Bima Journal of Science and Technology, Vol. 7 (4) Dec, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i4.542

The Effect of Stability Variation on the Antifungal Activities of Selected Chemical Agents

[1]*Otegwu T. C., [2] Durowaiye M. T. and [1]Adamu H.

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe, Nigeria

[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

Corresponding Author: temitegwu@gmail.com

ABSTRACTS

Aspergillus niger has been found to be one of the most prevalent pathogen in yam tuber (genus *Dioscorea*). The stability of active chemical agents were evaluated under the following environmental factors: temperature, pH and storage. The test combine antifungal agents: Benomyl/Sodium Benzoate (0.2/550µg/ml), Benomyl/Terbinafine hydrochloride (0.05/0.06µg/ml), Benomyl/Trichloroacetic acid (0.2/1.5µg/ml), Benomyl/Trifluoroacetic acid (0.01/8.5µg/ml) were investigated using the agar-well diffusion and agar dilution method against *Aspergillus niger* spores. The combined antifungal agents exhibited different degree of antifungal activities at 40 ºC, 70 ºC and 100 ºC, while at alkaline pH 9 the antifungal activities of the chemical agents were observed to display low zones of inhibition. There was no significant change at 90% confident limit in the antifungal activities of the four formulated fungicide combinations observed within the four weeks period of evaluation at both room temperature (25-27±2.0 ºC) and refrigerator (4°C and 7°C). The importance of the data from this study, enable recommended storage conditions, retest interval and shelf lives to be established under the influence of a variety of environmental factors, i.e. temperature, duration of storage and pH.

Keywords: Fungi, Antifungal Agents, Stability, *Aspergillus niger.*

INTRODUCTION

The world production of yam was estimated at 75.1 million tons of tuber in 2021, (75.1 million tons) of this came from West Africa, the main producers being; Nigeria, the world's largest yam producer with over 71% of world production, it is mostly grown in Benue, Taraba, Cross River, Adamawa, Delta, Ekiti, Imo, Edo, Kaduna, Ogun, Kwara, Ondo, Osun, Plateau, Niger and Oyo states; Côte d'Ivoire 8.1%; Benin 4.3% and Ghana 3.5% (FAOSTAT, 2021). Yam tuber is one of the oldest foods cultivated since 50,000 BC in Africa and Asia. In addition to these continents, yams also currently grow in the tropical and subtropical regions of North and South America. It's one of the most popular and widely consumed foods globally. They have been taken as major diets in many countries, notably those in South America, Africa, the Pacific Islands, and the West Indies (IITA, 2009). Yam is a good source of energy, because of high carbohydrate content (low in fat and protein) (Amusa *et al*., 2003). Yams also contain modest amounts of Vitamin B_1 (thiamin) and Vitamin C. Yams also provide bulk fiber, which are needed to make the intestines or bowels work properly. They are rich in nutrients including starch, crude protein (3.59to 8.93%), crude fiber (average 3.48%) amino acids (2.31 to 7.26%) and sugar (3.39%) (Omonigho and Ikenebomeh 2000; Zhang et al. 2014).

Otegwu et al., 2018 quoted that researchers discovered that yam could be used to treat disease like diabetes mellitus, to increase

DOI: 10.56892/bima.v7i4.542

coronary flows and prevent hyper-costerolemia therefore controls blood pressure because of the potassium in it. Yam has been reported to be used in dermatology and gastroenterology infection and also sources of progesterone and cortisone. Its medicinal use as a heart stimulant is attributed to its chemical composition, which consists of alkaloids of saponin and sapogenin (Amusa *et al.*, 2003). Yams are subjected to several diseases. There are different genera of fungi that have been reported in association with storage deterioration in yam tubers (Okigbo and Ikediugwu, 2000). The major microorganism causing diseases in yams are: - *Aspergillus flavus* Lark Ex Fr, *Aspergillus niger* Van Tiegh, *Botryodiplodia theobromae* pat, *Fusarium oxysporum* schlecht ex Fr, *Fusarium solani* (Mart). Sacc, *Penicillium chrysogenum* Thom Rhizoctinia sp, *Penicillium oxalicum* Curries and Thom, *Rhizopus stolonifer* (Enrend. ex Fr) Lind, *Rhizopus nodosus* N'amyslowski and *Trichoderma viride*. Per. ex S. F. Gray among others (Okigbo and Ikediugwu 2000, 2001, 2002; Okigbo 2004).

