-MST *Pennisetum glaucum*

	0.5 mM	1.0 mM	2.5 mM	5.0 mM
Urea	85.45	80.91	66.36	60.91
EDTA	70.91	88.18	52.73	113.64

CONCLUSION

This study established the physicochemical and kinetic properties of *Sorghum bicolor* and *Pennisetum glaucum* 3-MST. The biochemical parameters obtained confirmed that the enzyme 3-MST is present in the plants studied and indicates the presence of a powerful detoxification mechanism of cyanide as it functions in the conversion of cyanide to thiocyanate, thereby improving the survival of the animal feeding on plants containing cyanide.

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