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Seasonal impacts on antifungal activity and chemical composition of extracts from medicinal plants *Turraea holstii* and *Clausena anisata*

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Curative dependence on season of harvest for medicinal plants is an alleged claim by traditional health practitioners. This study aimed to verify these claims by investigating antifungal activity and chemical profiles of two traditionally used medicinal plant species: *Turraea holstii* and *Clausena anisata* harvested in the rainy and dry seasons, with a view of establishing appropriate the season for optimal activity. The antifungal activities were determined by Broth micro-dilution method, while chemical profiling of the extracts from the plant materials was done by gas chromatography (GC). Results indicated that extracts of plant materials harvested in dry season showed enhanced antifungal activity as compared to extracts of plant materials harvested in the rainy season, highest potency being 0.39 mg/mL, observed on dichloromethane fractions of both *T. holstii* and *C. anisata*. The GC chromatograms showed a general increase in the number and amount of chemical species for extracts of plant materials harvested in dry season as compared to extracts of plant materials harvested in the rainy season. Thus, it is concluded that because the dry season produces the best curative activity, harvesting should focus on this season.

Key words: Chemical profile, antifungal activity, extracts, seasonal impacts.

INTRODUCTION

Fungal diseases affect more than a bilion people worldwide, mostly in the tropic and subtropic regions (Bongomin et al., 2017). Factors contributing to fungal growth and dissemination are poor hygiene, immunodeficiency, sweating and poor nutrition, which are characteristics of tropical developing countries like Tanzania (Charles, 2009).

Management of these infections using traditional herbal remedies is common in developing countries, owing to easy access and affordability of phytomedicines, especially for the low income population. Previous reports have unveiled over a hundred plant species used in Tanzania to manage superficial fungal infections, some of which have been analyzed for their antifungal activity

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(De Boer et al., 2005; Hamza et al., 2006; Runyoro et al., 2006; Maregesi et al., 2008; Ochanga and Chacha, 2016; Mbunde et al., 2019).

Factors that are frequently varied to optimise activity of plant extract are usually extracting solvent and plant part (Hamza et al., 2006). However, time of plant material harvest is also a factor that influence efficacy, but this is often overlooked despite tales from traditional health practitioners that some herbs are more effective when harvested at specific times of the year (Hussain et al., 2010).

Turraea holstii Gurke (Meliaceae) is used traditionally in Tanzania to treat fungal infections, particularly fungal ulcers and oral candidiasis (Hamza et al., 2006). *Clausena anisata* (Willd) Hook (Rutaceae) stem bark, roots, and leaves are used in Tanzania against oral candidiasis and fungal infections of the skin (Hamza et al., 2006). The leaves of the two plants were used in formulating medicinal concoctions and their minimal effect in survival of the plant after harvest (Hamza et al., 2006; Hilonga et al., 2018). This study investigated seasonal effect on the chemical composition and antifungal activity of leaves of *C. anisata* (Rutaceae) and *T. holstii* from the Mafinga district of Iringa regions in Tanzania.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *C. anisata* and *T. holstii* were collected from Mafinga district of Iringa regions in Tanzania in the middle of the rainy season (on the 21st of March 2015); and middle of the dry season (on 29th of July 2015). Collections were made on the same locations for both seasons. The plants were identified by Mr Haji Omari Selemani, a botanist at the Department of Botany, University of Dar es Salaam. Voucher specimens number FM011/2005 for *C. anisata* and voucher specimens number FM012/2005 for *T. holstii*, are kept at the herbarium of the Institute of Traditional Medicine of the Muhimbili University of Health and Allied Sciences.

Extraction

For each plant material, 1 kg was soaked in 4 L of ethanol for 24 h then filtered, followed by subsequent removal of solvent using a rotary evaporator under vacuum. The process was repeated three times for each plant material to get the crude extracts. Fresh solvent was used for each of the repeated extraction. Volumes of sovent, soaking time and extraction enviroment were kept the same for each plant material (Bandar et al., 2013).

