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Anti-Contamination of Pathogenic Fungi on Post-Harvest Wheat Grains

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During growing seasons of 2012 and 2013, wheat plants were treated 30-day pre-harvesting, with three salts, *i.e*. sodium chloride, potassium chloride and sodium metasilicate and three antioxidants**,** *i.e*. salicylic acid, humic acid and ascorbic acid as well as four essential oils, *i.e*. garlic, thyme, cumin and eucalyptus, and the fungicide Tilt. Harvested grains were evaluated for contamination with *Alternaria alternata*, *Aspergillus niger* and *A. flavus* during storage at 20±5°C for six months. Results showed that all treatments significantly decreased the endograins contamination during storage. The highest efficacy was induced by garlic, sodium metasilicate, cumin and potassium chloride compared with the check treatment. Grains contamination (%) in oils treatments ranged from 0.0 to13.3, meanwhile it was 3.33 to 23.33 for salts and 7.77 to 17.77 for antioxidants. Also, results showed that the crude fungal filtrates of the three fungi completely inhibited the roots linear growth compared with the untreated check. Moreover, germination of grains treated with the three dilutions of fungal filtrates (1:1, 1:2 and 1:3) recorded 22.2, 55.5 and 88.8% with *A. flavus* and 33.3, 55.5, 88.8% with *A. niger* as well as 33.3, 44.4, 88.8% with *A. alternate*, respectively.

Keywords: Wheat grains, Pre-harvest treatments, antioxidants, oil extract, salts and Bio assay toxin

Wheat grains (*Triticum aestivum*) are contaminated mainly by *Aspergillus flavus* and *Aspergillus niger* resulting in a reduction of grain quality (Scudamore, 2005). Such fungi are known to produce mycotoxins as secondary metabolites in the infected grains during storage. Existent conditions such as: moisture content, temperature, storage period, contamination rate, broken grain and impurities, insect presence, oxygen rate, damages during harvest processing and grain transport may be interfered (Lazzari, 1997; Santos, 2002; Scussel, 2002; Garcia *et al.*, 2003; Scudamore, 2005 and Logrieco *et al.*, 2009).

Singh *et al.* (1992) found that essential oils of *Eucalyptus tereticornis, Ageratum conyzoides, Ocimum kilim* and *saccharum* inhibited the growth of *Alternaria alternata.*

Antioxidants are represented as safe materials for the environment. Benzoic acid, salicylic acid and tannic acid have direct antifungal activity on *Alternaria radicina* and *Alternaria tenuissima* on media (Galal *et al.,* 2000). However, $CaCl_2$ salt and Tecto fungicide gave the most suppressive effect to inhibit *Aspergillus niger* during storage at room temperature for up to 3 months in onion bulbs (Naffa and Shenoudy 2007).

Sodium metasilicate is known to play several improvements in plants, including a positive effect on reproduction, alleviation of metal toxicity and nutrient imbalance, provision of structural rigidity and increasing resistance against fungal diseases such as powdery mildews and root-rots (Bélanger, *et al.*, 1995). Sodium metasilicate has also shown potentiality for control of fungal diseases in cucumber (Chérif, *et al.*, 1992), muskmelon and zucchini squash (Menzies, *et al.*, 1992). Salicylic acid, ascorbic acid and sodium metasilicate decreased the rust severity (%) in morocco and sids-1 wheat cultivars (Tohamey and El-Sharkawy, 2014).

This study was designed to investigate the effectiveness of pre-harvest treatment of wheat grains with salts (sodium chloride, potassium chloride and silicon); antioxidants (salicylic acid, humic acid and ascorbic acid) at concentration of 1 and 1.5g/l; essential oil of garlic, thyme, cumin and eucalyptus at concentration of 10 and 20 m/l comparing with fungicide (Tilt) at concentration of 4.5 ml/l water on protecting wheat grains of fungal contamination after 6 months of storage at 20±5°C.

