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### **Original Research Article**

# Assessment of transaminase enzymes and effect of potassium iodide on its production in the yeast form of *Sporothrix schenckii*

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#### ABSTRACT

**Background:** The dimorphic fungus *Sporothrix schenckii* (*S. schenckii*) is the cause of sporotrichosis. Many fungi have transaminases, also known as aminotransferases, which are portions of proteins. However, nothing is known about this enzyme in *S. schenckii*.

**Aims:** The current study shows how potassium iodide (KI) affects the transaminases enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) produced by *S. schenckii's* yeast form in vitro. **Materials and Methods:** YNB (yeast nitrogen base) medium was used to create a master culture of *S. schenckii*, which was then incubated at 37°C (yeast). The amount of KI added to the YNB medium increased. Each container received one millilitre (mL) of the master culture suspension, which was then incubated at 37°C for different lengths of time – the early-log phase on day six, the mid-log period on day twelve, and the growth peak on day eighteen, respectively. A 5% homogenate was produced after centrifugation and used in the transaminases enzyme assay.

**Results:** On days 6, 12, and 18, the control specimen's mean aspartate aminotransferase level was 10.11  $\pm$  3.09, 10.36  $\pm$  2.33, and 17.62  $\pm$  4.27 IU, respectively. On days 6, 12, and 18, the test specimen's mean aspartate aminotransferase level ranged from 9.80  $\pm$  2.42 (KI 0.4 gramme %) to 19.59  $\pm$  3.9 IU (KI 0.2 gramme %), from 4.52  $\pm$  2.28 (KI 0.4 gramme %) to 28.46  $\pm$  4.88 IU (KI 0.2 gramme %), and from 4.50  $\pm$  1.02 (KI 0.8 gramme %) to 14.49  $\pm$  3.60 IU (KI 0.05 gramme %). On days 6, 12, and 18, the control specimen's mean alanine aminotransferase level was 10.70  $\pm$  3.82, 29.60  $\pm$  3.02, and 19.74  $\pm$  4.62 IU, respectively. Day 6, 12, and 18 saw variations in the test specimen's mean alanine aminotransferase level, which ranged from 11.40  $\pm$  3.04 (KI 0.1 gramme %) to 18.52  $\pm$  3.97 IU (KI 0.2 gramme %) to 12.54  $\pm$  1.92 IU.

**Conclusions:** The transaminase enzymes' low activity indicates that KI has an inhibiting effect on the growth of *S. schenckii* (yeast), which has caused a drop in the enzymes' activity.

The impact of KI on the lipids of *S. schenckii* may be monitored to comprehend the mechanism of action of KI in the future.

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#### 1. Introduction

Sporothrix schenckii is the fungus that causes sporotrichosis, or "rose handler's illness," and it can

infect both humans and animals."<sup>1,2</sup> It is found in nature all over the world, growing as a mould when dead or senescent plant matter is combined with it. Mould cells that enter a vulnerable host transform into budding yeast cells and remain in the infected tissue as such.<sup>3,4</sup> *S. schenckii* is

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a temperature-dependently dimorphic fungus because of the temperature-dependent transition between the hyphal and yeast forms.<sup>5</sup> Numerous clinical presentations of sporotrichosis, including lymphocutaneous, localised cutaneous, disseminated, mucosal, skeletal, and visceral, have been identified. Several fungi include transaminases, also known as aminotransferases, which are a component of protein.<sup>6</sup> Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), transaminases that cause sporotrichosis, have not been studied in relation to S. schenckii. Since the early 20<sup>th</sup> century, potassium iodide (KI) has been used traditionally to treat sporotrichosis with excellent results; nevertheless, the precise mechanism of action is still unknown.<sup>7-10</sup> The transaminases enzyme activity in a number of fungal isolates has been investigated by certain researchers.<sup>11,12</sup> They didn't look at the transaminase activity in S. schenckii or the impact of different concentrations of KI on it. Thus, the goal of the current investigation was to quantify the transaminases enzymes in S. schenckii yeast form and investigate the impact of KI at varying concentrations on the in vitro synthesis of these enzymes.

