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Incidence of Mycotoxigenic fungi Associated with Decayed Saccharrum officinarum and the Effect of Chlopyriphos insecticide on their mycelia Growth and Glutamic-oxaloacetic acid Activity.

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Abstract: *Forty-six fungal species belonging to thirty genera were isolated from sixty samples of Saccharrum officinarum collected from Sabo Sugarcane market, Ogbomoso, Nigeria. Aspergillus, Trichoderma, Mucor and Pythium were the most common genera on the two isolation media. The dominant species of Aspergillus were A. niger, A. flavus, A. ustus, A. terreus and A. wentii. Some species were dominant on 60g/l sucrose such as Aspergillus niger, A. flavus, Emericella nidulans, Trichoderma viride, Torula herbarum and Mamaria echinoeotryoides, while the dominant species on 20 g/l glucose were Mucor circinelloides, Aspergillus niger, Torula herbarum and Trichoderma viride. Mycotoxins including aflatoxins B1, B2, G1 and G2, zearalenone and diacetoxyscirpenol were detected in the examined samples of Saccharrum officinarum. The mycelial growth of A. flavus, A. niger, Fusarium moniliforme and Torula herbarum decreased significantly (P≥0.05) with the increase in Chlopyriphos concentrations, although 10 Part per millim (ppm) was less effective than the higher levels of the insecticide (50-250 ppm). Chlopyriphos stimulated the activity of Glutamic-oxaloacetic acid (Go-T) in A. flavus, F. moniliforme and T. harbarum, while the Go-T activity was inhibited in A. fumigatus with the Chlopyriphos treatments. The significant of the incidence of mycotoxigenic fungi associated with decayed samples of Saccharrum offioinarum and the effect of chlopyripos insecticide on their mycelia growth and Glutamic-oxaloacetic acid is herein discussed.*

Keywords: *Fungi, Mycotoxin, Saccharrum officinarum, Chlopyriphos*

I. Introduction

Saccharum officinarrum is one of the most important crops in the world because of its strategic position and immense uses in the daily life of any nation as well as for industrial uses aimed at nutritional and economic sustenance. Sugarcane contributes about 60% of the total world sugar requirement while the remaining 40% came from sugar beet, ([1]. It is a tropical crop that usually takes between 8 – 12 months to reach its maturity. Matured cane may be green, yellow, purplish or reddish considered ripe when sugar content is at its maximum [2, 3]. The African in area and sugar production was estimated as 1.2 million hectares with 72.1 million metric tons, respectively [4]. The important sugar-producing countries in the tropical Africa are Mauritius, Kenya, Sudan, Zimbabwe, Madagascar, Cote d'Ivoire, Ethiopia, Malawi, Zambia, Tanzania, Nigeria, Cameroon and Zaire. Nigeria is one of the most important producers of the crop with a land potential of over 500,000 hectares of suitable cane field capable of producing over 3.0 million metric tons of sugarcane [2]. Sugarcane plant (Saccharum officinarum) is the predominant crop used in sugar production in savannah zone of northern Nigeria and it is been transported to different communities in South Western Nigeria. Sugar present in the stem of Saccharum officinarum represents the main source for fungal growth. Dare and Fawole Curei and Girot [3] studied the seasonal fluctuations of soil and root surface fungi of sugarcane in the savannah zone of northern Nigeria. They isolated 73 species and 5 varieties representing 33 genera using glucose-, cellulose-and sucrose-Czapek's agar media. Since some molds can produce toxic metabolites (mycotoxins), proliferation of the organisms represents a potential health hazard [6]. The occurrence of mold from sugarcane and sugarcane juice are also common. According to Almas et al., [7]. Cladosporium, Curvularia, Fusarium, Helminthosporium, Pestalotia and Phoma are found in sugarcane caryopses. In sugarcane the microbial contamination found were mainly yeast [8]. The distribution of Aspergillus flavus and A. parasiticus in sugarcane field soils and on harvested sugarcane stem s were studied by Takahasho et al., [9] who found that aflatoxin production were 89% in 146 of 164 and of all the isolates 69% were A. flavus isolates. Aflatoxin G was produced by 40 % of A. flavus isolates. Aspergillus niger and A. flavus are the common allergens and may cause opportunistic invasive infections [10, 11] A. fumigatus is an ubiquitous mold and the most common cause of invasive aspergillosis in immune compromised patients [12]. Therefore, detection of fungal contaminants is essential to ensure safe and high quality food [13]. On the other hand, insecticides have been used to control insects and play a significant role in increasing crop production. They commonly affect some of the non-target organisms such as microbial

