

EFFECT OF STORAGE FUNGI ON THE DECAY OF CHILLI FRUITS

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Based on their frequency of the total isolates, ten spp. of storage fungi of chilli fruits were selected to observe their effect on decay, storing them at varying RH at 30±0.5°C for a period of 30 days. Consequently, it was found that all the spp of fungi inflicted discoloration of the pericarp and mouldiness, loss of capsaicin and dry wt besides lessening the strength of detachment of the pedicel from the pericarp. These changes were magnified proportionately with rise of RH level of storage. *Aspergillus flavus* was found to play the leading role to inflict above changes.

Keywords: Capsaicin; Chilli fruits; Detachment of pedicel; Loss in weight; Mouldiness; Pigments; Storage fungi;

Introduction

The fungi inflicting decay of dry chilli fruits during storage in India was reported earlier by collecting the stored and decayed samples from the markets of twenty places covering sixteen states¹. Among the symptoms, discoloration, detachment of pedicel from the fruit and breakage of the pericarp, and loss in capsaicin content were distinct. In the present paper ten common spp of fungi of stored chilli fruits were selected, to observe their effect on discoloration, loss in dry wt, loss in pigment, loss in capsaicin and detachment strength of the pedicel.

Materials and Methods

Freshly harvested chilli fruits var 'Sabaur Arun' was dried at 60° C for 20hr and desiccated for next 72 hr over fused calcium chloride in sealed desiccators. The lot was weighed and reweighed till the weight became constant. This was considered as the method for determining the moisture content of the fruits and method of their sterilization too. A triplicate lot of 20g of chilli fruits sterilized as above was set for all the noted particulars. The spore suspension of *Fusarium sporotrichoides* Sherb, *F. oxysporum* Schlecht, *Aspergillus candidus* Link, *A. nidulans* (Eidam) Wint, *A.flavus* Link ex Fries, *A. niger* Van Tieghem, *A. terreus* Thom, *Curvularia pallescens* Boedijn, *Memnoniella echinata* (Rivolta) Galloway and *Cladosporium cladosporioides* (Fres) de Vries was prepared growing them on Zapek Dox Agar medium for 7 days at 28±0.5° C.

The number of spores in sterilized distilled water was adjusted to 1x10⁵ /ml of suspension by counting with

haemocytometer. 20g lot of the sterilized chilli fruit was infested with 0.5ml of the spore suspension with specially designed atomiser keeping in sterilized desiccators to prevent dispersal of spores and maintain a closed container. Triplicate of fruit lot was prepared for each item of the parameter besides uninfested control. Both the lots were stored over 60, 70, 80 and 90% RH maintained by glycerol² in sealed desiccators at 30±0.5°C for 30 days. The equivalent moisture (EM) of the fruit was determined

by the formula $\frac{W_1 - W_2}{W_1} \times 100$ where W_1 = wt of stored

fruit at different RH, W_2 = wt of fruit after dryness. The EM at increasing RH was found to be 8.62, 12.93, 16.68 and 19.76%, respectively.

The symptoms of decay i.e discoloration (DC) of the pericarp of stored fruit and mouldy growth (MG) on the surface were recorded by visual observation and using magnifying glass (10X) and the result was tabulated (Table1). The loss in dry wt of the stored chilli fruit was calculated by drying the lot as noted earlier and the mean of triplicate was recorded (Table 2).

Extraction, identification and elution of pigments of the pericarp - One g powder of the pericarp dried as above was extracted with 100 ml of petroleum ether (B.P.60 - 80 °C) for 3 hr with Soxhlet extractor warming it on sand bath. The pigment extract was stored at 5°C in small conical flasks tightly sealed with polyethylene sheet. Thin layer chromatography was adopted using calcium carbonate as adsorbant and petroleum ether (B.P.60 - 80 °C) as solvent

Table 1. Various degree of discoloration of the pericarp and mycelial growth due to storage of fruits with fungi at varying RH at 30±0.5 °C for 30 days.