#### 2. Materials and Methods

This was an experimental investigation carried out in a tertiary care hospital's microbiology department. Although this study was approved by the institutional ethical committee, neither human nor animal subjects were used in it. An ATCC 14284 / MTCC 1359 standard strain of S. schenckii was obtained from the Institute of Microbial Technology located in Chandigarh, India. By subculturing mycelia or conidia on enriched culture medium, blood agar, the standard strain of Sporothrix schenckii (ATCC 14284) was able to shift from mould to yeast form.  $^{13-16}$  S. schenckii was sub-cultured from the slope of Sabouraud's dextrose agar (SDA) in 50 mL of YNB (Yeast Nitrogen Base, HiMedia, Mumbai) medium, which was placed in a screw-capped bottle and incubated at 37°C to create a master culture. After injecting the right amount of YNB medium on day seven, the growth of S. schenckii in the bottle was brought to 90% transmission at 540 nm, following the instructions provided by Bareja et al.<sup>6</sup> The resulting master culture was used for further study. The YNB medium was produced and then added to 150 screw-capped bottles with a 160 mL capacity in 50 mL aliquots. At progressively higher concentrations, KI was added to the YNB medium until the final concentrations of the medium were 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 grammes per cent. As a control, one bottle of YNB without KI was used. Fifty of the 150 bottles were utilised on each of the following three days: the sixth (early-log phase), the twelfth (mid-log period), and the eighteenth (peak of growth). Out of 50 bottles, 5 served as a control (no KI), while the remaining bottles were utilised to test different KI concentrations. For every KI concentration,

five bottles were used in an effort to lower error. Each bottle was inoculated with one millilitre of master culture suspension, which was then incubated at 37 degrees Celsius for varying durations. On the sixth, twelfth, and eighteenth days, respectively, the contents of the corresponding bottle were centrifuged at 6000 rpm for thirty minutes. After obtaining the deposit, it was centrifuged once more for 30 minutes at 6000 rpm in 5.0 mL of citrate buffer saline (0.15 mol/L sodium chloride, 0.015 mol/L sodium citrate, pH 7). To guarantee adequate washing, the procedure was carried out twice. This resulted in a deposit that was taken, dried in filter paper folds, weighed, and then finely pulverised in a tissue homogenizer. For the ensuing enzyme assay, a 5% homogenate was made from each weighted tissue in ice-cold distilled water. The Reitman and Frankel method was used to determine the transaminases enzymes, aspartate aminotransferase and alanine aminotransferase.<sup>17</sup> The following formula was used to estimate the enzymes AST and ALT in milligrammes weight of homogenate .:

Calculation

$$\begin{array}{rcl} \text{AST} & / & \text{ALT} & (\text{I.U} & / & \text{L}) &= & \frac{T-C}{S} & \times & 16.1 & \times \\ \frac{Vol. \, of \, homogenate}{0.2 \, ml} & \times & \frac{1}{Wt. \, of \, tissue} \end{array}$$

#### 2.1. Statistical analysis

SPSS (Statistical Package for Social Sciences) Version 15.0 statistical analysis software was used to do the statistical analysis. To examine the variances within and across research groups, the ANOVA (Analysis of Variance) test was employed. Every experimental mean was compared to the control mean using Dunnett's "t"-Test.

#### 3. Results

The enzymes aspartate aminotransferase and alanine aminotransferase were determined for three different days,  $6^{th}$ day (early-log period),  $12^{th}$  day (mid-log period) and  $18^{th}$  day (peak of growth) respectively.

# 3.1. Aspartate aminotransferase (AST) in the tissue S. schenckii (Yeast phase)

On day 6, a random zigzag trend of aspartate aminotransferase levels was observed with increasing concentration. The mean value was 10.11 IU at blank, 19.58 IU at KI 0.2 and 17.49 IU at KI 0.8 gram % concentrations (Table 1). On day 12, peak mean aspartate aminotransferase levels were observed at KI 0.2 gram % (28.46 IU) and minimum at 0.4 gram % (4.52 IU) concentration. With the limited data points, the trend was irregular and haphazard (Table 2). On day 18, a decreasing trend of aspartate aminotransferase levels was observed with increasing concentration. Mean value was 17.62 IU at blank which reached to 4.50 IU at concentration KI 0.8 gram % (Table 3).