GC analysis of crude extracts

GC analysis was performed on Varian CP-3800 Gas Chromatograph fitted with an autosampler. The procedure described by Gomathi et al. (2015) with minor adjustments was adopted. Nitrogen was used as a carrier gas. The crude extract (10 mg) was dissolved in 1 mL of ethyl acetate then filtered by Agilent Captiva Premium Syringe Filters before injecting 12.5 µL to the HP-5 column of 30 m length and 0.32 mm internal diameter µm. The injector temperature was set at 250°C and the carrier gas flow rate set at 18 psi. The oven temperature was programmed to rise from 70 to 180°C at a rate of 30°C min^{-1} (3.67 min), then 180 to 280°C at a rate of 4°C (25 min) and then left at 280°C for 3 min. Total elution time was 31.67 min. Flame ionization detector (FID) at 280 was used to detect the eluents.

Partitioning of extracts

The crude extracts of *C. anisata* from the two seasons were each disolved in 250 mL of 9:1 H_2O/MeOH. To separate the alkalodal fraction from non alkaloidal contents, alkaloids were converted to alkaloidal salts using method described by Kam et al. (1999). 5 mL of 2M HCl was added dropwise with stirring followed by vigorous shaking. The solution was then partitioned successively with pet-ether, dichloromethane and ethyl acete to obtain the non-alkaloidal pet-ether soluble fraction, dichloromethane soluble fraction and ethyacetate soluble fraction. The residual solution basefield with 7 mL of 2 M ammonia solution then partitioned with 250 mL dichloromethane, followed by ethylacetate to get the alkaloidal fractions.

The crude extract of *T. holstii* from each season was dissolved in water then partitioned sequencially with pet-ether, dichloromethane and ethylacetate to get pet-ether soluble fractions, dichloromethane soluble fractions, ethylacetate soluble fractions and residual water fractions.

Solvents were removed using rotary evaporator to get the dry fractions.

Antifungal assays

In-vitro antifungal tests on extracts and partitioned fraction were carried out using broth microdilution method (Moshi et al., 2009). The extracts samples were serially diluted in 96 well microtitre plates. Each well contained 100 µl of the test sample dissolved in dimethyl sulfoxide and 100 µl of inoculated brooth containing fungi to make final to concentrations of 25, 12.5, 6,25, 3.12, 1.56, 0.78, 0.39 and 0.19 mg/mL for each sample. These were incubated for 24 h at 37°C, after which 40 µl of 0.2% nitrotetrazolium chloride was then added to each well and the plates were read 2 h letter. Minimum inhibition conentrations (MICs) were recorded in the last wells that did not show blue coloration. Samples were duplicated in the 96 well microtitre plates. Dimethyl sulfoxide and Fluconazole were also included and treated in the same manner as samples. Fungi in the form of yeast, *Candida albicans* (ATCC 90028) and *Cryptococcus neofarmans* (ATCC 90112) were used as pathogens for antifungal tests.

RESULTS AND DISCUSSION

The observed antifungal activities for *T. holstii* and *C. anisata* extracts (Table 1) range from strong to weak activities, as compared to the recommended rating of antifungal activity which classify activity of MIC less than 0.5 mg/mL as strong activity, MIC of 0.6 to 1.5 mg/mL as moderate activity and MIC above 1.6 mg/mL as weak activity (Maregesi et al., 2008). Strong activity was observed for dichloromethane fractions of *T. holstii* harvested in dry season and dichloromethane fractions of *C. anisata* harvested in rainy season, both with MIC of 0.39 mg/mL. These activities validate the use of *T. holstii* and *C. anisata* extracts in treatment of fungal infections

Table 1. Results for comparison of antifungal results.

Extract/Fraction	Antifungal activity (MIC, mg/mL)				Season with superior activity
	Cryptococcus neoformans		*Candida albicans*		
	March-(RS)	July-(DS)	March-(RS)	July-(DS)	
T. holstii crude extract	3.12	3.12	3.12	6.23	RS (*C. albicans*)
C. anisata crude extract	3.12	1.56	0.78	0.78	DS (*C. neoformans*)
T. holstii pet-ether fraction	3.12	3.12	6.25	6.25	-
C. anisata pet-ether fraction	3.12	3.12	-	-	-
T. holstii DCM fraction	3.12	3.12	3.12	0.39	DS (*C. albicans*)
C. anisata DCM fraction	3.12	3.12	0.39	0.78	RS (*C. albicans*)
T. holstii Et Ac fraction	1.56	0.78	-	-	DS (*C. neoformans*)
C. anisata Et Ac fraction	1.56	0.78	3.12	3.12	DS (*C. neoformans*)
T. holstii water fraction	3.12	6.25	-	6.26	-
C. anisata alkaloidal fraction	3.12	3.12	6.25	3.12	DS (*C. albicans*)
Brooth	-	-	-	-	
DMSO (blank)	25.00	25.00	25.00	25.00	--
Fluconazole	0.0003	0.0003	0.0003	0.0003	-

