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ROLE OF HYDROLYTIC FUNGAL ENZYMES IN MAIZE GRAINS DETERIORATION AND PROTECTING FUNGAL GRAIN INVASION

By

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ABSTRACT: The five major natural storage maize grain fungi; ***Aspergillus flavus,*** Link ex Fr., ***A. niger,*** van Tiegh, ***Cephalosporium maydis, Corda., Fusorium moniliforme,*** J. Ve Diagn and ***Penicllium expansum,*** Link, which previously caused decreasing in germinability, moisture content, 100 seed weight, protein % and oil% were tested for cellulose and starch decomposing ability ***in vitro***.

Data revealed that the tested fungi were able to elaborate cellulose and starch degrading enzymes. ***A.niger*** and ***A. flavus*** exhibited the maximum cellulolytic (C_1 and C_x) and amylolytic activity, followed by ***P. expansum*** and ***F. moniliforme***, while ***C. maydis*** was relatively weak in this respect. Increasing the incubation period was accompanied with an increase in the cellulose and starch decomposing ability.

Antifungal activity of organic acids, plant extracts and essential oils was evaluated ***in vitro*** and seed – protecting treatment was studied under (***in vivo.***) conditions.

Essential oils of ***Cinnamomum zeylanicum*** and ***Syzygium aromaticeum*** completely inhibited the mycelial growth of all tested fungi while ***Nigella sative*** oil has poor negative effect. Acetic acid 10% or propionic acid 10% caused the greatest inhibitory action on the fungi, but it was lower than the previous treatment.

Concerning with plant extracts, all of them were less effectiveness than essential oils or organic acids. ***Allium sativum*** extract was the highly negative effect on the fungi.

Application of the organic acids, i.e. acetic acid or propionic acid (5 or 10% w.v.) as grains soaked for 10 menutes or the grains mixed with the three essential oils at 1000mg essential oils / 1kg maize grains was done before storing grains.

The seed treating with essential oils of ***C. zeylanicum*** or ***S.***

aromaticum completely inhibitd the kernel (cvs) fungal invasion, while ***Allium sativum*** essential oil was the least effective when compared with the others.

Acetic acid or propionic acid (5% and 10% conc.) were effective in almost, eliminating the seed fungal invasion and the two organic acids at 5% conc. caused the lower effects than 10% concentration.

Authors recommend that grains must be soaked in acetic acid or propionic acid at 10% for 10 menutes or mixed the seeds with market essential oils of ***Cinnamomum zeylanicum*** (Cinnamon) or ***Syzygium cromaticum*** (clove) at 1000 mg/ kg maize grains, to prevent fungal invasion. This is a new beneficial method for protecting grains against fungal invasion instead of using fungicides.

INTRODUCTION

Maize (***Zea mays***) is concetered one of the promising cereal crops in Egypt. Fungi represent one of the major factors which usually induce discoloration and deterioration of grains during harvest and storage. It also causes great reduction in grain weight, quality characters, seed viability and seed germination (El. Shazly ***et al***, 2007).

The specificity of the storage fungi appears to be based on its ability to produce the hydrolysis enzymes and/or mycotoxins. There were a strong correlation between the high pathogenic potential of both storage fungi as a grain invaders and their high hydrolysis activity and aflatoxin level (Zedan, 1991 and Zedan and Arab, 1994).

Recent studies (Mellon ***et al***, 2002 and Subash ***et al***, 2005) reported that the utilization of the three major corn reserve materials, starch, triglycerides (refined corn oil) and zein (storage protein), by ***A. flavus, A. niger, A. versicolor, Penicillium citrinum, F. solani*** and others were monitored ***in vitro*** over a 7-day fermentation. Medium composition in initially approximated proportions in matur corn kernels changed little over the first 18 h. Subsequently, they found that hydrolysis of both starch and triglycerides occurred simultaneously with peak concentration of glucose and free fatty acids on day 2 of fermentation period. Aflatoxin B_1 production increased after 36 h. with peak at o fourth day. Maximum cellulolytic activity was recorded for ***A. niger*** and ***F. solani,*** while ***Aspergillus versicolor*** exhibited high amylolytic and gelatinolytic activity, whereas ***Penicillium citrinum*** showed only high amylolytic

activity.