On day 6, mean aspartate aminotransferaselevel of control specimen was  $10.11 \pm 3.09$  IU. The mean AST levels of test specimen were ranged from 9.80  $\pm$  2.42 (KI 0.4 gram %) to 19.59± 3.9 IU (KI 0.2 gram %). On day 12, mean aspartate aminotransferase level of control specimen was  $10.36 \pm 2.33$  IU. For test specimen mean levels were ranged from  $4.52 \pm 2.28$  (KI 0.4 gram %) to  $28.46 \pm 4.88$  IU (KI 0.2 gram %). On day 18, mean aspartate aminotransferase level of control specimen was 17.62 ± 4.27 IU. For test specimen, mean values were ranged from  $4.50 \pm 1.02$  (KI 0.8 gram %) to  $14.49 \pm 3.60$  IU (KI 0.05 gram %) (Table 4). By comparing all the three days, in general there was decrease in the activity of enzyme with increase in duration of incubation  $6^{th}$  to  $18^{th}$  day (Table 5, Figure 1). No deposit obtained at the concentration KI1.6, 3.2, 6.4 and 12.8 gram%.

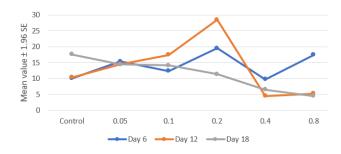


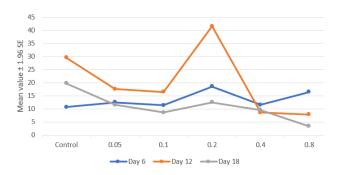
Figure 1: Expression of mean aspartate aminotransferase (AST) in *S.schenckii* (yeast) atdifferent time intervals

# 3.2. Alanine aminotransferase (ALT) in the tissue S. schenckii (yeast phase)

On day 6, with increasing concentration, a random trend of mean alanine aminotransferase levels was observed with peak value at KI 0.2 gram % concentration and minimum value at blank (Table 6). On day 12, with increasing concentration, a decreasing trend of mean alanine aminotransferase levels was observed except KI 0.2 gram % concentration (41.56 IU). The mean alanine aminotransferase value peaked at KI 0.2 gram % and was found to be minimum at KI 0.8 gram % concentration (Table 7). On day 18, with increasing concentration, the mean values of ALT were showed a declining trend except KI 0.2 gram %. The mean value was maximum at blank and minimum at KI 0.8 gram % concentration (Table 8).

On day 6, mean alanine aminotransferase level of control specimen was  $10.70 \pm 3.82$  IU. For test specimen the mean alanine aminotransferase levels were ranged from  $11.40 \pm 3.04$  (KI 0.1 gram %) to  $18.52 \pm 3.97$  IU (KI 0.2 gram %). On day 12, mean alanine aminotransferase level of control specimen was  $29.60 \pm 3.02$  IU. For test specimen mean alanine aminotransferase levels were ranged from  $7.82 \pm 1.50$  (KI 0.8 gram %) to  $41.56 \pm 4.56$  IU (KI 0.2 gram %).

On day 18, mean alanine aminotransferase level of control specimen was 19.74  $\pm$  4.62 IU. For test specimens, mean alanine aminotransferase values were ranged from 3.33  $\pm$  0.70 (KI 0.8 gram %) to 12.54  $\pm$  1.92 IU (KI 0.2 gram %) (Table 9). By comparing all the three days, in general there was an increase in the activity of enzyme alanine aminotransferasewith increase in duration of incubation 6<sup>th</sup> to 12<sup>th</sup> day in control and test concentrations except KI 0.4 and 0.8 gram % followed by a decrease in the mean value of control as well as in test concentrations (Table 10, Figure 2). No deposit obtained at the concentration KI1.6, 3.2, 6.4 and 12.8 gram%.