population ranging from inhibitory to stimulatory effects [14]. Chlopyriphos is a broadspectrum insecticide and commonly used in sugarcane to control the white fly [15]. The effect of insecticides on fungal growth and enzymes activity has been studied by different workers [16, 17, 18]. Therefore the objective of the present study was to evaluate the occurrence of fungal Species and Mycotoxins from Decayed Sugarcane (Saccharrum officinarum) in Ogbomoso, Nigeria, as well as evaluate the effects of chlopyriphos insecticide on their mycelia growth and glutamic-oxaloacetic acid enzyme activity.

II. Materials And Methods

Collection of Saccharrum officinarum Samples

Sixty samples of (Saccharrum officinarum) stems were collected during two seasons (raining and dry 2013) from the Sabo sugarcane market, Ogbomoso, Oyo state, Nigeria. Each sample was represented by 15 decayed stem parts. The samples were transferred directly to the Pure and Applied Biology laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria for fungal isolation, while mycotoxin analysis was determined using the standard kit at laboratory of Institute of Agriculture Research and Training (IAR&T) More plantation Ibadan, Nigeria.

Isolation and identification of fungi from Saccherrum officinarum.

The stem-samples were washed with sterilized distilled water. Each stem was cut into segments (0.5 cm long) by knife, and then each segment was cut into four equal parts. These segments were placed on the surface of two solidified media, glucose (20 g/l) and sucrose (80 g/l) Czapek's agar to which chloromphnicol was added as a bacteriostatic agent [19]. Five Petri plates of tested medium were used for each stem sample. Plates were incubated at 25oC for one week. For the recovery of aquatic fungi, stem segments from the collected samples were placed in Petri-dishes; 100 mm in diameter (6 replicates). The segments in each Petri-dish were then covered with sterile distilled water (20 ml) and 12 sterilized sesame seeds were introduced into each Petri-dish, as employed by [19]. The growing fungi were identified, counted as numbers per segment. The identification of fungal genera and species was performed according to [20]. Fungal species recovered from stem samples were purified on suitable media such as glucose-peptone-agar, malt-extract-agar, potato-dextrose-agar, potato-dextrose-yeast-agar, sabouraud's-dextrose-agar and Czapek's-medium.

Mycotoxin extraction from Saccherrum officinarum.

The mycotoxin extraction and cultification was done at Institute of Agriculture and Training (IAR&T) Ibadan, Oyo state, Nigeria. Following the method of [18] Fifty grams of decayed stems of each sugarcane sample were transferred to 500 ml Erlenmeyer flasks containing 150 ml of chloroform each and placed in a shaker (200 rpm) for 16 hours, then filtered through filter paper (Whatman No. 1). The chloroform extract was dried over anhydrous sodium sulphate. The remaining stem samples were dried at 50^{o}C over night, followed by re-extraction by 150 ml of 90% methanol-water. Chemical detection of mycotoxins. Thin layer chromatography (TLC) technique was carried out using precoated with Silica Gel type 60, F254 (MERCK, Germany). Aflatoxins B1, B2, G1 & G2; ochratoxins A & B; sterigmatocystin; citrinin; T-2 toxin; diacetoxyscirpenol (DAS); zearalenone; moniliformin and fusarin C were used as standards. The developing solvent systems used were methanol - chloroform (v/v 3 : 97), ethyl acetate-hexane (v/v, 70 : 30), ethanol - chloroform (v/v, 5 : 95) and toluene - acetone - methanol (v/v/v, 50 : 30 : 20). The developed plates were then viewed under UV light (254 and/or 366 nm) and sprayed with reagents for identification according to [21].