The organic acids, plant extracts and essential oils were applied to prevention the seed – borne fungi because it a safety for human and animal health and to avoid hazards of fungicides. Blanehard ***et al*** (2001) compared the efficacy of propionic and two recently mixtures. They found that propionic acid emerged as the most effective on linear growth of storage fungi on PDA media and as a consistent preservative.

They also evaluated some essential oils for growth of the storage fungi and its aflatoxin production. They found that the clove oil (eugenol) was the most inhibitory to the growth of ***A. parasiticus*** and ***F. moniliforme,*** followed by cinnamon (cinnamic aldehyde). Eucalyptus oil (cineole) did not effect on the fungal growth. Also, the oils reduced the aflatoxin contamination of the grins. The feasibility of implementing this results to control the storage fungal invasion of the stored grains was recorded by (Chatterjee, 1990, Montes-Belmont and Carvajal, 1998. Juglal ***et al,*** 2002, Sonia ***et al***, 2003 and Velluti ***et al***, 2004.

The major goal of this study was adding more informations about the hydrolysis enzymes and their role in maize grain deterioration and determination the antifungal activity of some organic acids, essential oils, gaves a new method to prevent the storage fungal grains by some substances with out polluting the environment.

MATERIAL AND METHODS

Source of the causal organisms:

Pure isolates of ***Aspergillus flavus***, ***A. niger***, ***Cephalosporium maydis***, ***Fusarium moniliforme*** and ***Penicillium expansum*** were previously isolated from the storoge maize grains cvs at Sakha and El-Gemmeiza Agricultural Experimental Stations, Egypt. Seed health were previously tested by the Blotter filter paper, PDA and Wort agar medium salted with 10% (W.V.) of sodium chloride (Smith, 1969). The International Rules for Seed Testing Association (ISTA, 1993) were used for detect seed – borne mycoflora (El- Shazly *et al.*, 2007).

Hydrolysis enzymes activity of the storage maize fungi:

In order to obtain more information of the role of ***Aspergillus flavus***, ***A. niger, Cephalosporium maydis, Fusarium moniliforme*** and ***Penicillium expansum*** in maize grain deterioration, they were tested for

their enzymatic activity ***in vitro***.

A- Cellulolytic (C1 and Cx) activity:

Cellulolytic (C_1 and C_x) activity was studied by using Tolboys and Buschs, medium 1970 in which glucose was replased by filter paper and carboxymethyl cellulose (CMC), respectively. Cellulase (C_1) activity was determined as the percentage of loss in weight of filter papers in medium. Cellulase (C_x) activity was measured by estimation of the loss in viscosity of the substrate in Ostwald viscometer at 30^0C, as described by Echandi ***et al.*** (1957).

B- Starch-hydrolyzing activity:

Starch-hydrolyzing activity for the tested storage fungi was estimated using Czapek's agar medium containing 0.5% soluble starch as a sole carbon source (Collins, 1969).

Seed treatment:

a) Plant aqueous extracts preparation:

The extraction technique was carried out according to Wilson *et al.*, (1997). Seven dried plant materials (as shown in Table 1) were grounded into fine powder in a high speed micromill and soaked in distilled water at rate 1:1 (W.V) for 24 hours. Then the mixture was filtered through double layers of cheesecloth and centrifuged at 1200 rpm for 30 mim., and sterilized using membrane filter paper of pore size of 0.22 µm. The crude solution was considered as 100% concentration **(Arjunan *et al.*, 1994).**

b) Some organic acids and plant essential oils:

Two acids i.e acetic acid and propionic acid at concentration (0.5 and 10.0%).