RS: Rainy season; DS: dry season; DCM: dichloromethane; Et Ac: ethyl acete.

and uncover the two plants as potential source of compounds which can be used as templates for discovery of new fungal medication.

The highest difference being observed for *T. holstii* dichloromethane fraction on *C. albicans*, where the activity observed for dry season sample was 0.39 mg/mL, being three folds stronger than the activity observed for the sample harvested in rainy season, 3.12 mg/mL. This can be attributed to the increase in the observed number chemical species in dry season as compared to the rainy season, where both *T. holstii* and *C. anisata* had four extra peaks for chemical species in the dry season (Table 2 and Figures 1 to 4). The findings are supported by Harbowy and Balentine (1997), who observed in their study of tea chemistry, that biosynthesis of phenolic compounds in tea leaves is effectively induced by sunlight, noticing decreased concentrations of total phenolics in shaded tea flushes as compared to those exposed to the sunlight. Similar findings were also observed in the study of chemical contents of *Jatropha curcus* during summer and winter seasons, where it was noticed that the plant contains more phenols, tannins and free amino acids during summer (Tomar et al., 2015). On the basis of this information, the dry season which has more sunlight is expected to have more chemical contents than the rainy season, as observed in the results. The differences are not only observed in the number of chemical species, but also the amounts of the chemical species observed in both seasons. A chemical species corresponding to the peak at t_R 21.8 appears in *C. anisata* is observed in both rainy and dry seasons, but the intensity observed in dry season extract (6.91%) is about three times than one observed in rainy season extract (2.35%). This implies that the compound represented by this peak is found in large amounts in dry season. It can therefore be concluded that medicinal plants harvested in dry season when there is more sunlight are likely to have more chemical contents and higher medicinal potency than those harvested in rainy or cloudy seasons.

Conclusion

The extracts from *T. holstii* and *C. anisata* harvested in dry season showed enhanced antifungal activity and increased chemical content. This makes the dry season to be ideal season for harvesting the plants if optimal effectiveness is desired when used in managing fungal infections. Isolation, identification and antifungal studies of the compound from the two plants extracts obtained from the dry season are recommended for the future.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Table 2. Comparison of GC chromatograms for extracts of *T. holstii* and *C. anisata* harvested in rainy season (March) and dry seasons (July).

T_R (min)	*T. holstii extract*		T_R (min)	*C. anisata extract*	
	% on March extract-Rainy season	% on July extract-Dry season		% on March extract-Rainy season	% on July extract-Dry season
1.07*	88.7	88.7	1.07*	88.7	88.7
1.81	0.16	0.09	1.10	4.56	-
3.90	0.22	0.09	1.79	0.10	-
4.05	0.41	0.25	4.09	0.10	-
4.10	0.18	-	4.29	0.13	0.08
4.24	-	0.11	4.38	-	0.11
4.29	0.25	0.12	4.44	0.32	0.28
4.39	0.15	0.22	4.53	0.08	0.24
4.45	0.35	0.60	4.64	-	0.22
4.49	0.50	0.44	5.00	0.11	0.09
4.54	0.31	0.25	5.15	-	0.07
4.64	0.30	0.63	5.33	-	0.08
4.85	-	0.13	5.52	0.08	-
5.12	0.20	0.11	5.63	0.06	-
5.15	0.15	-	5.85	-	0.06
5.33	0.24	0.26	5.93	-	0.26
5.69	0.40	0.23	7.01	-	0.06
5.77	0.15	0.03	7.18	-	0.08
5.82	0.16	0.10	7.44	-	0.08
5.93	0.43	0.69	7.71	-	0.09
6.08	-	0.12	9.98	-	0.06
6.3	0.23	0.23	8.72	0.31	0.23
6.55	0.18	0.04	9.22	0.19	0.08
6.72	0.28	0.07	11.08	0.86	0.70
7.00	0.15	0.19	11.36	0.08	-
7.30	-	0.13	11.47	0.36	0.25
7.45	0.30	0.19	11.88	0.10	-
7.72	0.20	0.21	12.00	0.34	0.10
7.97	0.23	0.09	15.78	0.10	0.20
8.26	0.18	0.20	19.32	-	0.22
8.74	0.65	0.68	20.07	0.08	0.10
11.10	1.09	0.85	20.16	-	0.08
11.38	-	0.17	21.80	2.35	6.91
11.48	0.20	0.59	23.00	0.07	0.06
11.91	-	0.26	23.89	0.15	-
14.07	0.43	0.87	25.11	0.10	-
18.07	0.25	0.40	25.87.	0.11	-
19.94	0.57	1.13	28.90	0.24	0.34
21.75	-	0.35	30.46	-	0.05
23.87	-	0.06	-	-	-
24.87	-	0.12	-	-	-
25.85	0.84	-	-	-	-
28.14	0.30	-	-	-	-
28.55	0.15	-	-	-	-
30.4	0.18	0.09	-	-	-
31.11	0.33	0.13	-	-	-