A report by Ala'a 2008 highlighted the ability of a hypersensitive chemical compound in an enclosed system to retain its physical, chemical, microbiological and toxic qualities is referred to as the stability of the compound. Some of the factors that can make the antimicrobial activities of chemical compounds unstable are: environmental factors like air (oxygen & carbon dioxide), other chemicals, light, heat, water (hydrolysis) and duration of material storage and before usage. All these factors have reported to lead to the instability of the chemicals agents (Oyi *et al.,* 2007).

Decomposition in hydrolysis usually occurs in chemicals, if not in conducive temperature, pH and also in the presence of water, while oxidative reaction are strongly influenced by environmental factors such as light and metal ions to trigger off reaction. The rate of the hydrolysis depends on the quantity of water present, degree of temperature and the pH.

Aulton (2000) has also reported that deterioration of some thermo liable agents can occur as a result of the increase rate of chemical reaction since they can only withstand a particular range of temperature. This could affect the chemical agents, therefore, there is need to investigate the effect of these factors on the activities of combined antifungal agents on the fungus, *Aspergillus niger*.

MATERIALS AND METHODS

Test Organism

The microorganism (*Aspergillus niger*) used in this study was isolated from yam in the Department of Pharmaceutics. & Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Determination of Minimum Inhibitory Concentration (MIC) Using Agar Dilution Method

Ten milliliters (10 mls) of double strength Sabouraud dextrose agar was melted and mixed aseptically with 10mls volume of varying concentration of the test anti-fungal agents such as Benomyl Viz; 8000, 4000, 2000, 1000, 500, 250, 125, 62.50, 31.25, 15.63, 7.81, 3.91, 1.95, 0.97, 0.49, 0.245, 0.12, 0.06, 0.03 and 0.015 (µg/ml). Terbinafine hydrochloride Viz; 1600, 800, 400, 200, 100, 50, 25, 12.50, 6.25, 3.12, 1.57, 0.78, 0.39 (µg/ml). Trichloroacetic acid Viz; 20, 10, 5, 2.50, 1.25, 0.63 and 0.31 (µg/ml). Sodium Benzoate Viz; 80000, 40000, 20000, 10000, 5000, 2500, 1250, 625, 312.50, 151.25, 78.13, 39.06, 19.53, 9.77 and 4.88 (µg/ml). Trifluoroacetic acid Viz; 75, 40, 20, 10, 5, 2.50 and 1.25 (µg/ml). Each admixture was aseptically poured into sterile plates and

DOI: 10.56892/bima.v7i4.542

allowed to set. The standardized spores of test fungus (10^6cfu spores/ml) were aseptically inoculated (10.0 µl) in duplicates on sterile filter paper disc plated at equidistance on the SDA test antifungal plates. The inoculated organisms were allowed to diffuse for a period of 30 minutes. The plates were then incubated at 30ºC for 7days. The first lowest concentration that showed no growth of inoculated test fungi spores was considered as the MIC of the test anti-fungal agent (Shettima *et al*., 2000 & Shettima *et al*., 2005).

Determination of Combined MIC of Test Antifungal Agents

Varying concentrations of the test anti-fungal agents (e.g. Sodium Benzoate 100-500µg/ml; Terbinafine hydrchloride 0.4-1.2µg/ml; Trichloroacetic acid 2-6µg/ml and Trofluoroacetic acid 0.6-1.5µg/ml) in 5 mls volume each were mixed with fixed concentration of another test anti-fungal agents (Benomyl 0.05-0.3µg/ml) in same 5 mls. Each of these admixtures in 10 ml volume was mixed with melted 10ml volume of sterilized double strength of Sabouraud Dextrose Agar (SDA) at 45ºC aseptically in a Petri dish and was allowed to set.

Ten microliter (10µl) of standardized fungi spores (106 cfu/ ml) were inoculated on asterilized duplicate filter paper discs aseptically placed at equidistance on the test anti-fungal agents contained in the SDA.