Determination the Effect of Chlopyriphos Insecticide on Mycelial Growth and Glutamic- oxaloacetic transaminase.

The effect of the insecticide Chlopyriphos on mycelial growth and glutamic-oxaloacetic transaminase (Go-T) activity were studied using most commonly occurring fungal species namely; Aspergillus flavus, A. niger, Fusarium moniliforme and Torula herbarum. Fifteen Erlenmeyer flasks (250 ml), each containing 50 ml Czapeck's-Dox liquid medium, were used for each fungus. Triplicate flasks served as control, in which media were amended with 10, 50, 150, 200 and 250 (part per million) of the chloropyriphos insecticide. Each flask was inoculated with 1 ml of the spore suspension obtained from seven days old cultures (Czapeck's-Dox medium) of the required fungus. The flasks were then incubated at 27oC for seven days, after which the mycelial filtrates were collected from the flasks by Buchner filtration using hardened filter papers, washed several times with sterile distilled water and weighed glutamic-oxaloacetic transaminase (Go-T) was determine following the method of [22], in which ten milligrams of the fresh mycelia were mixed and homogenized with 1.0 ml phosphate buffer. The extracts were clarified by centrifugation for 15 min at 8000 ×g and then analyzed for Go-T as described by [23] using the transaminases kit (Quimica Clinica Aplicada S.A.).