Materials and Methods

Field treatments:

Wheat plants (cvs. Morocco and Sids-1), grown in Etay El-Barood station, Behera Governorate, were sprayed 30-day before harvest with salts (sodium chloride, potassium chloride and sodium metasilicate); antioxidants**, (**salicylic acid, humic acid and ascorbic acid) at two concentrations (1 and 1.5g/l) and essential oils (garlic, thyme, cumin and eucalyptus) at concentration of (10 and 20 m/l) as well as one fungicide (Tilt 4.5 ml/l water). Salts, antioxidants, essential oils and the fungicide used in this study were obtained from Chemical Industrial Development Company (CID), Egypt. Tested plot size was 1/400 of feddan (about 100 m^2 containing 6 rows with 20 cm between rows). Each row was sown by 5 g wheat grains.

Contamination assessment:

Wheat grains were harvested from the field at a rate of 1.5 kg/feddan (3 replicates, ½ kg each) and packed in bags of burlap cloth then stored for 6 months at 20±5^o C and 14% RH (room conditions). Samples were tested for mycoflora status by plating sterilized wheat grains on PDA media. Plates were incubated at 29±1^o C for 6 days, depending on the fungal growth within the tested samples. Pure cultures of colonies were obtained by individually transferring fungal spores on fresh PDA plates. Contamination (%) was calculated according to Spalding and Reeder (1974) as follows:

$$\text{Contamination (\%)} = \frac{\text{Number of contaminated grains}}{\text{Total number of grains tested}} \times 100$$

Bioassay of the fungal toxicity on grain germination and linear growth of roots:

Preparation of culture filtrates of tested *A. alternata*, *A. flavus* and *A. niger* isolates was carried out in flasks (250 ml.) each contained 100ml of sterilized potato dextrose broth (PDB) medium, inoculated with disk (5-mm-diam.) of any of the

three tested fungi and incubated at 25±2°C for 10 days, then filtered through two layers of cheese cloth. The toxicity of the fungal filtrates was evaluated in Petri dishes, each containing 5 maize seeds/plate grown upon three layers of sterilized blotters amended with 10 ml of one of three dilutions (1:1, 1:2 or 1:3 fungal filtrate: water), then incubated at room temperature (25-28°C) for 2 weeks when seed germination (%) and length of root were determined. Five Petri dishes were used as replicates for each treatment.

Statistical analysis:

All data obtained were subjected to the analysis of variance using the MSTAT Statistical Software and comparison was calculated using L.S.D. at 0.05 as described by Gomez and Gomez (1984).

Results

Data presented in Tables (1 and 2) show that all tested pre-harvest salt treatments during 2012 and/or 2013 seasons significantly decreased naturally fungal contaminations on wheat grains. Sodium metasilicate was the most effective treatment to reduce the contamination on wheat grains (cv. Morocco) at two tested concentrations. The efficacy of high concentration (1.5 g/l) of sodium metasilicate has resulted in the highest efficacy on contamination (94.0% and 91.5%) followed by potassium chloride (91.1and 82.6), sodium chloride (77.6 and 73.9) on cv. Morocco and Sids-1, respectively, during season 2012. The same results were recorded in seasons 2013, when fungal contamination reached 95.5 and 89.9% in case of sodium metasilicate, followed by 92.5 and 81.2% for potassium chloride and 80.6 and 72.5% for sodium chloride on cv. Morocco and sids-1, respectively.

Table 1. Effect of pre-harvest salt treatments at season 2012 on wheat grain contamination with fungi after storage for 6 months at 20±5°C

Treatment	Concentration (g/l)	Tested wheat cultivar			
		Morocco		Sids-1	
		P*	E**	P	E
Sodium chloride	1.0	23.3	68.7	22.2	71.0
	1.5	16.7	77.6	20.0	73.9
Potassium chloride	1.0	13.3	82.1	16.7	78.3
	1.5	6.7	91.1	13.3	82.6
Sodium metasilicate	1.0	10.0	86.7	11.1	85.5
	1.5	4.4	94.0	6.7	91.5
Tilt 4.5 ml/l water		20.0	73.3	23.3	69.6
Control		74.4	0.00	76.7	0.0
L.S.D. at 0.05% for: Treatment (T):		0.92		0.32	
Conc.(C) :		0.80		0.28	
T x C :		1.61		0.56	

* P = Percentage of contamination.