These were allowed to diffuse into the SDA for 30 minutes. They were then incubated at 30ºC for 5 days. Control was set up, i.e. SDA plates without test antifungal agents, but inoculated with the standardized fungi spores. The lowest mixed concentration of test-antifungal agents that showed no growth was taken as combined anti-fungal agents' minimum inhibitory concentration (MIC). The Fractional Inhibitory Concentration (FIC) was calculated using the MICs for each pair of antifungal, according to Ting-Chao (2010).

Determination of Effect of varying pH Values on the Antifungal Activities of the Chemical Agents

Three different pH values (pH 3, 7, and 9) were used, which was obtained and maintained using pH buffers powder, confirmed with pH meter. The buffer powder was added to the Formulated product which is in 10mls volume with sterile distilled water. The content was allowed to stand for 30minutes.The standardized cultures of the test fungi spores was used to flood the SDA plates which was allowed to dry at 37 ºC in a sterile incubator. Using the cup plate method, a sterile cork borer (6mm diameter) was used to make a hole in each of the agar plates. The bottom sealed with two drops of the melted SDA at 45 ºC. Hundred microliter (0.1ml) of the fixed concentration of the formulated products (Benomyl / Sodium benzoate (0.2/550µg/ml), Benomyl/Terbinafine hydrochloride (0.05/0.06µg/ml), Benomyl/ Trichloroacetic acid (0.2/1.5µg/ml) and Benomyl/ Trifluoroacetic acid (0.01/8.5µg/ml) and sterile distilled water (which served as control) was then dispensed into the holes using micropipette. This was allowed to diffuse into the agar at room temperature for one hour after which it was incubated at 30 ºC for 48 hours. The zones of inhibition were then measured to the nearest millimeters. This procedure was carried out in duplicates and the same method used for the other pH values.

Determination of Effect of Varying Temperature on the Activities of the Chemical Agents

Same procedure was carried out as in the case of pH study above. The formulated product was dissolved in sterile distilled water, giving a known concentration of the test agent. The set up was maintained at different

DOI: 10.56892/bima.v7i4.542

temperatures (Room temperature, 40 ºC, 70 ºC, and 100 ºC) in the water bath for 30 minutes. Hundred microliter (0.1ml) of the product solution was aseptically transferred into the already made holes in the standardized test fungal spores suspension flooded sterile SDA plates and incubated at 30 ºC for 48 hours. Positive and negative controls were also set up. The zones of inhibition of the test organism were then measured using a well calibrated meter rule.

Determination of Effect of Length of Storage Time on the Antifungal Activities of the Chemical Agents

A solution of the formulated products was prepared in 10 ml volume with sterile distilled water at intervals day 1, day 2, day 7, week 2, week 3 and week 4. At the various intervals, solution of the formulation prepared was aseptically assessed for antifungal activity using the agar well diffusion method. Hundred microliter (0.1ml) of the product solution was aseptically dispensed into the bored hole in the SDA containing the test organism. The plates were then allowed to stand for 1 hour for diffusion. Positive and negative controls were set up and the plates were incubated at 30 ºC for 48 hours and the results of the zone of inhibition taken appropriately.

Statistical Analysis

Measures of Central Tendency, which is part of Descriptive Statistical Analysis was used in analyzing the results from this study.

RESULTS

Minimum Inhibitory Concentration

The minimum inhibitory concentration investigation values in Table 1 below shows that all the test antifungal agents singly displayed inhibitory effect on the test phytopathogenic fungi spores (*Aspergillus niger*), in this order: - Benomyl (0.49 µg/ml) been the most effective followed by Terbinafine Hydrochloride (1.57 µg/ml), Trichloroacetic acid (2.50 µg/ml), Trifluoroacetic acid (10.00 µg/ml) and Sodium Benzoate (625.00 µg/ml) which is least effective.

Table 1: Minimum Inhibitory Concentration summary of the different single chemical compounds

Chemical compound	MIC (µg/ml)
Benomyl	0.49
Sodium benzoate	625.00
Terbinafine hydrochloride	1.57
Trichloroacetic acid	2.50
Trifluoroacetic acid	10.00

Minimum Fungicidal Concentration

The results of minimum fungicidal concentration (MFC) of the other chemical compounds against *Aspergillus niger* phytopathogenic spores (Table 2 below) shows the following order viz: Benomyl (5µg/ml) been the most effective followed by Terbinafine Hydrochloride (6.25µg/ml), Trichloroacetic acid (10.00µg/ml), Trifluoroacetic acid (10.00µg/ml) and Sodium Benzoate (20,000.00 µg/ml) the least effective.