III. Results And Discussion

The result on the fungi associated with decayed Saccharrum officinarum samples using glucose or sucrose as carbon source is presented in Table 1 and Fig. 1. These results showed that numbers of fungi were greatest on 20 g/l glucose medium than 80 g/l sucrose medium, while, the number of species isolated on sucrose medium was more diverse than that recovered on glucose medium. In this respect, twenty-six species belonging to eighteen genera were collected from the forty samples of sugarcane on glucose-medium, while thirty-eight species belonging to twenty-six genera were collected on the sucrose-medium. A total of forty-six fungal species belonging to thirty genera were isolated from the sixty samples of sugarcane on both glucose and sucrose media during raining and dry seasons. Sixteen out of these species were isolated on both tested media, while ten species were obtained only on glucose medium and twenty-two species were isolated only on sucrose medium. The dominant genera on the two types of media were Aspergillus, Trichoderma, Mucor, Mammaria, Torula and Cephalosporium. Fungi recovered on glucose-agar medium. Twenty-six species belonging to eighteen genera were recovered from sixty decayed sugarcane samples in Sabo Market, Ogbomoso, Nigeria on glucose- Czapek's agar at 25^{o}C (Table 1). Aspergillus was the dominant genus representing 95% of the samples constituting 45.8% of the total number of fungi. It was represented by seven species of which A. flavus (8.78%), A. niger (30.4%), and A. ustus (4.4%) were of high occurrence. These Aspergillus species were also recovered from Saccharrum officinarum leaves, stem, bagasse and juice by [24, 26]. The remaining Aspergillus species were of moderate to rare occurrence on 20~5% of the samples. These species namely, A. awamori (1.1% of the total number of fungi), A. terreus (0.6%), A. wentii (0.4%) and A. japonicus (0.2%). Trichoderma, Emericella, and Torula species were the second in occurrence. These were recovered from 85%, 75% and 90% of the tested samples and represented by high occurrence by 19.4%, 11.9% and 7.9% of the total fungal, respectively. Each was represented by one species namely, Trichoderma viride, Emericella nidulans and Torula herbarum. Colletotrichum dematium was of high occurrence, it appeared in 45% of the Saccharrum officinarum samples and constituted 3.93% of the total fungal; this agrees with the result of Abdel-Hafez et al., [27]. Eurotium chevaliere, Mucor heimalis, Pythium intermedium and Pilobolus sp. were of moderate occurrence (40%, 40%, 30% and 35% of the samples) and constituted 1.5%, 1.4%, 1.3% and 1.8% of the total fungal population, respectively. Each of Gliomastix cerealis and Melanospora fallax were of low occurrence (20% of the samples), representing 1.0% and 0.6% of total fungi, respectively. The remaining species were of rare occurrence (5~10% of the samples) constituting 0.7~0.1% of the total fungi. These species were Apodachlya brachynema, Achlya megasperma, A. americana, Allomyces macrogynous, Curvularia tetramera, Cunninghamella elegans, Moncilium mucidum, Synecephalostrum racemosum and Rhizopous stolonifer. Several researches reported that some strains of these fungi produced several toxic metabolites [28, 29]. Fungal genera and species recovered on sucrose agar. Thirty-six species belonging to twenty-four genera were recovered from sixty Saccharrum officinarum samples on 60 g/l sucrose- Czapek's agar at 25^{o}C (Table 1). Abdel-Hafez et al., [26] isolated forty-six species and two varieties belonging to twenty genera from sixthy Saccharrum officinarum juice samples on glucose-, sucrose- and cellulose-Czapek's agar at 28^{O}C. The dominant genera were Aspergillus (eight species), Trichoderma (one specie), Mucor (four species), Torula (two species) and Cladosporium (one specie). They occurred in samples at rates 0.5~85.0% of the total samples investigated. In this respect, Almas et al., [7] recorded that Aspergillus, Eurotium and Penicillium were the most common genera in dried fruits using 20% sucrose-Czapek's agar at 28^{o}C. These results almost agree with the findings of Megalla et al., [30] who noted that Aspergillus and Penicillium were the most common in tropical foodstuffs, respectively. The most dominant species were Aspergillus awamori, A. niger, A. ustus, A. flavus, A. versicolor, A. oryzae and A. terreus. Trichoderma (80% of the samples) was second to Aspergillus and was represented by one species namely, T. viride which constitutes 16.1% of the total count of the isolates. Mucor came third and it was represented by four species namely, M. circinelloides, M. heimalis, M. plumbeus, and M. racemosus constituting 0.2~7.6% of the total fungi recovered (Table 1). Torula was represented by two species namely, T. herbarum and T. grisea constituting 4.9% and 0.6% of the total fungi recovered, respectively. Cladosporium herbarum, Cunninghamella elegans, Saccharomyces spp., Fusarium moniliforme and Cephalosporium curtipes were of moderate occurrence, they comprised 1.25~3.49% of the total fungi recovered. The following genera namely, Curvularia tetramera, Mammaria echinoeotryoides, Penicillium luteum and Pythium intermedium were represented by low occurrence and constituting 1.1~2.3% of the total fungi recovered. The remaining genera and species were represented by rare occurrence (5.0~10.0%); with a frequency of 0.1~0.8%. Saccharomyces spp. appeared only on the sucrose medium. The result on the seasonal fluctuation of the fungal species is presented in Table 1 and Fig. 1. The result showed that twenty-six species appeared on glucose medium in both raining and dry seasons Table 1 and Fig. 1. The seasonal fluctuation of these species on glucose medium revealed that nine species appeared in dry, seven species in raining and ten species in both seasons Table 1 and Fig. 1. On the other hand, thirty-eight fungal species were recovered on sucrose medium in both raining and dry seasons Table 1 and Fig. 1. Nineteen species of them were isolated in winter, ten species in summer and nine species isolated in both seasons on sucrose medium. Generally, forty-six fungal species were recovered from the two seasons on the two