*Solvent peak (ethyl acetate).

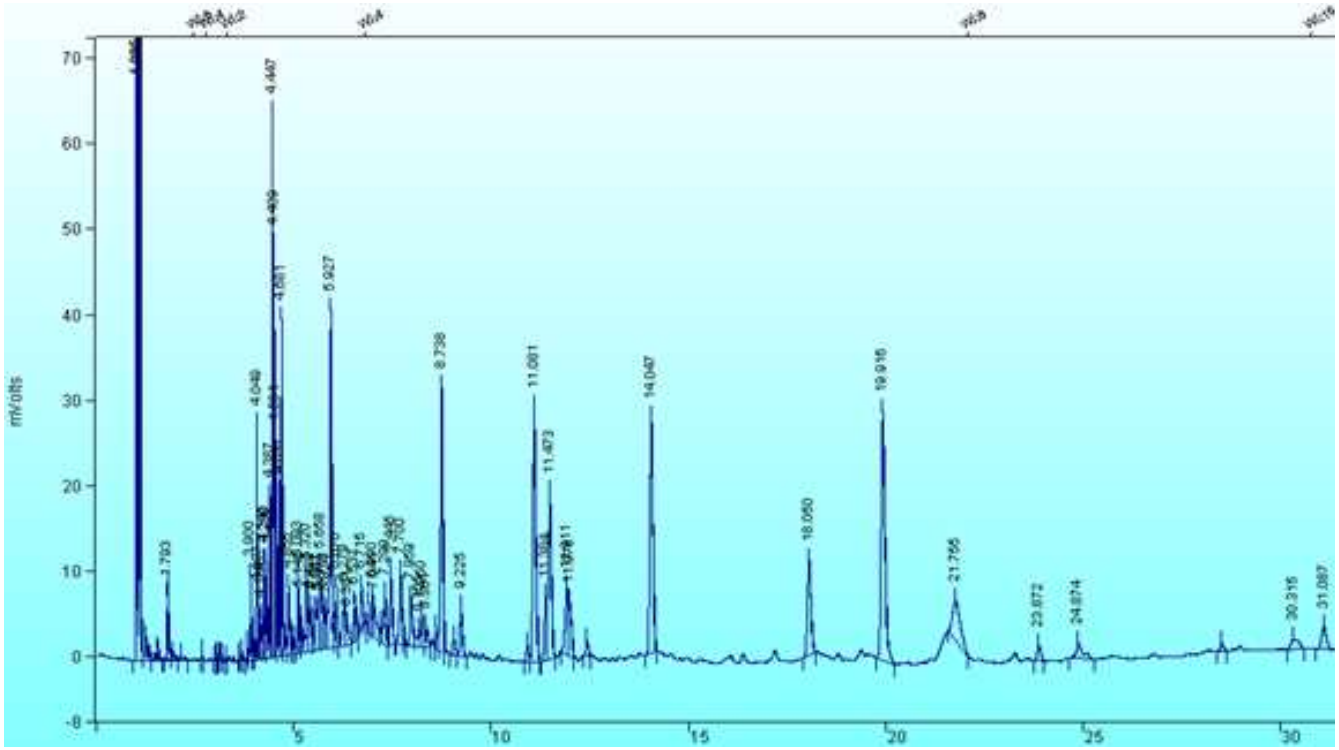

Figure 1. GC chromatogram of *T. holstii* crude extract harvested in dry season.