Three market plant essential oils of ***Cinnamomum zeylanicum*** (Cinnamon), ***Syzygium aromaticum*** (clove) and ***Allium sativum*** (garlic).

All the previous prepared concentrations were tested on the fungal growth and seed protecting treatment against the fungal pathogens associated with maize grains.

1- Antifungal assay (Disk Diffusion Assay):

Filter paper disks (Whatman No. 1. 10 mm diameter) separately saturated by different plant extracts, plant essential oils and the two concs.

of organic acids were placed on growth plates with (PDA) medium which were inoculated with each fungus separately. The plates were incubated at 28^0C.

Table (1): Botanical classification and main active principles of the spices:

No	Scientific name	Family	Common name	principle
1	*Allium sativum* L	Liliaceae	Garlic	Allicin
2	*Allium cepa* L	Liliaceae	Onion	Thiopropanal – S- oxide
3	*Eucalyptus globules* L.	Myrtaceae	Camphor	Cineole
4	*Melia azdirachta* L.	Meiliaceae	Chinaberry	Tetranitoterpenoid (Meliatoxins)
5	*Nigella sativa* L.	Ranunculaceae	Nigella	Nigellone
6	*Syzygium aromaticus* L.	Myrtaceae	Clove	Eugenol
7	*Cinnamomum zeylanicum* Nees	Lauraceae	Cinnamon	Cynnamic aldehyde

As the fungal mycelium grow across the culture medium, any zone of growth inhibition surrounding the disk could be detected (Thornberry, 1950 and Ghosh *et al.*, 1978). The filter paper disk saturated with distilled sterilized water was used as control. The antifungal activity was shown on inhibition percent of fungal growth as the following equation: % Inhibition = A-B/ A x 100, where, A = Diameter of untreated fungus and B = Diameter of treated fungus.

2-Effect of seed treatments on pathogenic fungi *in vivo*:

Grains of three maize grains cvs. which were sensitive to the fungal infection (***A. flavus, A. niger, F. moniliforme and P.expansum***) of single–crosshybrid 129 (S.C.129), triple–cross 324 (T.C. 324) obtained from El-Gemmeizea A. E. S. and triple cross 352 (T.C. 352) obtained from Sakha A.E.S.

One hundred grains were soaked in each concentration (0.5 and 10.0% w.v) of organic acids i.e. acetic acid and propionic acid for 10 minutes. Also, the maize grains mixed with the three plant essential oils at 1000 mg essential oil/1kg maize grains. Treated grains subjected to the blotter test (ISTA, 1993) as previously mentioned. The incidence of observed fungi was recorded using a stereobinocular microscope. Water soaked grains were served as control. Percentages of the fungi under investigation were recorded as previously mentioned.

RESULTS AND DISCUSSION

Hydrolysis enzymes activity of the storage maize grain fungi:

In order to know more informations of the ability of the ***Aspergillus flavus, A.niger, Cephalosporium maydis, Fusarium maniliforme*** and ***Penicillium expansum*** to invade maize grains and their ability to produce certain hydrolsis enzymes ***in vitro*** and some physiological studies were used in these experiments.

Cellulolytic enzymes activity:

Data given in Table (2) indicate that all the tested fungi possess higher cellulolytic activity ***in vitro Aspergillus niger, A. flavus*** exhibited the highest level of C_1 anzyme activity (69.0and 66.9%) and (78.0 and 73.3%) decomposed cellulose after 15 and 30 days incubation period, respectively. Whereas, ***P. expansum*** and ***F. moniliforme*** revealed a moderate level activity for the same enzyme. ***Cephalosporium maydis*** ranked third level where it showed the lowest percentage, being 51.8 and 52.3% respectively.

On the other hand, culture filterate of ***A. niger*** and ***A. flavus*** exhibited the highest percentage of cellulolytic activity (C_x), being 86.4 & 85.3% and 94.4 & 91.4% after 7 and 14 days incubation period, respectively, ***P. expansum*** and ***F. moniliforme*** revealed a high level activity, while ***C. maydis*** ranked the third level in the two incubation periods.