Table 2: MFC Summary of the different single chemical compounds

Chemical compound	MFC (µg/ml)
Benomyl	5.00
Sodium benzoate	20,000.00
Terbinafine hydrochloride	6.25
Trichloroacetic acid	10.00
Trifluoroacetic acid	20.00

Effect of the Combined Antifungal Agents

When these test antifungal agents were combined; better antifungal activities were observed with lower concentration because of the synergistic, additive or antagonistic effect of the combinations Benomyl/Sodium Benzoate, Benomyl/Terbinafine Hcl, Benomyl/Trichloroacetic Acid and Benomyl/Trifluoroacetic Acid. This involves

Bima Journal of Science and Technology, Vol. 7 (4) Dec, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i4.542

the calculation of the fractional inhibitory concentration (FICs) of the single and combined MICs of the test antifungal agents against *Aspergillus niger*. Table 3 below shows an example of one of the calculation of FIC of Benomyl and Terbinafine Hydrochloride. And Table 4 is the summary of Combined Antifungal Agents Inhibitory activities Against *Aspergillus niger* Spores (10^6 cfu/ ml) of all the combinations.

Table 3: Synergistic Inhibitory activities of Benomyl and Terbinafine Hydrochloride against *Aspergillus niger* spores (10^6 cfu/ ml)

MIC of Benomyl (0.49 µg/ml) (C2)		**MIC of Terbinafine HCl (1.57 µg/ml) (C2)**		
Benomyl conc. in admixture (C1)	FIC	Terbinafine HCl in admixture (C1)	FIC	ΣFIC
0.30	0.61	0.40	0.25	0.86
0.20	0.41	0.40	0.25	0.66
0.15	0.31	0.40	0.25	0.56
0.10	0.21	0.40	0.25	0.46
0.05	0.10	0.80	0.51	0.61
				ΣFIC= 3.15

ΣFIC = 3.15= 0.63 (mean of FIC)

------ -----

n 5

FIC = C1/C2

C1 = concentration of antifungal agent present in the inhibitory combination

C2 = concentration of antifungal agent which would produce the same effect as the combination when acting alone.

FIC = Fractional Inhibitory Concentration of the combined antifungal agents.

Table 4: Summary of combined antifungal agents inhibitory activities against *Aspergillus niger* spores (10^6 cfu/ ml)

Combined Antifungal agents	**Mean of FIC**	**Inference**
Benomyl/Sodium benzoate (0.2/550µg/ml)	1.41	Additive
Benomyl/Terbinafine HCl (0.05/0.06µg/ml)	0.63	Synergistic
Benomyl/Trichloroacetic acid (0.2/1.5µg/ml)	1.21	Additive
Benomyl/Trifluoroacetic acid (0.01/8.5µg/ml)	0.80	Synergistic

Key: FIC>4=Antagonistic

FIC=1-4=Additive

FIC<1=Synergistic

Stability Study

This allows evaluation of active chemical agent's stability or drug products stability under the influence of a variety of environmental factors such as temperature, humidity, pH and light. Data from these studies enable recommended storage conditions, retest interval and shelf lives to be established.

Effect of pH

From this research work/study, the antifungal effect of the antifungal agents is affected with change in pH (3, 7 and 9). Benomyl / Sodium Benzoate (0.2/550µg/ml), Benomyl/Terbinafine Hydrochloride (0.05/0.06µg/ml) and Benomyl/Trifluoroacetic Acid (0.01/8.5µg/ml) all show better antifungal effect at pH 7.