Fungus spp	Discoloration and mycelial growth	RH(%)			
		60	70	80	90
<i>A. flavus</i>	DC	OC	O	P	FP
	MG	-	+	++	+++
<i>A. niger</i>	DC	OC	C**	***	***
	MG	-	+	++	+++
<i>A. nidulans</i>	DC	R	OC	O	P
	MG	-	-	+	++
<i>A. candidus</i>	DC	OC	OC	O	P
	MG	-	-	+	++
<i>A. terreus</i>	DC	OC	O	P	FP
	MG	-	+	++	+++
<i>F. sporotrichoides</i>	DC	OC	O	P	FP
	MG	-	+	++	+++
<i>F. oxysporum</i>	DC	OC	O	P	FP
	MG	-	+	++	+++
<i>C. pallescens</i>	DC	OC	OC	P	P
	MG	-	+	++	+++
<i>M. echinata</i>	DC	OC	OC	O	P
	MG	-	+	++	+++
<i>C. cladosporioides</i>	DC	OC	O	P	FP
	MG	-	+	++	+++
Control	DC	ND	ND	OC	OC
	MG	-	-	-	-

DC = Discoloration, OC = Off- coloration, R= Red, O = Orange, P = Pink, FP = Faint pink, ND = No discoloration, MG = Mycelial growth, - = Absent, + = Present, increasing number of + sign indicates increasing degree of mycelial growth, ** = Pericarp slightly blackened, *** = Pericarp blackened.

for ascending chromatography modifying the column chromatography of Laszto Chohnoly³. 20µl concentrated pigment solution was spotted and the set of the chromatography was kept in the dark to prevent fading of colour of the pigment and run was continued for 4hr. The spots of Capsorubin (red, hRf 26), Capsanthin (pink, hRf 36), Carotene (orange, hRf 53) and Xanthophyll (yellow, hRf 91) were scrapped with scalpel, extracted with solvents and read at 486, 504, 425 and 425 nm, respectively^{4,5}. The O.D. was recorded (Table3) as measure of concentration of the pigment in the pericarp.

Determination of capsaicin-Capsaicin was measured

Table 2. Loss in dry wt of chilli fruit due to storage of fruits with fungi at varying RH (%) at 30±0.5 °C for 30 days (Expressed as mean per cent wt)

Fungus spp	RH (%)				CD Value
	60	70	80	90	
<i>A. flavus</i>	10.85	14.76	23.11	30.22	0.196
<i>A. niger</i>	7.52	13.37	18.68	24.80	0.137
<i>A. nidulans</i>	8.54	10.94	14.39	19.56	0.143
<i>A. candidus</i>	10.37	13.78	19.20	24.11	0.185
<i>A. terreus</i>	9.38	12.81	15.16	18.66	0.169
<i>F. sporotrichoides</i>	9.78	12.88	17.39	22.53	0.095
<i>F. oxysporum</i>	9.48	12.58	16.61	23.25	0.158
<i>C. pallescens</i>	9.26	12.27	14.79	18.76	0.184
<i>M. echinata</i>	8.76	12.01	17.97	23.73	0.208
<i>C. cladosporioides</i>	9.26	12.40	16.78	23.53	0.193
Control	2.27	2.59	3.02	3.68	0.342

CD at 60% RH = 0.114

extracting from the placenta of the fruit which is its sole organ. 100mg of dry powder was extracted with 25ml of acetone in Soxhlet extractor for 3 hr⁶ and final volume of the extract was made to 100ml with ethyl acetate after evaporating acetone at 50 °C at reduced pressure and quantitatively read at 430nm preparing calibration curve with standard solution of capsaicin⁷. The loss of capsaicin (%) due to storage fungi was calculated with the help of the amount in the control (Table 4).

Detachment strength of the pedicel -One of the symptoms of decay is the complete detachment of the pedicel from the fruit or vulnerable to easy detachment by slight jerk. The strength of detachment of the pedicel was measured by a self-designed instrument consisting of a pan made of very thin tin foil hanged with three fine copper wire. Fixed length (1.5 cm) of the pedicel was tied with the wire of 5 cm length and wt from lower to higher was placed over the pan. The wt at which the pedicel was found detached from the fruit was noted (Table 5).

Results and Discussion

The EM of the stored fruit increases with rise of the RH (%) level of storage. Two types of symptoms of decay of the stored fruit was noticeable in the pericarp of chilli due to storage fungi. One was DC and another as MG. Almost all the fungi inflicted off- coloration at 60 % RH, orange DC at 70, Pink DC at 80 and pink to faint pink at 90% RH. *F. sporotrichoides*, *F. oxysporum*, *A. flavus*, *A. terreus* and *C. cladosporioides* caused faint pink DC. The mouldiness became evident above 60% RH and its degree stretched with increase in the RH level. The control fruit expressed no mouldiness and no discoloration except off - coloration at 80 and 90% RH (Table1). The loss in dry

Table 3. Loss of pigments of the pericarp of chilli fruits stored with fungi at varying RH (%) at 30±0.5 °C for 30 days (expressed as O.D.)