**Figure 2:** Expression of mean alanine aminotransferase (ALT) in *S.schenckii* (yeast) at different time intervals

#### 4. Discussion

An amino group is moved from an amino acid to an alpha-ketoacid during transamination. Amination of ketoacids produced by the metabolism of fat, protein, and carbohydrates produces amino acids in tissues.<sup>11,12</sup> Few research have been conducted to ascertain the aspartate aminotransferase and glutamate dehydrogenase enzyme activities in different fungal isolates (Fusarium solani, Pisolithus tinctorius, oxysporum, Fusarium Hebeloma westraliense, Laccarialaccata, and Scleroderma *verrucosum*) by electrophoresis.<sup>18,19</sup>They investigated the transaminase enzyme's activity in several fungi. They didn't look at the transaminase activity in S. schenckii or the impact of different concentrations of KI on it. This investigation looked at the quantification of the transaminases enzyme and the impact of KI at varying concentrations on the yeast phase of the dimorphic fungus S. schenckii that is present in the host tissue. On days 6, 12, and 18 of incubation for yeast forms, which included early log, mid log, and exponential phases of growth, the activity of the transaminases enzymes AST and ALT was measured.

#### 4.1. Aspartate aminotransferase (AST)

In the yeast phase the control specimen's mean aspartate aminotransferase level was  $10.11 \pm 3.09$  IU on day 6. The

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Conc. of KI (gram %)	Ι	П	III	IV	$\mathbf{V}$	Mean (IU)
Control /Blank	8.24	6.38	9.46	13.82	12.66	10.11
0.05	15.46	18.28	14.64	19.22	9.42	15.40
0.1	12.24	15.68	17.66	9.22	7.26	12.41
0.2	19.46	25.82	20.34	15.82	16.5	19.58
0.4	9.24	7.82	7.82	10.48	13.64	9.80
0.8	17.3	13.64	16.58	20.42	19.54	17.49

Table 1: Expression of AST in S. schenckii (yeast) on 6<sup>th</sup> day

### Table 2: Expression of AST in S. schenckii (yeast) on 12<sup>th</sup> day

Ι	II	III	IV	V	Mean(IU)
10.24	13.46	8.48	11.82	7.82	10.36
14.28	12.76	16.82	19.28	9.68	14.56
17.46	19.76	14.2	20.5	15.28	17.44
28.3	30.64	22.46	35.28	25.64	28.46
4.56	2.34	2.34	5.68	7.68	4.52
5.24	3.46	4.36	4.36	9.2	5.32
	14.28 17.46 28.3 4.56	10.24         13.46           14.28         12.76           17.46         19.76           28.3         30.64           4.56         2.34	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

Table 3: Expression of AST in S. schenckii (yeast) on 18<sup>th</sup> day

Conc. of KI (gram %)	Ι	II	III	IV	V	Mean(IU)
Control /Blank	17.64	15.34	20.78	22.54	11.82	17.62
0.05	12.64	19.26	16.46	14.28	9.82	14.49
0.1	14.24	10.76	13.48	17.62	14.82	14.18
0.2	11.28	14.62	8.42	9.44	13.26	11.40
0.4	6.24	4.46	8.72	5.62	7.62	6.53
0.8	4.44	2.82	5.32	5.32	4.62	4.50

Table 4: Expression of mean AST in *S. schenckii* (yeast) at different time intervals (n=5 for each concentration)

Conc. of KI	Γ	Day 6		]	Day 12			Day 18	
(gram %)	Mean(IU)	SD	''p''	Mean (IU)	SD	''p''	Mean (IU)	SD	"p"
Control	10.11	3.09		10.36	2.33		17.62	4.27	
0.05	15.40	3.85	0.089	14.56	3.69	0.169	14.49	3.60	0.301
0.1	12.41	4.33	0.741	17.44	2.73	0.008	14.18	2.47	0.225
0.2	19.59	3.97	0.001	28.46	4.88	< 0.001	11.40	2.58	0.008
0.4	9.80	2.42	1.000	4.52	2.28	0.031	6.53	1.67	< 0.001
0.8	17.50	2.67	0.011	5.32	2.26	0.074	4.50	1.02	< 0.001

Significance of difference as compared to control (Dunnett's t-test has been used)

Table 5: Comparison of change in mean AST levels in S. schenckii (yeast) at different concentrations