Table (2): The activity of cellulolytic enzymes (C1 and Cx) of the tested fungi after two incubation periods.

Fungi	Relative C_1 enzyme activity after*		Relative C_x enzyme activity after**	
	15 days	30 days	7 days	14days
Aspergillus flavus	66.9	73.7	85.3	91.4
Aspergillus niger	69.0	78.0	86.4	94.4
Cephalosporium maydis	51.8	59.3	74.5	81.7
Fusarium moniliforme	55.9	63.3	75.1	82.2
Penicillium expansum	57.5	63.6	77.9	83.4
L.S.D at 5%	3.57	2.93	5.2	1.95

* = The percentage of loss in the weight of filter papers in the medium

** = The percentage of loss in viscosity of 1.2% C MC solution – PH 6.2

Generally, increasing the incubation period was accompanied with an increase in the cellulose decomposing ability. These results are in line with those obtained by Fahim *et al* (1982), Ibrahim *et al* (1986), Zedan (1991), El – Refai *et al* (1994), Krikstaponis *et al* (2001), Subash *et al* (2005) and Abo El-Dahab (2006) who found that maximum cellulolytic activity was recorded for *A. niger, A. versicolor, A. flavus, F. moniliforme, P. expansum* and *Cephalosporium majdis.* Therefore, there might be a correlation between the high pathogenic potential of both fungi as a grain invadors and their high cellulolytie activity.

Starch hydrolyzing activity:

It is obvious (Table 3) that all tested fungi were active starch-hydrolyzers. *A. flavus* and *A. niger* were the most active fungi. While, those of *C. maydis F. moniliforme* and *P. expansum* were low in this respect. The hydrolyz area increased with the increasing of incubation period. These results are in agreement with those obtained by Fahim *et al* (1982), Zedan (1991), Sauer and Pomeranz (1992) Christen *et al* (2002), Mellon *et al* (2002), Subash *et al* (2005) and Abo El-Dahab (2006) who mentioned that *A. flavus* and *A. niger* were able to produce amylase in a starch – medium. It was also found that the hydrolyzed area increased by increasing incubation period.

Table (3): Average diameter of the hydrolyzed starch area (mm.) caused by the tested fungi after tow incubation periods.

Fungi	Average diameter of the hydrolyzed starch area (mm.)	
	48 hours	96 hours
Aspergillus flavus	42	82
Aspergillus niger	33	64
Cephalosporium maydis	22	40
Fusarium moniliforme	24	41
Penicillium expansum	26	45
L.S.D. at 5%	6.18	7.15

Finally, the tested fungi were able to invade and discolour the germs of stored grains. The extent of germ discoloration or deterioration was not proportional with the magnitude of invasion or decrease in germination. These findings suggest that the reduction in seed germination, 100 seed weight, protein, oil and starch seed content depend

not only on germ invasion but also may be principally attributed to the interior changes in the grain constituents during fungal invasion. The high dynamic power of fungi in invading maize grains during storage may be correlated with their high enzymatic activity. Therefore, it may be stated that those fungi play an active role in grain deterioration.

Seed treatments:

The antifungal activity of organic acids, plant extracts and essential oils were evaluated under ***in vitro*** conditions and seed – protecting treatment carried out under ***in vivo*** conditions.

A- Antifungal activity (*in vitro*):

Data presented in (Table 4) show that the antifungal activity of propionic acid and acetic acid at 5 and 10 % concs. was moderately, which was less than 80.0% inhibition value. Acetic acid at 10% caused the highest inhibition with ***P. expansum, F. moniliform*** and ***C. maydis*** (77.9, 77.6 and 73.1), respectively while, propionic acid at 10% conc. gave the highest antifungal effect on ***F. moniliforme*** (77.1 %). These results are in accordance with those obtained by Mazzani, *et al* (1998), Kavita and Reddy (2000), Moreno- Martinez, ***et al*** (2000), Blanchard, ***et al*** (2001) and Sonia ***et al*** (2004) who reported that ***P. expansum*** was more sensitive to organic acids, followed by ***F. moniliforme*** but ***A. niger*** ranked the final in this respect.