Bima Journal of Science and Technology, Vol. 7 (4) Dec, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i4.542

Table 5: Effect of varying pH values on the antifungal activities of the chemical agents

	Zone of inhibition (mm)		
Combination	**pH 3**	**pH 7**	**pH 9**
Benomyl / Sodium benzoate (0.2/550µg/ml)	4.00	10.00	3.50
Benomyl/Terbinafine hydrochloride (0.05/0.06µg/ml)	20.00	26.00	14.50
Benomyl/Trichloroacetic acid (0.2/1.5µg/ml)	3.50	NZ	NZ
Benomyl/Trifluoroacetic acid (0.01/8.5µg/ml)	NZ	4.50	2.50
– control	– – –	– – –	– – –
+ control	+ + +	+ + +	+ + +

Key: NZ = no zone
– = no growth
+ = presence of growth

Effect of Temperature

Results of the effect of temperature on the antifungal activity of the different antifungal compounds (Table 6) shows that at the temperature range of room temperature, 40ºC, 70ºC and 100ºC, the antifungal activity was significantly reduced at 95% confident limit. This reduction depends on each combination: - Benomyl/Terbinafine Hydrochloride (0.05/0.06µg/ml) antifungal activity reduces with increase in temperature, this is in line with the work of Moldovan & David 2014, the increase of temperature at 75ºC resulted in an accelerated degradation and destabilization of any chemical agent.

Table 6: Effect of varying temperature on the antifungal activities of the chemical agents

	Zone of inhibition (mm)			
Combination	**Room**	**40ºC**	**70ºC**	**100ºC**
Benomyl / Sodium benzoate (0.2/550µg/ml)	15.50	NZ	NZ	NZ
Benomyl/Terbinafine hydrochloride (0.05/0.06µg/ml)	40.00	35.00	31.30	26.00
Benomyl/Trichloroacetic acid (0.2/1.5µg/ml)	6.50	NZ	NZ	NZ
Benomyl/Trifluoroacetic acid (0.01/8.5µg/ml)	7.00	NZ	NZ	NZ
– control	– – –	– – –	– – –	– – –
+ control	+ + +	+ + +	+ + +	+ + +

Key: NZ = no zone
– = no growth
+ = presence of growth

Effect of Storage

There was no significant change at 90% confident limit in the antifungal activities of the four formulated fungicide combinations observed within the four weeks period of evaluation at both room temperature (25 - 27±2.0 ºC) and refrigerator (4°C and 7°C) (Tables 7 and 8) Observation from the combinations of Benomyl/Terbinafine Hcl (0.05/0.06µg/ml), showed a higher antifungal activity than Benomyl/Sodium benzoate (0.2/550µg/ml), Benomyl/Trichloroacetic acid (0.2/1.5µg/ml) and Benomyl/Trifluoroacetic acid (0.01/8.5µg/ml).

Bima Journal of Science and Technology, Vol. 7 (4) Dec, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i4.542

Table 7: Effect of storage on the antifungal activities of the chemical agents. (Room Temperature-25 -27±2.0 ºC)

Zone of inhibition (mm)						
Combination	**Day 1**	**Day 2**	**Day 7**	**Week 2**	**Week 3**	**Week 4**
Benomyl/Sodium benzoate (0.2/550µg/ml)	5.50	5.50	5.50	4.50	4.50	3.50
Benomyl/Terbinafine HCl (0.05/0.06µg/ml)	35.00	27.50	22.50	21.00	21.00	21.00
Benomyl/TCA (0.2/1.5µg/ml)	5.00	5.00	4.50	4.50	4.50	4.50
Benomyl/TFA (0.01/8.5µg/ml)	6.00	6.00	5.00	4.50	4.50	4.50
– control	– – –	– – –	– – –	– – –	– – –	– – –
+ control	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

Key: – = no growth
+ = presence of growth
TCA = Trichloroacetic acid
TFA= Trifluoroacetic acid

Table 8: Effect of storage (4º C-7 ºC Refrigerator)

	Zone of inhibition (mm)					
Combination	**Day 1**	**Day 2**	**Day 7**	**Week 2**	**Week 3**	**Week 4**
Benomyl/Sodium benzoate (0.2/550µg/ml)	6.50	6.50	5.00	4.50	4.50	4.50
Benomyl/Terbinafine HCl (0.05/0.06µg/ml)	35.00	25.00	23.50	21.00	20.00	19.50
Benomyl/TCA (0.2/1.5µg/ml)	4.50	4.50	4.50	4.00	3.50	3.50
Benomyl/TFA (0.01/8.5µg/ml)	5.50	5.50	5.50	5.00	4.00	3.50
– control	– – –	– – –	– – –	– – –	– – –	– – –
+ control	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