Conc. of KI	Day 6	to Day 12		Day	6 to Day 1	8	Day	12 to Day	18
(gram %)	Mean Change(IU)	SD	''p''	Mean Change (IU)	SD	''p''	Mean Change (IU)	SD	"p"
Control/Blank	0.25	4.53	0.907	7.51	4.78	0.025	7.26	4.39	0.021
0.05	-0.84	2.88	0.550	-0.91	2.86	0.515	-0.07	4.19	0.971
0.1	5.03	5.50	0.110	1.77	6.28	0.562	-3.26	3.44	0.102
0.2	8.88	6.60	0.040	-8.18	3.56	0.007	-17.06	5.22	0.002
0.4	-5.28	0.53	< 0.001	-3.27	2.62	0.050	2.01	2.64	0.163
0.8	-12.17	2.37	< 0.001	-12.99	1.99	< 0.001	-0.82	2.26	0.463

Paired 't'-test used.

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Conc. of KI (gram %)	Ι	II	III	IV	$\mathbf{V}$	Mean(IU)
Control/Blank	10.12	13.24	7.56	15.82	6.76	10.70
0.05	9.82	14.24	12.24	14.24	11.76	12.46
0.1	11.26	16.38	11.26	9.84	8.28	11.40
0.2	23.44	18.68	21.24	13.84	15.42	18.52
0.4	9.82	14.48	11.76	8.24	13.28	11.51
0.8	18.24	12.46	16.24	19.44	15.82	16.44

**Table 6:** Expression of ALT in S. schenckii (yeast) on  $6^{th}$  day

# Table 7: Expression of ALT in S. schenckii (yeast) on 12<sup>th</sup> day

Conc. of KI (gram%)	Ι	II	III	IV	V	Mean(IU)
Control/Blank	25.46	29.64	33.66	30.82	28.42	29.60
0.05	17.24	18.46	20.82	16.74	14.62	17.57
0.1	16.34	13.62	20.68	18.24	13.62	16.50
0.2	44.68	38.72	41.36	35.82	47.24	41.56
0.4	8.62	5.82	10.24	9.62	8.62	8.58
0.8	7.42	9.82	8.62	5.82	7.42	7.82

## Table 8: Expression of ALT in S. schenckii (yeast) on 18th day

Conc. of KI (gram	Ι	II	III	IV	V	Mean(IU)
%) Control/Blank	22.24	19.82	25.62	17.62	13.42	19.74
0.05	11.24	19.82	10.82	9.64	11.24	19.74
0.1	8.86	7.24	8.86	11.68	6.48	8.62
0.2	12.24	10.82	15.34	13.48	10.82	12.54
0.4	9.24	7.24	10.62	11.82	8.86	9.556
0.8	3.24	2.48	4.44	3.24	3.24	3.32

Table 9: Expression of mean ALT in S. schenckii (yeast) at different time intervals (n=5 for each concentration)

Conc. of KI	Day 6				Day 12			Day 18	
(gram %)	Mean (IU)	SD	"p"	Mean (IU)	SD	"p"	Mean (IU)	SD	''p''
Control	10.70	3.82		29.60	3.02		19.74	4.62	
0.05	12.46	1.86	0.831	17.58	2.28	< 0.001	11.56	1.96	< 0.001
0.1	11.40	3.04	0.996	16.50	3.05	< 0.001	8.62	2.00	< 0.001
0.2	18.52	3.97	0.002	41.56	4.56	< 0.001	12.54	1.92	< 0.001
0.4	11.52	2.53	0.991	8.58	1.69	< 0.001	9.56	1.75	< 0.001
0.8	16.44	2.67	0.028	7.82	1.50	< 0.001	3.33	0.70	< 0.001

Significance of difference as compared to control (Dunnett's t-test has been used)

Table 10: Comparison of change in mean ALT levels in S. schenckii (yeast) at different concentrations