Concerning with plant extracts, all of them were less effectiveness than organic acids caused a moderate antifungal activity against the tested fungi, which were less than 70.0%. ***Allium sativum*** caused the highly negative effect on ***F. moniliforme***, ***P. expansum*** and ***C. maydis*** (66.4, 66.0 and 64.2 %), respectively. These result are in agreement with Kshemkslyani, ***et al*** (1990). Pai and Platt (1995), Hao ***et al*** (1998), Yin and Tsao (1999) and El-Sherbiny (2001) who found that garlic juice, onion extract and Eucalyptus had negative effects on fungal growth, sporulation, spore germination and aflatoxin production of ***A. flavus***. Chalfoun, ***et al*** (2004) studied the effect of ten powdered spice plants on mycelial growth and sporulation of ***A. niger*** and ***Eurotium repens***. Clove completehy inhibited the mycelial growth of the tested fungi. The other spices; cinnamon, garlic, thyme, ment, anise, oregano and onion were in a decreasing order promising antifungals. Basil did not show a pronounced fungistatic effect. Cinnamon and anise totally inhibited the production of B_1 and B_2 aflatoxin. Basil inhibited the synthesis of aflatoxin starting.

Table (4):Antifungal activity of some aqueous plant extracts, essential oils and organic acid on culture growth of tested fungi.

Treatments		Inhibition %					Average
	Fungi	*Aspergillus flavus*	*Aspergillus niger*	*Cephalosporium maydis*	*Fusarium moniliforme*	*Penicillium expansum*	
Organic acid	Acetic acid 10%	68.1	67.2	73.1	77.6	77.9	72.9
	Acetic acid 5%	61.3	62.6	64.7	86.3	74.2	66.2
	Propionic acid 10%	69.4	62.6	72.6	77.1	73.1	71.0
	Propionic acid 5%	58.6	59.0	63.6	65.6	71.0	63.5
Plant extracts	*Allium cepa*	62.1	47.8	52.1	58.3	60.3	56.1
	Allium sativum	59.3	54.9	64.2	66.4	66.0	62.2
	E. globules	57.4	41.9	48.6	69.0	54.1	54.2
	Melia azdirachta	51.9	49.9	43.2	61.5	56.9	52.8
Essential oils	*C. zeylanicum*	100	100	100	100	100	100
	S. aromaticum	100	100	100	100	100	100
	Allium sativum	58.1	56.7	65.1	67.9	66.9	62.9
	E. globules	61.4	50.7	50.7	70.3	55.6	57.4
	Nigella sativa	7.6	9.7	25.6	32.6	6.3	16.4
Average		62.7	58.7	63.3	70.4	66.3	64.3

L.S.D. at 5% fungi= 1.56
L.S.D. at 5% treatment= 1.95
L.S.D. at 5% interaction= 4.24

Our of the essential oils, ***Cinnamomum zeylanicum*** and ***Syzygium aromaticum*** completely inhibited all the tested fungi (100% inhibition). ***Eucalyptus globules*** was moderate effect, which ranged antifungal activity from 50.7 – 70.3% effectiveness. ***Nigella sativa*** has poor negative effect on the tested fungi, ranged from (6.3- 32.6 %). These results are in accordance with those obtained by Chatterjee (1990), Pai and Platt (1995), Juglal ***et al.*** (2002) and Sonia ***et al.*** (2003) who evaluated of different essential oils on the growth of certain Aspergilli and ***F. moniliforme***. They found that the clove oil (eugenol) was the most inhibitory effect on the growth of ***A. parasiticus*** and ***F. moniliforme,*** followed by cinnamon (cinnamic aldehyde). Neem and Eucalyptus oil (sineole) did not effect on fungal growth. The feasibility of implementing

these rsults in the fungal infection control. These results clearly suggest that commonly occurring mycotoxigenic fungi can be controlled with clove oil (eugenol). Thus, spice oil successfully inhibited the growth of ***A. parasiticus*** and ***F. moniliforme***, required to production of fumanisins and prevented the synthesis of aflatoxin starting