tested media. Nineteen out of these species were isolated only in dry and twelve species were isolated only in raining, while seventeen species were isolated in both seasons. The result on visual estimation of mycotoxin production by some fugal associated with decay Saccharum officinarum samples is presented in Table 2. The result showed that Fusarium moniliforme produced zearalenone and diacetoxyscirpenol toxins. In addition These results were in agreement with those of Leitao et al., [31]. Fusarium was recorded as zearalenone producer in some tropical food [32, 33]. Thin layer chromatography analysis revealed the significant amounts of aflatoxins B1, B2, G1 and G2 in the tested samples produced by A. flavus (Table 2). Thin layer chromatography analysis revealed the significant amounts of aflatoxins B1, B2, G1 and G2 in the tested samples produced by A. flavus (Table 2). These aflatoxins particularly B1 are associated with acute poisoning of animal and human [7], lack of appetite, weight loss, unthrifitness, neurological abnormalities, jaundice of mucous membrane, convulsions and death [33], causes damage of chromosomes [34] and carcinogenic for human liver [35]. Based on visual estimation, when the sugarcane samples were subjected to aflatoxin screening, A. niger, A. ustus and Emericella nidulans were not toxin producers. Similarly, the isolated Colletotrichum dematium was also not toxin producer. The result on fungi associated with Saccharrum officinarum sample are visual estimation of mycotoxin is presented in Table 2. Colletotrichum falcatum but not from healthy or wounded sugarcane. The result on the effect of different concentrations of the insecticide Chlopyriphos on mycelial growth and Go-T activity in A. flavus, A. fumigatus, F. moniliforme and T. harbarum is presented in Table 3. Generally, the growth of mycelium decreased significantly ($P \geq 0.05$) with the increase in Chlopyriphos concentrations in all tested fungi. Although 10 ppm was less effective than the higher levels of the insecticide. Highly significant ($P \geq 0.05$) decrease in mycelial fresh weight was observed at 100~250 ppm of Chlopyriphos in all studied fungi, as compared with the control treatment. At low level (10 ppm) of Chlopyriphos, mycelial growth decreased significantly ($P \geq 0.05$) in F. moniliforme and T. herbarum while the reduction in mycelial growth of A. flavus and A. fumigatus did not statistically differ from the control treatment. The results revealed that the fungal growth decreased with the increase in pesticide concentrations, which is in agreement with Abd-Elaah [36] who found that Chlopyriphos sharply reduced the growth of Saprolegnia ferax, Achlya proliferoides and Dictyuchus sterilis. The effect of insecticides on the inhibition of mycelial dry weight of Aspergillus fumigatus and Fusarium moniliforme was also observed by Botelho and Moriteiro [37]. They reported that the rate of inhibition to be also influenced by the type of the fungus, age of the mycelium and concentration of the pesticides. The results indicated that the Chlopyriphos treatments stimulated the Go-T activity in A. flavus and inhibited it in A. fumigatus, as compared with the respective control values (Table 4). The activity of Go-T in F. moniliforme was higher than that of the control at 10~150 ppm of the insecticide, while it was inhibited at the higher level (250 ppm). In T. herbarum, the lowest level of Chlopyriphos (10 ppm) greatly stimulated the Go-T activity, while its activity decreased with the increase of Chlopyriphos concentration. The above results revealed that the insecticide Chlopyriphos stimulated the activity of Go-T in A. flavus, F. moniliforme and T. herbarum especially at low doses. The inhibitory effect was prominent in case of A. fumigatus, indicating that this fungus was more sensitive to this insecticide than the other tested fungi This findings therefore [17] suggest that microorganisms can develop the ability of degrade pesticides either by enzyme induction or by mutation.

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Table 1: Fungi isolated from Decayed Saccharrum officinarum on 20 g/l glucose and 80 g/l sucrose Czapeck's agar media