Fungus spp	Pigments*	RH(%)			
		60	70	80	90
<i>A. flavus</i>	CR	0.058	0.052	0.042	0.027
	CS	0.030	0.025	0.018	0.009
	CT	0.015	0.012	0.007	0.001
	XP	0.010	0.007	0.004	0.000
<i>A. niger</i>	CR	0.60	0.055	0.044	0.029
	CS	0.032	0.027	0.020	0.011
	CT	0.016	0.014	0.008	0.002
	XP	0.012	0.010	0.006	0.001
<i>A. nidulans</i>	CR	0.063	0.056	0.047	0.032
	CS	0.032	0.028	0.020	0.012
	CT	0.017	0.014	0.009	0.003
	XP	0.012	0.009	0.006	0.002
<i>A. candidus</i>	CR	0.061	0.055	0.046	0.031
	CS	0.033	0.028	0.021	0.011
	CT	0.016	0.013	0.008	0.002
	XP	0.011	0.008	0.005	0.001
<i>A. terreus</i>	CR	0.062	0.058	0.047	0.033
	CS	0.034	0.029	0.022	0.012
	CT	0.017	0.015	0.010	0.003
	XP	0.012	0.009	0.007	0.002
<i>F. sporotrichoides</i>	CR	0.062	0.057	0.048	0.032
	CS	0.036	0.032	0.025	0.015
	CT	0.018	0.015	0.010	0.003
	XP	0.012	0.010	0.007	0.002
<i>F. oxysporum</i>	CR	0.063	0.057	0.048	0.033
	CS	0.035	0.032	0.024	0.014
	CT	0.017	0.014	0.010	0.003
	XP	0.012	0.010	0.006	0.001
<i>C. pallescens</i>	CR	0.061	0.056	0.046	0.031
	CS	0.033	0.028	0.021	0.012
	CT	0.017	0.015	0.009	0.002
	XP	0.012	0.010	0.005	0.001
<i>M. echinata</i>	CR	0.062	0.058	0.046	0.032
	CS	0.032	0.028	0.021	0.011
	CT	0.017	0.014	0.009	0.001
	XP	0.012	0.009	0.005	0.001
<i>C. cladosporioides</i>	CR	0.061	0.057	0.046	0.032
	CS	0.031	0.027	0.020	0.010
	CT	0.016	0.014	0.009	0.002
	XP	0.013	0.011	0.006	0.001
Control	CR	0.070	0.069	0.067	0.064
	CS	0.045	0.044	0.043	0.042
	CT	0.021	0.021	0.020	0.019
	XP	0.014	0.014	0.013	0.012

* CR = Capsorubin, CS = Capsanthin, CT = Carotene, XP = Xanthophyll

Table 4. Loss of capsaicin of the placenta of chilli fruits stored with fungi at varying RH (%) at 30±0.5 °C for 30 days (expressed as mean per cent wt)

Fungus spp	RH (%)				
	60	70	80	90	Mean
<i>A. flavus</i>	5.12	6.75	8.49	10.68	7.76
<i>A. niger</i>	4.35	5.18	6.76	8.42	6.17
<i>A. nidulans</i>	3.68	4.36	5.51	7.18	5.18
<i>A. candidus</i>	3.82	4.56	5.83	7.52	5.43
<i>A. terreus</i>	3.98	4.78	6.15	7.87	5.69
<i>F. sporotrichoides</i>	4.44	5.63	7.25	8.68	6.50
<i>F. oxysporum</i>	4.32	5.47	6.90	8.35	6.26
<i>C. pallescens</i>	4.02	4.92	5.98	8.14	5.76
<i>M. echinata</i>	3.81	4.52	5.82	7.16	5.33
<i>C. cladosporioides</i>	4.28	5.34	6.85	8.48	6.23
Control	0.62	0.78	1.12	1.35	0.96

CD = 0.882

Table 5. Strength of the detachment of the pedicel of chilli fruit due to its storage with fungi at varying RH (%) at 30±0.5 °C for 30 days (expressed as mean wt in g)