B- Seed protecting – treatment *(In vivo):*

It is obvious (Table 5) that the tow organic acids as seed treatments were effective in almost, for eliminating the seed borne fungi of all the tested cultivars. Also, the two organic acids at 5% conc. caused the lower effects than the 10% conc. on all cultivars. Results in (Table 4) indicate that acetic acid and propionic acid at 10% conc. were approximately equal effects gaving 100% efficiency except propionic acid which gave only 95.7% and 88.9% efficiency against ***A. niger*** and ***A. flavus*** on cv. T.e. 324, respectively. Also, gave efficiency controlling ***F. moniliforme*** 96.2% on cv. T.e. 352. Similar results were obtained by Sholberg and Gaunce (1996), Mazzani ***et al*** (1998), Kavita and Reddy (2000), Moreno-Martinez ***et al*** (2000), Blanchard ***et al*** (2001) and Sonia ***et al*** (2004) who found that the maize grains treated with acetic acid, propionic acid, sodium propionate or benzoic acid gave temporary control and prevented the growth of storage fungi (Aspergilli, Penicillia and Fusaria) inhibited the kernel invasion with an aflatoxin contamination and had adverse effect on the germination and viability of groundnut and maize seeds.

Also, data presented in Table (4) indicated that the essential oils of ***Cinnamomum zeylanicum*** and ***Syzygium aromaticm*** were approximately aqual effects giving 100% efficiency except ***Cinnamomum zeylanicum*** gave only 96.2% efficiency controlling ***F. moniliforme*** on cv. T.c. 352. ***Allium sativum*** was the least effective when compared with other essential oils. These results are in line with those obtained by Mukherjee and Nandi (1997), Montes Belmont and Carvajal (1998) Juglal ***et al*** (2002), Sonia ***et al*** (2003), Chalfoun ***et al***.(2004) and Velluti ***et al*** (2004) who tested the antifungal activity of certain plant essential oils for inhibition of fungal infection and mycelial growth in postharvest maize grains during storage. The authers found that the oils of ***Cinnamomum zeylanicum***, ***Syzygium aromaticun*** and ***Allium sativum*** inhibit the ***in vivo*** mycelial growth of established seed – borne infections and the production of B_1 and B_2 aflatoxin. It could be suggested that these plants could prove useful for preserving maize from fungal infection.

Table (5): Effect of tow chemical organic treatments and three essential oils on four maize seed-borne pathogens of cultivars S.C 129, T.C 324 and T.C 352.

Cultivars	Fungi	Treatments															
		Control		Acetic acid 10%		Acetic acid 5%		Propionic acid 10%		Propionic acid 5%		*Cinnamomum zeylanicum*		*Syzygium aromaticum*		*Allium sativum*	
		No	% Eff	No	% Eff	No	% Eff	No	% Eff	No	% Eff	No	% Eff	No	% Eff	No	% Eff
S.C 129	*A. flavus*	16	0	0	100	0	100	0	100	2	87.5	0	100	0	100	7	56.3
	A. niger	20	0	0	100	6	70.0	0	100	4	80	0	100	0	100	9	55.0
	F. moniliforme	29	0	0	100	4	86.2	0	100	3	89.7	0	100	0	100	13	55.2
	P. expansum	14	0	0	100	2	85.7	0	100	2	85.7	0	100	0	100	8	57.1
T.C 324	*A. flavus*	18	0	0	100	2	88.9	2	88.9	3	83.3	0	100	0	100	8	55.6
	A. niger	23	0	0	100	4	82.6	1	95.7	3	87	0	100	0	100	10	56.5
	F. moniliforme	27	0	0	100	3	88.9	0	100	4	85.2	0	100	0	100	11	59.3
	P. expansum	16	0	0	100	5	68.8	0	100	6	62.5	0	100	0	100	7	56.3
T.C 352	*A. flavus*	20	0	0	100	2	90	0	100	2	90	0	100	0	100	8	60.0
	A. niger	25	0	0	100	4	84	0	100	5	80	0	100	0	100	11	56
	F. moniliforme	26	0	0	100	3	88.5	1	96.2	5	80.8	1	96.2	0	100	12	53.8
	P. expansum	18	0	0	100	3	83.3	0	100	4	77.8	0	100	0	100	7	61.1