**E = Efficacy (%)= (Control - treatment) / (Control) x 100

Table 2. Effect of pre-harvest salt treatments at season 2013 on wheat grain contamination with fungi after storage for 6 months at 20±5°C

Treatment	Concentration (g/l)	Tested wheat cultivar			
		Morocco		Sids-1	
		P*	E**	P	E
Sodium chloride	1.0	21.1	71.6	23.8	69.6
	1.5	14.4	80.6	21.1	72.5
Potassium chloride	1.0	11.2	85.1	17.8	76.8
	1.5	5.5	92.5	14.1	81.2
Sodium metasilicate	1.0	8.9	88.1	13.3	82.6
	1.5	3.3	95.5	7.8	89.9
Tilt 4.5 ml/l water		17.8	76.1	20.0	73.9
Control		74.8	0.0	76.7	0.0
L.S.D. at 0.05% for: Treatment (T):		0.31		0.33	
Conc.(C) :		0.27		0.29	
T x C :		0.54		0.58	

* , ** As described in footnote of Table (1).

Data presented in Tables (3 and 4) indicate that all pre-harvest treatments of essential oils at concentrations of 10 or 20 ml/l (during 2012 and 2013 seasons), significantly increased the efficacy of both tested cultivars 6 months after storage at 20±5°C, compared with the control treatment. Garlic at 20 ml/l completely prevents the fungal contamination on both tested cultivars. In case of cumin, the highest efficacies (%), *i.e.* 94.0 and 95.7% for Morocco and Sids-1, respectively, were recorded in seasons of 2012 and 2013.

Table 3. Effect of pre-harvest essential oil treatments at season 2012 on wheat grain contamination with fungi after storage for 6 months at 20±5°C

Treatment	Concentration (m/l)	Tested wheat cultivar			
		Morocco		Sids-1	
		P*	E**	P	E
Garlic	10	3.3	95.5	3.3	95.7
	20	0.0	100.0	0.0	100.0
Thyme	10	10.0	86.6	11.1	85.5
	20	7.8	89.6	8.9	88.4
Cumin	10	5.5	92.5	6.7	91.3
	20	3.3	94.0	3.3	95.7
Eucalyptus	10	11.1	85.1	10.0	87.0
	20	8.9	88.1	7.8	89.9
Control		74.4	0.0	76.7	0.0
L.S.D. at 0.05% for: Treatment (T):		0.40		0.14	
Conc.(C) :		0.29		0.10	
T x C :		0.57		0.20	

* , ** As described in footnote of Table (1).

Table 4. Effect of pre-harvest essential oil treatments at season 2013 on wheat grain contamination with fungi after storage for 6 months at 20±5°C

Treatment	Concentration (m/l)	Tested wheat cultivar			
		Morocco		Sids-1	
		P*	E**	P	E
Garlic	10	4.4	94.0	0.0	100.0
	20	0.0	100.0	0.0	100.0
Thyme	10	11.1	85.1	10.0	87.0
	20	8.9	88.1	7.8	89.9
Cumin	10	6.7	91.0	5.5	92.7
	20	4.4	94.0	3.3	95.6
Eucalyptus	10	13.3	82.1	10.0	87.0
	20	7.8	89.6	6.7	91.3
Control		74.4	0.0	76.7	0.00
L.S.D. at 0.05% for: Treatment (T):		0.40		0.38	
Conc.(C) :		0.28		0.27	
T x C :		0.57		0.53	

* , ** As described in footnote of Table (1).