Key: – = no growth
+ = presence of growth
TCA = Trichloroacetic acid
TFA= Trifluoroacetic acid

DISCUSSION

The antifungal activities of test chemical agents: Benomyl/Sodium benzoate (0.2/550µg/ml), Benomyl/Terbinafine hydrochloride (0.05/0.06µg/ml), Benomyl/Trichloroacetic acid (0.2/1.5µg/ml) and Benomyl/Trifluoroacetic acid (0.01/8.5µg/ml) on phytopathogenic *Aspergillus niger* spores in Zaria, Nigeria were investigated, and was discovered that these test agents all have antifungal effects on the isolate spores. The MIC of these test agents individually was Benomyl 0.49 µg/ml, Terbinafine hydrochloride 1.57 µg/ml, Trichloroacetic acid 2.50 µg/ml, Trifluoroacetic acid 10.00 µg/ml and Sodium Benzoate 625.00 µg/ml.

The study showed that at varying pH ranges, temperature and storage period investigated; there were varying changes observed in the antifungal activities.

All the combined chemical agents, Benomyl/Sodium benzoate (0.2/550µg/ml), Benomyl/Terbinafine hydrochloride (0.05/0.06µg/ml), Benomyl/Trichloroacetic acid (0.2/1.5µg/ml) and Benomyl/Trifluoroacetic acid (0.01/8.5µg/ml), were observed to have the highest antifungal activities at pH7 and least activities at pH 9 (Alkaline medium). But Benomyl/Trifluoroacetic acid had no antifungal effect at acidic medium (pH 3). pH is one of the factors that affect/influence the survival and rate of growth of microorganism,

DOI: 10.56892/bima.v7i4.542

changes in the pH can affect the effectiveness of the chemical agents and its ability to continue with cell surfaces active sites. The degree of ionization of agents, either acidic or alkaline, depends on the pH. The non-ionized molecule is the active state and the alkaline pHs which favour the formation of ions of such compounds will decrease the activity. Others reveal cidal activity as the pH rises and best used under alkaline conditions (Franklin and Snow, 2013).

The results of effect of varying temperature, 40°C, 70°C and 100°C, on the antifngal activities of the four chemical agents reveals that Benomyl/Terbinafine hydrochloride shows a consistent gradual reduction in antifungal activity with increase in temperature, noting the zone of inhibition (mm) as shown in Table 7 above. While Benomyl/Sodium benzoate, Benomyl/Trichloroacetic acid and Benomyl/Trifluoroacetic acid showed no effect at all at temperature 70°C and 100°C. All show greatest effect at room temperature. In all the chemical agents, decreased antifungal activities observed at high temperatures may be due to gradual chemical destruction at higher temperature which can lead to degradation which in turn affects the antifungal activity of the agents (Timothy *et al.,* 2010). This reduction depends on each combination: - Benomyl/Terbinafine hydrochloride (0.05/0.06µg/ml) antifungal activity reduces with increase in temperature, this is in line with the work of Moldovan and David (2014), the increase of temperature at 75ºC resulted in an accelerated degradation and destabilization of any chemical agent.

Again from the result of this research, the storage of the combined test antifungal agents: Benomyl/Terbinafine hydrochloride (0.05/0.06µg/ml), Benomyl/Sodium benzoate (0.2/550µg/ml), Benomyl/Trichloroacetic acid (0.2/1.5µg/ml) and Benomyl/Trifluoroacetic acid (0.01/8.5µg/ml) at both room temperature (25-27 ± 2.0°C) and refrigerator (4°C and 7°C) did not show significant changes at 90% confident limit in the antifungal activities of the four formulated fungicide combinations observed within the four weeks evaluation period.

Observation from the results of the effect of storage at both ambient temperature and refrigerator on the antifungal activities of the chemical agents against test antifungal spores generally showed no significant difference. This further confirms the effect of varying temperature in this work which show greatest effect at room temperature.