*** Number of infected seeds**

**** Relative effectiveness to control treatment = $\frac{\text{Control} - \text{Number of infected seeds} \times 100}{\text{Control}}$**

To summarize, it can be concluded that, of three tested maize seed cultivars for efficiency controlling to the ***A. flavus, A.niger, F. moniliforme*** and ***P. expansum*** infection, the authers recommend that soaking the maize grains in acetic acid or propionic acid at 10% for 10 menuts, or mixing the seeds with essential oils market of ***Cinnamomum zeylanicum*** (Cinnamon) or ***Syzygium aromaticum*** (Clove) at 1000mg essential oil/kg maize seeds.

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دور الإنزيمات الفطرية المحللة في تلف حبوب الذرة الشامية والوقاية من غزو الفطريات للحبوب

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أجري هذا البحث على بعض الفطريات المعزولة من حبوب الذرة الشامية المخزونة وهي الفطريات: اسبرجيللس فلافس، أسبرجيللس نيجر، سيفالوسبوريم مايبديس، فيوزاريوم مونيليفورم، بنسيليوم أكسبانسم والتي سببت نقصاً في كل من إنبات البذور والمحتوى المائي للحبوب، ووزن ١٠٠ حبه والنسبة المئوية للبروتين والزيوت بها، وكان هذا النقص مطرداً مع طول فترة التخزين. وقد أجريت هذه الدراسة في محاولة لمعرفة دور الإنزيمات الفطرية المحللة لكل من السيليلوز والنشا في تلف تلك الحبوب.

كل الفطريات المختبرة لها القدرة على إفراز الإنزيمات المحللة وأظهرت الفطريات اسبرجيلس نيجر واسبرجيلس فلافس أعلى نشاط إنزيمي متبوعة بالفطريات المختبرة الأخرى، وقد ارتبطت زيادة النشاط الإنزيمي إيجابياً بطول فترة التحضين.

درس النشاط المضاد للفطريات في المعمل، وذلك بواسطة بعض الأحماض العضوية والمستخلصات النباتية والزيوت الطيارة، وقد أظهرت الزيوت الطيارة للقرفة وفصوص الثوم تأثيراً مضاداً تاماً للفطريات المختبرة وكان الزيت الطيار لحبة البركة أقلها في هذا الشأن كذلك أظهر الحامض العضوي اسيتيك، البروبيونيك بتركيز ١٠% نشاطاً مضاداً عالياً ولكن بتأثير أقل من الزيوت الطيارة ـ أيضاً أظهرت المستخلصات النباتية تأثيراً أقل مما سبق وكان أعلاها هو مستخلص الثوم.

تم تقييم المعاملات السابقة في الوقاية من الغزو الفطري للحبوب وقد ظهر أن خلط حبوب الذرة بالزيوت الطيارة لنبات القرفة أو الثوم بنسبة ٨٠٠مجم/ كيلوجرام من الحبوب أدي إلي المنع التام للغزو الفطري للحبوب. كذلك فإن نقع الحبوب في حامض أسيتيك أوبروبيونيك تركيز ١٠% لمدة ١٠ دقائق أدي إلي نفس النتيجة. ويمكن أن نوصي باستخدام هذه المعاملات لوقاية الحبوب من الغزو الفطري بدلاً من استخدام المبيدات الفطرية.