Conc. of KI	Day	6 to Day	12	Day	6 to Day 1	18	Day	12 to Day	18
(gram %)	Mean Change (IU)	SD	"p"	Mean Change (IU)	SD	"p"	Mean Change (IU)	SD	"p"
Control/Blank	18.90	4.84	0.001	9.04	6.22	0.031	-9.86	4.61	0.009
0.05	5.12	2.74	0.014	-0.90	2.33	0.437	-6.02	2.73	0.008
0.1	5.10	4.78	0.076	-2.78	3.97	0.192	-7.88	2.25	0.001
0.2	23.04	4.97	< 0.001	-5.98	4.01	0.029	-29.02	5.51	< 0.001
0.4	-2.93	3.85	0.164	-1.96	4.10	0.345	0.97	0.82	0.058
0.8	-8.62	4.08	0.009	-13.11	2.50	< 0.001	-4.49	1.74	0.004

Paired 't'-test used.

test specimens' average AST levels varied between 9.80  $\pm$  2.42 (KI 0.4 gramme %) and 19.59  $\pm$  3.9 IU (KI 0.2 gramme %). With the exception of KI 0.4 gramme%, the mean aspartate aminotransferase level of test specimens was greater than the mean control level. With the exception of KI 0.2 and 0.8 gramme%, statistical analysis revealed no discernible variation in mean AST levels between the concentrations and the control. The mean value of the test values at both of these doses was considerably greater than the control group's (Table 4). The control specimen's mean aspartate aminotransferase level on day 12 was  $10.36 \pm 2.33$ IU. The mean values of the test specimen varied between  $4.52 \pm 2.28$  (KI 0.4 gramme%) and  $28.46 \pm 4.88$  IU (KI 0.2 gramme%). With the exception of KI 0.4 and 0.8 gramme%, the mean aspartate aminotransferase level of test specimens was greater than the mean control level. For every test group, with the exception of KI 0.05 and 0.8 gramme percent, the mean difference from control was statistically significant (Table 4). The control specimen's mean aspartate aminotransferase level was  $17.62 \pm 4.27$  IU on day 18. The mean values for test specimens varied between 4.50  $\pm$  1.02 (KI 0.8 gramme %) to 14.49  $\pm$  3.60 IU (KI 0.05 gramme %). The mean value was considerably lower for all test concentrations, commencing at KI 0.2 gramme percent, than for the control group (p<0.05) (Table 4). When comparing the three days, it was generally seen that the aspartate aminotransferase enzyme activity increased as the incubation period increased from the sixth to the twelfth day, and that the enzyme activity decreased on the eighteenth day, with the exception of the control. In contrast, mean AST levels were shown to be declining at KI 0.05, 0.4, and 0.8 gramme % and increasing at control, KI 0.1, and 0.2 gramme % between days 6 and 12. For KI 0.2, 0.4, and 0.8 gramme percent, the improvement was likewise noteworthy (Table 5). For the control and KI 0.1 gramme%, mean AST levels increased between days 6 and 18, but mean AST levels decreased for all other concentrations during that time. The range of the mean change was  $7.51 \pm 4.78$  IU (control) to  $-12.99 \pm 1.99$ (KI 0.8 gramme %). In terms of statistics, the shift was similarly noteworthy for the control, KI 0.2, 0.4, and 0.8 gramme percent concentrations. For both the control and KI 0.4 gramme% groups, a rise in mean AST levels was seen between days 12 and 18. There was a decrease in the test group's mean AST levels at every other concentration. With the exception of the KI 0.2 gramme concentration and the control group change, none of the other changes were statistically significant (p>0.05) (Table 5). On day six, the mean aspartate aminotransferase level for every test concentration-aside from KI 0.4 gramme percent was higher than the mean control level. On day 12, a rise in mean AST was also noted, reaching KI 0.2 gramme percent. With the exception of the control, it showed that the aspartate aminotransferase enzyme was hyperactive on days 6 and

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12 and declined on day 18. (Tables 4 and 5). Conversely, mean aspartate aminotransferase was found to be reduced on day 12 and elevated on day 18 for KI 0.4 gramme % at KI 0.4 and 0.8 gramme %. However, on day 18, the exponential phase of growth, the mean value of all these test concentrations was lower than that of the control (Table 5). It suggests that KI inhibits the growth of *S. schenckii*, which in turn causes aspartate aminotransferase activity to decrease.