General Analysis	Glucose-medium				Sucrose-medium			
	TC	TC %	F %	Season*	TC	TC %	F %	Season*
A. awamori Nakazawa (Usami)	16	1.1	20.0	R&D	28	2.5	40.0	R&D
A. niger Van Tieghem	433	30.4	95.0	R&D	286	25.6	85.0	R&D
A. ustus (Bain.) Thom & Church	63	4.4	70.0	R&D	8	0.7	20.0	D
A. flavus Link	125	8.8	80.0	R&D	61	5.5	60.0	D
A. versicolor (vuill.) Tiraboschi	-	-	-	-	19	1.7	20.0	R&D
A. oryzae (Ahlb) Cohn	-	-	-	-	4	0.4	10.0	D
A. terreus Thom	8	0.6	15.0	D	6	0.5	20.0	D
A. terricola Marchal	-	-	-	-	2	0.2	5.0	D
A. wentii wehmer	5	0.41	0.0	D	-	-	-	-
A. japonicus Saito	2	0.1	5.0	R	-	-	-	-
Apodachlya brachynema (Hildebrand)	9	0.6	10.0	D	-	-	-	-
Achlya megasperma (Humphrey)	4	0.3	10.0	R	2	0.2	5.0	R
Achlya americana (Humphrey)	2	0.1	5.0	R	-	-	-	-
Allomyces macrogynous (Emerson &	5	0.4	10.0	R	-	-	-	-
Willson)	-	-	-	-	2	0.2	5.0	D
Acremonium furcatum F.et V.Moreau	2	0.1	5.0	R	21	1.9	15.0	R&D
Curvularia tetramera (Mckinney) Boedijn	8	0.6	10.0	D	17	1.5	30.0	R
Cunninghamella elegans Lendner	-	-	-	-	28	2.5	30.0	R
Cladosporium herbarum Link ex Fr.	56	3.9	45.0	R	6	0.5	10.0	D
Colletotrichum dematium (Pers.ex Fr.)	-	-	-	-	16	1.4	25.0	R&D
Cephalosporium curtipes (Saccardo)	169	11.9	75.0	R&D	28	2.5	25.0	R&D
Emericella nidulans (Edam.) Vuill	21	1.5	40.0	D	-	-	-	-
Eurotium chevaliere Mangin					14	1.3	25.0	D
Fusarium moniliforme Shled.	-	-	-	-	29	2.6	40.0	D
Mammaria echinoeotryoides Cesati	-	-	-	-	2	0.2	5.0	R&D
Mortierella polycephala Coemans	9	0.6	20.0	D	4	0.4	5.0	D
Melanospora fallax Zukal	6	0.4	10.0	R	-	-	-	-
Moncillium mucidum W. Gams	-	-	-	-	23	2.1	20.0	R
Mucor circinelloides Van Tiegh	20	1.4	40.0	R&D	83	7.4	50.0	R
M. heimalis Wehmer	-	-	-	-	2	0.2	5.0	D
M. plumbeus Bon	-	-	-	-	85	7.6	70.0	R
M. racemosus Fres.	14	1.0	20.0	D	-	-	-	-
Gliomastix cerealis (Kart.) Dickinson	113	7.9	90.0	R&D	55	4.9	20.0	D
Torula herbarum Link ex Fr.	-		-	-	-			
T. grisea	276	19.4	85.0	R&D	180	16.1	80.0	R&D
Trichoderma viride Pers.ex Fr.	-	-	-	-	10	0.9	10.0	R
Stemphylium piriforme Wallroth	-	-	-	-	39	3.5	30.0	R&D
Saccharomyces spp.	-	-	-	-	12	1.1	20.0	R
Penicillium luteum (Zukal)	26	1.8	35.0	R&D	-	-	-	-
Pilobolus sp Van Tieghem.	19	1.3	30.0	R&D	16	1.4	20.0	D
Pythium intermedium (de Bary)	-	-	-	-	2	0.2	5.0	D
Py. aphanidermatium (Drechsler & water	-	-	-	-	4	0.4	10.0	R
house)	3	0.2	5.0	D	5	0.5	10.0	D
Verticillium tenerum Link	-	-	-	-	4	0.4	5.0	D
Rhizopous stolonifer (Fhrenb) Lindat	-	-	-	-	4	0.4	5.0	D
Humicola fusco-atra Traaen	-	-	-	-	2	0.2	5.0	R
H. grisea Traaen								
Helminthosporium sativum (Pammel, King and Bakke)								

TC: total counts, TC%: percentage of total counts, F%: frequency of occurrence.
*R= Raining and D = Dry
- = Not Isolated

Table 2: Visual estimation of mycotoxins in Saccharrum officinarum samples

Fungal species	Toxin production							
	Aflatoxins				Zearalenone		Diacetoxy-scripenol	
	B1	B2	G1	G2				
Fusarium moniliforme			-	-	-	-	+	+
Aspergillus flavus			+	+	+	+	-	-
A. fumigatus			-	-	-	-	-	-
A. solani			-	-	-	-	-	-
Emericella nidulans			-	-	-	-	-	-
Colletotrichum dematium			-	-	-	-	-	-