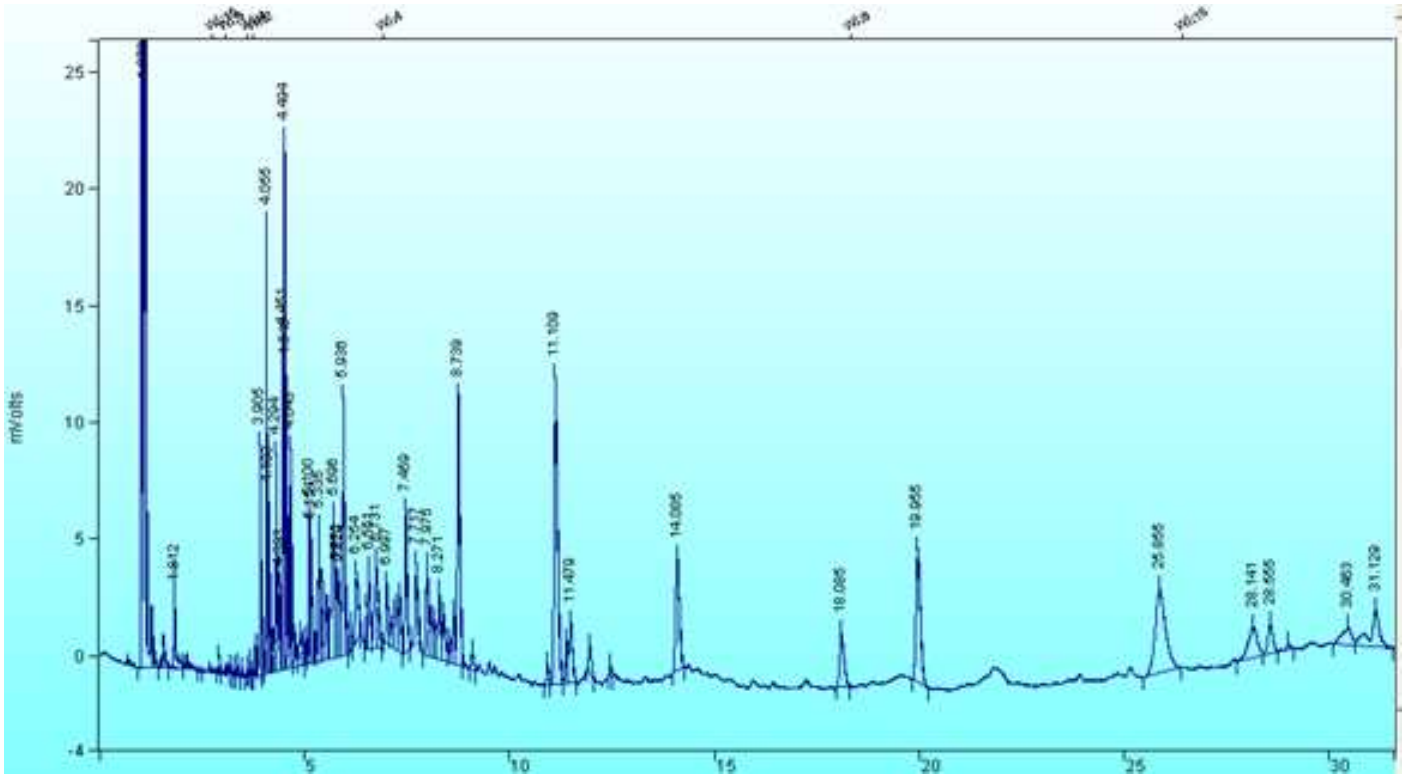

Figure 2. GC chromatogram of *T. holstii* crude extract harvested in rainy season.