#### 4.2. Alanine aminotransferase (ALT)

On day six of the yeast phase, the control specimen's mean alanine aminotransferase level was 10.70 ± 3.82 IU. The test specimens' mean levels of alanine aminotransferase varied between  $11.40 \pm 3.04$  (KI 0.1 gramme %) and 18.52 $\pm$  3.97 IU (KI 0.2 gramme %). Every test concentration had mean values that were higher than the control. In terms of statistics, all concentrations showed no discernible variation in mean alanine aminotransferase levels when compared to control, with the exception of KI 0.2 and 0.8 gramme percent. The mean value of the test values at both of these doses was considerably greater than the control group's (Table 4). The control specimen's mean alanine aminotransferase level on day 12 was 29.60 ± 3.02 IU. Alanine aminotransferase levels in test specimens varied between 7.82  $\pm$  1.50 (KI 0.8 gramme %) and 41.56  $\pm$  4.56 IU (KI 0.2 gramme %). For each test group, the mean difference from control was statistically significant. The mean values at all test concentrations, with the exception of KI 0.2 gramme%, were considerably lower than the control, whereas the mean value at KI 0.2 concentration was significantly higher. The control specimen's mean alanine aminotransferase level on day 18 was 19.74 ± 4.62 IU. Alanine aminotransferase mean levels for test specimens varied from 3.33  $\pm$  0.70 (KI 0.8 gramme %) to 12.54  $\pm$ 1.92 IU (KI 0.2 gramme %). Table 9 shows that the mean result for each test concentration was considerably less than the control's (p<0.05). On days 6 and 12, mean alanine aminotransferase levels increased for the control group at concentrations of KI 0.05, 0.1, and 0.2 grammes%, while they decreased for the group at concentrations of KI 0.4 and 0.8 grammes%, respectively. The control, KI 0.05, 0.2, and 0.8 gramme%, showed a significant change as well (Table 10). Only the control group showed an increase in mean alanine aminotransferase levels between days 6 and 18, whereas all other concentrations showed a decrease in mean levels of the enzyme. The range of the mean change was -0.90  $\pm$  2.33 IU (KI 0.05 gramme %) to -13.11  $\pm$ 2.50 (KI 0.8 gramme %). In terms of statistics, the shift was similarly noteworthy for the control, KI 0.2, and 0.8 gramme% concentrations. All groups saw a drop in mean levels between day 12 and day 18, with the exception of KI 0.4 gramme%. The range of the extent of change was  $0.97 \pm 0.82$  IU (KI 0.4 gramme %) to  $-29.02 \pm 5.51$ 

(KI 0.2 gramme %). All other changes were statistically significant (p<0.05), with the exception of the change at KI 0.4 gramme percentage (Table 10). On day six, the mean alanine aminotransferase level was higher than the mean control level for all test concentrations. On day 12, KI 0.2 gramme% also showed an elevated mean ALT level. It showed that the alanine aminotransferase enzyme was hyperactive on days 6 and 12, and that it was hypoactive on day 18 (Tables 9 and 10). Comparing day 18 to day 12, a drop in mean values was seen for all test concentrations and the control. The mean values of all test concentrations were lower than the control on day 18, the exponential phase of growth (Tables 9 and 10). It shows that the enzyme is degrading automatically and that KI is inhibiting the growth of S. schenckii, which has caused the enzyme alanine aminotransferase to become less active.

#### 5. Conclusion

In this investigation, the mean value of aspartate aminotransferase (yeast phase) for all test concentrations, beginning at KI 0.2 gramme%, was considerably lower than that of the control (p<0.05). The mean value of alanine aminotransferase (yeast phase) was considerably lower in all test specimens compared to the control group (p<0.05). It suggests that KI has an inhibiting effect on the growth of *S. schenckii* (yeast), which has reduced transaminase activity. This response, in conjunction with additional defence systems within the body, may be how KI works to cure sporotrichosis. The effect of KI to the lipids of *S. schenckii* may be observed to understand the mechanism of action of KI in the future.

#### 6. Ethical Approval

The study was approved by the Institutional Ethics Committee vide letter no. GU/HREC/2015/1049.

#### 7. Source of Funding

None.

#### 8. Conflict of Interest

None.

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