+ = Present,
- = Absent

Table 3: The effect of different concentration of Chlopyriphos on mycelial growth on fungi associated with decay of Saccharrum officinarum

Name of pathogenic fungi	Mycelial weight of fungi (g) at Different rate (ppm) of chlopyriphos						
	0.0	10	50	100	150	200	250
Aspergillus fumigatus	4.50	03.10	02.60	02.00	01.60	01.20	1.00
Aspergillus flavus	6.00	4.83	03.71	03.41	02.41	02.00	01.50
Aspergillus solani	10.10	08.34	07.00	05.70	04.53	04.00	02.71
Torula herbarium	12.43	10.26	08.46	06.33	05.00	04.41	03.53
LSD 0.05	01.11	01.18	01.00	01.13	0.08	0.03	0.02

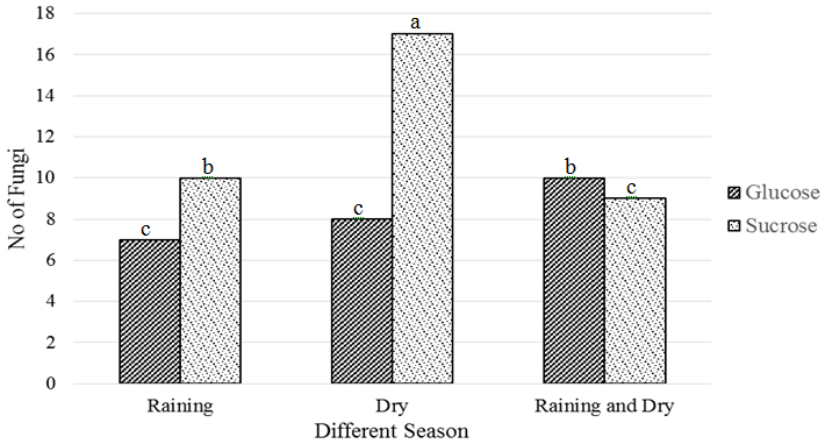
Table 4: Mycelial fresh weight and Glutamic-oxaloacitic acid (GO-T) content in the presence of different concentrations of Chlopyriphos

Conc. (ppm)	A. fumigatus		A. flavus		F. moniliforme		T. herbarium	
	Fresh weight (mg)	GOT µ/l	Fresh weight (mg)	GOT µ/l	Fresh weight (mg)	GOT µ/l	Fresh weight (mg)	GOT µ/l
0.0	4.1	14.0	5.7	8.0	8.0	8.0	7.2	14.0
10	3.8	10.0**	4.0	9.0	5.4*	11.0**	4.7*	18.0**
50	2.6**	10.0**	3.1**	11.0**	4.3**	9.5*	4.0**	21.4*
100	1.8*	9.0**	2.0**	14.5**	2.3**	9.5*	2.0**	24.0*
250	1.1**	8.5**	0.9**	17.2	1.5**	11.7	1.1**	27**
LSD 0.05	1.13	1.90	1.60	1.01	2.26	1.35	2.10	5.29
0.01	1.48	2.49	2.10	1.32	2.96	1.77	2.75	6.93

*, **: Significant and highly significant values as compared with the control treatment.

Table 5: The effect of Chlopyriphos on glutamic-oxaloacetic content of commonly occurring Fungi Associated with Decayed Saccharum officinarum.

Name of fungi	GOT(Unit/Gfw) At Different Concentration of Chlopyriphos (ppm)				
	0.0	10	50	100	250
Torula herbarum	15.03	18.11	21.78	24.00	27.89
Fusarium moniliforme	15.61	17.30	19.36	22.00	24.76
Aspergillus fumigatus	15.61	11.00	09.31	06.93	04.71
Aspergillus flavus	15.00	20.03	23.38	27.88	30.50
LSD 0.05	00.02	00.71	0.04	0.03	0.05



Data having similar alphabet are not significantly different at 5% level of probability using Duncan Multiple Range Test (DMRT)

Fig 1: Effect of Seasons on Numbers of Fungi Associated with Decayed Saccharrum officinarum