Data presented in Tables (5 and 6) indicate that all pre-harvest treatments of antioxidant (during 2012 and 2013 seasons), significantly decreased naturally fungal contaminations (%) on the tested wheat grains, when compared with the control treatment. The highest efficacies (89.6 and 88.1%) were obtained by the pre-harvest treatment during the two seasons, respectively, with Ascorbic acid followed by salicylic acid and humic acid on cv. Morocco. Moreover, the highest efficacies (89.9 and 91.3%) were obtained by the pre-harvest treatment during the two seasons, respectively, with salicylic acid followed by ascorbic acid (88.4 and 89.9%) and humic acid (86.9 and 88.4%) at concentration of 1.5 g/l.

Table 5. Effect of pre-harvest antioxidant treatments at season 2012 on wheat grain contamination with fungi after storage for 6 months at 20±5°C

Treatment	Concentration (g/l)	Tested wheat cultivar			
		Morocco		Sids-1	
		P*	E**	P	E
Salicylic acid	1.0	14.4	80.6	13.3	82.6
	1.5	8.9	88.1	7.8	89.9
Humic acid	1.0	17.8	76.3	16.7	78.7
	1.5	11.1	85.1	10.0	86.9
Ascorbic acid	1.0	16.7	77.6	14.4	81.2
	1.5	7.8	89.6	8.9	88.4
Control	-	74.4	0.0	76.7	0.0
L.S.D. at 0.05% for: Treatment (T):		5.84		5.95	
Conc.(C) :		4.13		4.21	
T x C :		8.25		8.42	

* , ** As described in footnote of Table (1).

Table 6. Effect of pre-harvest antioxidant treatments at season 2013 on wheat grain contamination with fungi after storage for 6 months at 20±5°C

Treatment	Concentration (g/l)	Tested wheat cultivar			
		Morocco		Sids-1	
		P*	E**	P	E
Salicylic acid	1.0	13.33	82.09	11.11	85.51
	1.5	10.00	86.75	6.66	91.31
Humic acid	1.0	16.66	77.62	13.33	82.61
	1.5	11.11	85.08	8.88	88.42
Ascorbic acid	1.0	17.77	76.31	16.66	78.72
	1.5	8.88	88.07	7.77	89.86
Control	-	74.44	0.00	76.66	0.00
L.S.D. at 0.05% for: Treatment (T):		0.44		0.03	
Conc.(C) :		0.31		0.02	
T x C :		0.62		0.04	

* , ** As described in footnote of Table (1).

Data in Table (7) indicate that the crud filtrate of the three pathogens cused complet inhibition of grains germination (%) and the linear growth of roots (cm) compared with the control (100-10%), respectively.

Table 7. Effect of different treatment concentrations on grains germination (%) and linear growth of roots (cm)

Treatment	Grains germination (%)			Linear growth of roots (cm)		
	A. niger	*A. flavus*	*A. alternata*	*A. niger*	*A. flavus*	*A. alternata*
Crud	0.0	0.0	0.0	0.0	0.0	0.0
1:1	33.3	22.2	33.3	3.0	2.0	3
1:2	55.5	55.5	44.4	6.0	5.0	4.4
1:3	88.8	88.8	88.8	7.3	6.8	8.2
Control	100	100	100	10	10	10

D i s c u s s i o n

During the two tested seasons (2012 and 2013), sodium metasilicate has the highest efficacy followed by potassium chloride and sodium chloride. These results are in harmony with those obtained by Chérif *et al*. (1992) and Epstein (1999). Sodium metasilicate may act by eliciting biochemical defense reactions, including the accumulation of lignin, phenolic compounds and pathogenesis-related proteins in the infected plants. Generally, salt treatments showed significant effects on wheat moulds under the natural infection conditions than the untreated check. Singh *et al*. (1992) stated that the essential oils of *Eucalyptus tereticornis, Ageratum conyzoides*

and *Ocimum kilimandscharicum* inhibited the growth of *Alternaria alternata*, *Fusarium oxysporum*, *Colletotrichum truncatum* and *Helminthosporium* sp. Prusky (1988) reported that the effectiveness of any given antioxidant in the plant depends on which free radical involved, how and where it is generated and where the target of damage is. Thus, while in one particular system an antioxidant may protect against free radicals, in other systems it could have no effect at all or, in certain circumstances, an antioxidant may even act pro oxidant that generates toxic oxygen species. Ascorbic acid plays an important role in reducing levels of activated oxygen radicals in plant cell.