Fungus Spp	RH (%)				
	60	70	80	90	Mean
<i>A. flavus</i>	4.08	3.22	2.12	1.05	2.61
<i>A. niger</i>	4.29	3.68	2.47	1.22	2.91
<i>A. nidulans</i>	4.88	4.02	3.03	1.68	3.40
<i>A. candidus</i>	4.62	3.75	2.75	1.47	3.14
<i>A. terreus</i>	4.77	3.86	2.98	1.75	3.34
<i>F. sporotrichoides</i>	4.67	3.92	2.82	1.36	3.19
<i>F. oxysporum</i>	4.70	3.89	2.95	1.53	3.26
<i>C. pallescens</i>	4.65	3.72	2.86	1.58	3.20
<i>M. echinata</i>	4.41	3.42	2.30	1.21	2.83
<i>C. cladosporioides</i>	4.56	3.87	2.68	1.21	3.08
Control	5.78	4.90	4.09	3.26	4.51
Mean value	4.67	3.84	2.84	1.57	-
Before storage	6.37	-	-	-	-

CD = 0.191, F(Column) 53.551, F (rows) 1115.56

wt. of chilli fruits due to fungi increased with increase in the RH level of storage. Thus the loss was minimum at 60% RH and maximum at 90% RH. *A. flavus* caused maximum loss and *A. niger* the minimum. The control fruit showed negligible loss (Table 2). *A. flavus* inflicted maximum degree of DC while *F.oxysporum* and *A. nidulans* the minimum. It is noteworthy that all the storage fungi reduced the amount of individual pigment (Table 3). Maximum loss in capsaicin was recorded due to *A. flavus* and minimum due to *A.candidus*, *A.nidulans*, *A.terreus*, *C. pallescens* and *M. echinata* (Table 4) imparting statistically alike. The loss increased with rise in RH level. The detachment strength of the pedicel was minimum due to *A. flavus* (Table 5). Summarily, it appear

that *A. flavus* plays leading role in the decay of stored chilli fruits in storage if all the parameters are taken into consideration.

Proportionate increase in EM of the fruit stored under increasing RH is expected due to absorption of the water vapour more at its high concentration. It is axiomatic that EM plays important role in the growth of the storage fungi on the stored seeds, more the EM more the growth and inversely less EM less growth. Similar case is observed in chilli fruits in storage. The colour of the pericarp is normally red while white or complete discoloration shows the most advanced stage of decay progressing in degree as Red → off coloration → orange → pink → faint pink → straw colour → white¹. This increasing discoloration, thus indicates the degree of decay. Off - coloration of seed coat of soybean has earlier been reported due to *Phomopsis*². Previously it has been reported that coupled with the white discoloration, there is mouldy growth inside the fruit and accumulation of spore dust, besides loss in capsaicin. The growth of the fungi at one spot of the pericarp and depigmentation to distant part, as observed here and absence of pigment globules in the cells of the pericarp of, stored fruit, normally present in uninfested control, indicates enzymic degradation³. These changes and loss in the detachment strength of the pedicel and even its detachment and breakage of the pericarp into pieces, are the manifestations of high degree of decay of the stored fruit.

The growth of fungi on the pericarp and its mouldiness point out the enzymic dissolution of the polymers including cuticle¹⁰, cellulose and pectic substance¹¹⁻¹³. Cellulose and pectic substance are expected to be dissolved resulting in attenuation of the detachment strength of the pedicel.

The loss in dry wt is expected due to dissolution of the polymeric wall, capsaicin and other parts of the fruit and utilization of the dissolution product by storage fungi. The loss of capsaicin, the bitter principle of chilli is expected due to its decomposition as has earlier been reported as enzymic degradation of alkaloid in *Strychnos*¹⁴. The maximum loss of pigment by *F. sporotrichoides*, *F. oxysporum*, *A. flavus*, *A. terreus* and *C. cladosporioides* indicates their ability to degrade the pigment with rapid rate. Responsible for maximum loss in dry wt and capsaicin content, depigmentation and reduction in the detachment strength due to *A. flavus* points out the ability of this fungus as multidirectional and the sum total is the maximum degree of decay of chilli fruits. Negligible loss in the above items in the control fruit might be due to degradation by ambient environment.

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