CONCLUSION

In Conclusion, the test compounds/chemicals were found to be effective at temperature 27ºC (room temperature) and 40ºC, but decreases as the temperature increased to 70 ºC and 100 ºC. The combined antifungal agents were found to show the highest antifungal activities at pH 7. And within four weeks of storage of these test agents at an ambient temperature (25 – 27 ± 2.0 ºC) and Refrigerator (4 ºC -7 ºC), the antifungal activities were found to be relatively stable i.e. no significant changes in the observed antifungal activities.

REFERENCES

Ala'a R. Alfayez and Zainab Al-mulla (2008). A Presentation Transcript on 'Stability tests for pharmaceutical products.' Sited on 1st July 1, 2013.

Amusa, N.A., Adegbite, A.A., Muhammed, S. and Baiyewu, R.A. 2003: Yam diseases and its management in Nigeria: *Academic Journals Citation: African Journal of Biotechnology* 12:

Aulton M. E. (2000). Tropical Preparation Pharmaceutics. The science of dosage

DOI: 10.56892/bima.v7i4.542

form design. Edited by M. E. Aulton. Churchhill-Livingstone. P.318-429.

FAO (2021). Food and Agricultural Organization. FAOSTATDATA. FAO, Rome, Italy.

Franklin T. J. and Snow G. A. (2013). Biochemistry of Antimicrobial Action. *Springer New York*, NY 2, P 56-75.

IITA, (2009) IITA Annul report. International Institute of Tropical Agriculture.

Moldovan B. and David L. (2014). Influence of Temperature and Preserving Agents on the Stability of Cornelian Cherries Anthocyanins. Molecules Journals 19(6), 8177-8188

Okigbo, R. N and Ikediugwu, F. E. O. (2000). Studies of biological control of postharvest rot of yams (Dioscorea spp.) with *Trichoderma viride. Journal of Phytopathology*, 148(6), 351 355.

Okigbo, R. N and Ikediugwu, F. E. O. (2001). Studies of biological control of tuber surface mycoflora of yam *Dioscorea rodundata*. *Tropical science*, 41(2), 85-89.

Okigbo, R. N and Ikediugwu, F. E. O. (2002). Evaluation of water losses in different region of yam (*Dioscorea spp*.) tuber in storage. *Nigeria Journal of Experimental Applied Biology* 3: 317-321

Okigbo, R. N. (2004) A review of biological control methods for post-harvest yams (Dioscorea spp.) in storage in South Eastern Nigeria KMTL. *Science Journal* 4:207-215.

Omomnibho S. E. and Ikenebomeh M. J. (2000). Effect of temperature treatment on the chemical composition of pounded white yam during storage. Food chem. 71:215-220

Otegwu T. C., Adeshina G.O. and Ehinmidu J. O. (2018) "Antifungal Activity of Selected Chemical agents against Phytopathogenic Fungi Spores". *European Journal of Medicinal Plants*. 25(2): 1-7; Article no. EJMP. 38306 ISSN: 2231-0894, NLM ID: 101583475

Oyi, A. R., Onaolapo, J. A., Haruna, A. K. and Morah, C. O. (2007) Antimicrobial screening and stability studies of the crude extract of *Jatropha curcas* linn. Latex. (Euphobiaceae) *Nigerian J. Pharm. Sci.* 6(2): 41-43

Shettima, A. S.; Bonire, J.J. & Ehinmidu, J. O. (2005). Antifungal activity of some synthesized ditriphenyltin and triphenyltin carboxylates. *Journal of tropical Biosciences* 5 (2): 47-50

Shettima, A. S.; Garba, M. G.; Chouthury, M. K.; Haruna , A. K.; Bonire, J.J.; Ehinmidu, J. O. and Ado, S. A. (2000). Synthesis of selected triorganotin cinnamates ligated pyridine and studies of their antifungal activity. *Journal of medical and Allied Sciences* Vol. 3 No. 1&2

Timothy, J. S., Alison, M. A. and Davies, J. (2010) Understanding the chemical basis of drug stability and degradation. *The Pharmaceutical Journal* Pg 75.

Ting-Chao, C. (2010). Drug combination studies and their synergy quantification using the Chou-Talalay. *Method. Cancer Resarch* 70: 440-446

Zhang Z., Gao W., Wang R. and Huang L. (2014). Changes in main nutrients and medicinal composition of Chinese yam (*Dioscorea opposite*) tuber during storage. Journal of Food Science and Technology, 51, 2535-2543.