Mycotoxins are secondary metabolites produced by fungi in their filtrate of various generations when they grow on agricultural products before or after harvest or during transportation or storage. Aflatoxins are one of the most potent mycotoxin that occurs naturally by several *Aspergillus* spp. Several surveys had been carried out in world to generate the basic information about the mycoflora and mycotoxin contamination of cereals (Halt, 1994; Vrabcheva *et al.*, 1996 and Carlos *et al.*, 2000). Barros *et al.* (2005) isolated more *A. flavus* strains (73%) from peanuts than the wheat grains (13%). The efficacy of antioxidants to control 8 mycotoxigenic fungal strains, including *A. flavus*, *A. ochraceus*, *A. terreus*, *Penicillium citrinum*, *P. purpurogenum* and *Stachybotrys atra*, was investigated by Giridhar and Reddy (2001). They reported the genus *Alternaria* to produces more than 70 mycotoxins and phytotoxins, but only few of them are occur naturally in foodstuffs. Moreover, Battilani *et al.* (2009) stated that *A. alternata* is considered as the most important toxin producing species.

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مكافحة تلوث حبوب القمح بالفطريات الممرضة بعد الحصاد

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تتعرض حبوب القمح أثناء التخزين للتلوث ببعض الفطريات مثل *Alternaria alternata, Aspergillus niger* and *A. flavus*. ويهدف هذا البحث إلى اختبار رش بعض المعاملات ٣٠ يوما قبل الحصاد بأملاح (كلوريد الصوديوم و كلوريد البوتاسيوم والصوديوم ميتا سليكات) ومضادات أكسدة (حمض الهيوميك والسلسيلك والاسكوربيك) والزيوت (الثوم والزعتر والكمون والكافور) لمكافحة تلوث حبوب القمح الداخلية بالفطريات لمدة ٦ أشهر تخزين على درجة الحرارة ٢٠±٥ درجة مئوية. وأظهرت النتائج أعلى فعالية الثوم ، والصوديوم ميتا سليكات ، والكمون وكلوريد البوتاسيوم مقارنة بالكنترول وكانت الصوديوم ميتا سليكات وزيت الثوم وحمض الاسكوربيك أعطى معنوية لخفض فاقد تلوث الحبوب المخزنة خلال عامى ٢٠١٢ / ٢٠١٣. وأوضحت النتائج أيضا أن المعاملة بمستخلص الزيوت وتراوحت نسبة التلوث في المدى (٠.٠: ١٣.٣) والأملاح (٣.٣٣: ٢٣.٣٣) ومضادات الأكسدة (٧.٧٧: ١٧.٧٧) ومن ناحية أخرى بالتقدير الحيوي للنسبة المئوية للانبات وطول الجذير للذرة المختبرة للراشح الفطري للثلاث فطريات فكان طول الجذير للراشح الخالى صفر% مقارنة بالكنترول (١٠ سم) للثلاث فطريات. وكانت نسبة الإنبات للحبوب للتخفيفات الرواشح الفطرية (١: ١، ١: ٢، ١: ٣) (٢٢.٢ ، ٥٥.٥ ، ٨٨.٨٪) مع فطر *A. flavus* (٣٣.٣ ، ٥٥.٥ ، ٨٨.٨٪) مع *A. niger* و (٣٣.٣، ٤٤.٤، ٨٨.٨٪) مع *A. alternata* بالترتيب ، مقارنة بمعاملة الكنترول (١٠٠% إنبات).