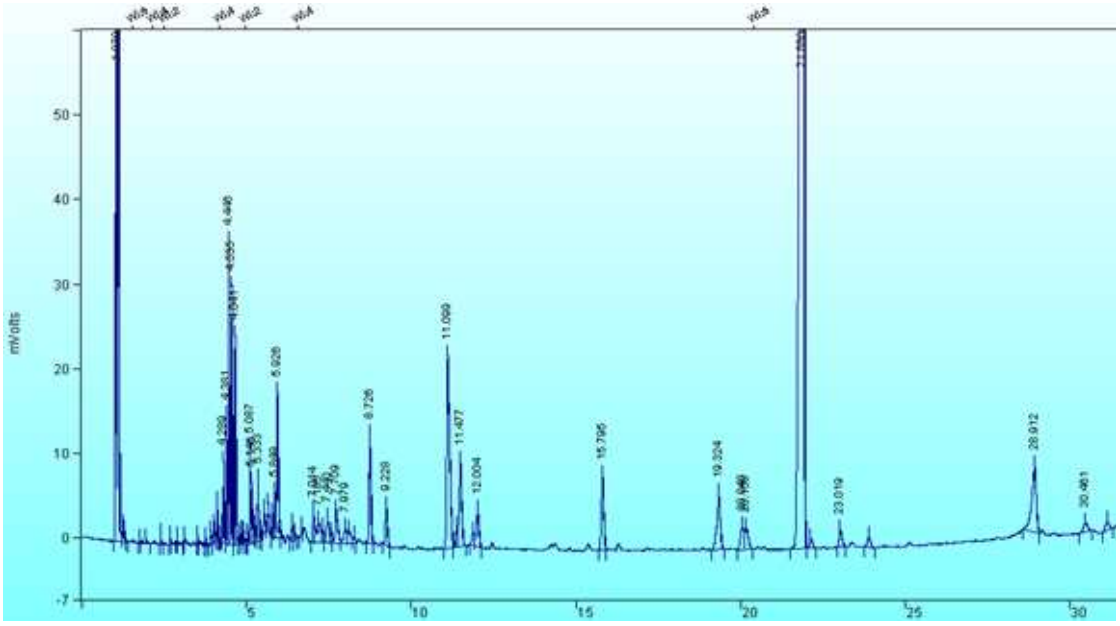

Figure 3. GC chromatogram of *C. anisata* crude extract harvested in dry season.

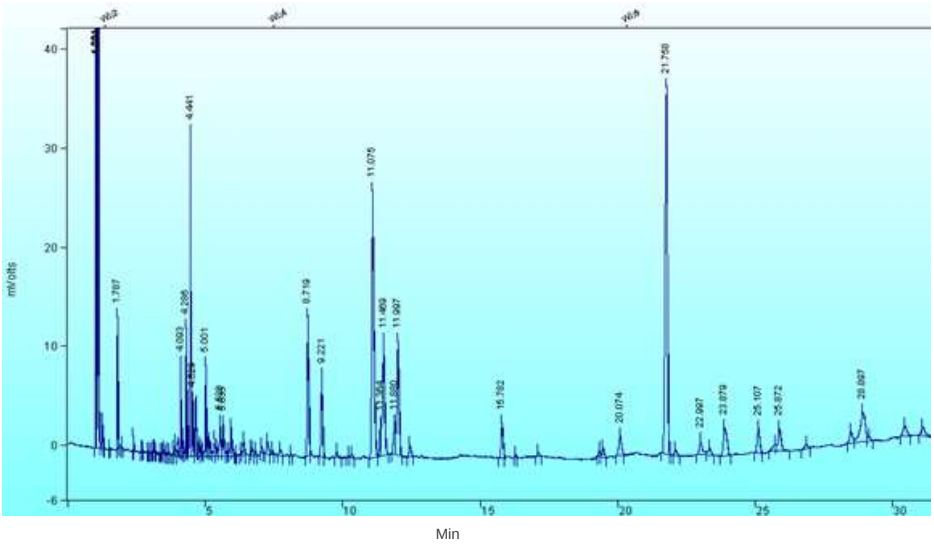

Figure 4. GC chromatogram of *C. anisata* crude extract harvested in rainy season.

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