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Occurrence and Control of *Alternaria alternata, Penicilliun citrinum* and *Aspergillus flavus* Mycotoxins in Broad Bean Seeds by Benzoic and Sorbic Acids

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ABSTRACT

Infected broad bean seeds were collected from Noubaria region during the season 2017-2018. Fungi isolated and purified from collected seeds were identified as *Aspergillus flavus, Penicilliun citrinum* and *Alternaria alternata.* The occurrence frequencies of the isolated fungi were 9.08, 15.16 and 75.76 %, respectively. Production potentials oftoxin by *Alternaria alternata* and *P. citrinum* isolates, as estimated by the Plug Agar method, were 23100 and 33700 ng/g, respectively. Production of aflatoxins by *A. flavus* isolates not detected. Sorbic and benzoic acids were showed to be more effective in reducing the growth of *A. alternata* and *P. citrinum* than metalaxyl and ridomyl fungicides. *A. alternata* was less sensitive to sorbic acid than benzoic acid. All the tested treatments significantly reduced the growth of *A. alternata* growth and alternariol (AOH) production; however, a higher efficiency ratio (99.86%) was realized by benzoic acid. Similar inhibition of growth and citrinin (CTN) production was found on *P. citrinum*. Metalaxyl was less efficient in inhibiting AOH and CTN toxins than the other tested treatments. The efficiency of benzoic and sorbic acids to reduce AOH and CTN production was higher by soaking seed treatment. Seeds inoculated with the tested fungi, then treated with sorbic or benzoic acid found to be healthy with normal color, texture and morphology.

INTRODUCTION

Broad bean (*Vicia faba*)is a common staple food in the Egyptian diet. The crop is exposed to infection with many fungal diseases, including those incited by mycotoxin-producing fungi, i.e., *Alternaria alternata* and *Penicillium citrinum*. Based on estimations of the Food and Agriculture Organization (FAO) of the United Nations, approximately 25% of the world's food crops are affected by mycotoxin producing fungi and global losses of food stuffs due to mycotoxins are in the range of 1000 million tons per year.

Alternaria is a common genus with a number of species that can invade crops at the pre and post-harvest stage and cause considerable losses due to the rotting of fruits and vegetables. Some species are fairly specific to particular crops (Broggi *et al*., 2007).

The black mould *Alternaria alternata* produces a wide diversity of mycotoxins which are of particular health concern. *Alternaria* species have the ability to produce more than 70 toxins, which play important roles in food safety since some of them are harmful to humans and animals (Laura Escrivá *et al*., 2017). Since no maximum allowable limits are set for *Alternaria* toxins in food and feed, prevention of *Alternaria* infestations and mycotoxin spoilage is the only way to avoid health risks (Brzonkalik *et al*., 2011, Hickert *et al*., 2016, Puntscher *et al*. 2019a). *A. alternata* is probably the most important mycotoxin-producing species which occurs on cereals, sunflower seeds, oilseed rape, olives, various fruits (Whitlow and Hagler, 2013; Zhao *et al*., 2015; Puntscher *et al*. 2019b). The principle *alternaria* mycotoxins that have been shown to occur naturally are tenuazonic acid, alternariol monomethyl ether, alternariol, altenuene, and altertoxin I (Guo *et al*., 2004 and Fox and Howlett, 2008; Gotthardt *et al*., 2019). AAL-toxins are produced by *A. alternata* f. sp. *Lycopersici*, a rarely occurring pathotype of *A. alternata*, and are structurally related to fumonisins (Logrieco *et al*., 2009).

Citrinin is a mycotoxin isolated from *Penicillium citrinum.* It was found to be produced by a variety of other fungi that are found or used in the production of human foods, such as grain, cheese, cake and red pigments (Gordon *et al*., 2010). Citrinin acts as a nephrotoxin in all species in which it has been tested, but its acute toxicity varies (Bennet and Klich, 2003).Citrinin induces mitochondrial permeability pore opening and inhibits respiration by interfering with complex I of the respiratory chain. Citrinin can permeate through the human skin (Boonen *et al*., 2012).

The inhibition of fungal growth and mycotoxins production can be achieved by physical, chemical and biological treatments (FAO, 1997). Synthetic chemical fungicides are applied to control fungal growth and mycotoxins production, but they have not been found very effective against mycotoxins. Again, their use in plant protection was being de-emphasized due to mammalian toxicity and stimulation of resistance in pathogens stemming from their inappropriate or excessive applications (Tripathi and Dubey, 2004 and Enyiukwu *et al.*, 2014, Ben Taheur *et al*., 2019). Heat treatment significantly reduced the concentration of alternariol, alternariol monomethyl ether and tenuazonic acid in *A. alternata* contaminated sunflower flour; however, the heat-treated material produced toxic effects when fed to rats (EFSA, 2011). These drawbacks reported in the literature necessitated the search for alternatives such as biological control and plant-based pesticides. Weak acids were also used as preservatives in the food and feed to prevent fungal spoilage. The most common weak acid preservatives are sorbic acid, benzoic acid and propionic acid (Eeckhout *et al.*, 2013).

Benzoic acid and sorbic acid salts are widely used as food preservatives (Mroueh *et al*., 2007) and prevention and control of mould growth and mycotoxin production in cereals (Eeckhout *et al.*, 2013). Metalaxyl-M and Ridomyl MZ 72 are systemic fungicides that inhibit protein synthesis in fungi by interference with the synthesis of ribosomal RNA. They are used as foliar spray to control diseases caused by air and soil-borne fungi and to prevent mycotxin production (Singh *et al.*, 2013). According to the available literature, production of *Alternaria* toxins in broad bean seeds by *Alternaria alternata* as well as reduction of mycotoxins concentration by treatment broad bean seeds with weak acids such as benzoic or sorbic acid derivatives, according to the available literature, were not studied. There are few or no relevant toxicity data on *Alternaria* toxins. alternariol and alternariol monomethyl are genotoxic and phytotoxic (AFSSA, 2009 and EFSA, 2011).

So, the main target of this study was to isolate and identify the most important mycotoxin producing fungi from edible broad bean seeds, determine their occurrence frequencies and test the effect of seed treatment with benzoic and sorbic acids on the growth and mycotoxin production by these fungi as an alternative method to the application of fungicides.

MATERIALS AND METHODS

Broad bean seeds were collected from broad bean yield cultured in season 2017-2018 and brought to Plant pathology laboratory at Agricultural Botany Department, Faculty of Agriculture, Saba Basha, Alexandria University.

Seed Health Testing and Frequency of The Isolated Fungi:

Surface sterilization of 400 seeds was carried out using sodium hypochlorite 70%, sterilized deionized at ratio 1: 4, according to ISTA (1996). After sterilization process, seeds were placed on sterilized 9 cm diameter filter papers in Petri plates, moistened with 3 ml of distilled water each. Plates were incubated in a growth room at 25±2 °C for 8 days under alternative light and dark 12 hours each. Each treatment was replicated thrice (Christensen, 1957). After 8 days of incubation fungal species found growing on the surface of seeds were purified using the single spore technique (Hansen, 1926 and Javaid *et al*., 2006) or hyphal tip technique (Riker and Riker, 1936) and maintained on potato dextrose agar (PDA) slants. Isolates were then identified according to St. Germain and Summerbell (1996), Hoog *et al*. (2000), Simmons (2007) and Tiwari *et al*. (2011). Occurrence frequency percentage (FP%) was determined according to Javaid *et al*. (2006), Mossini and Kemmelmeir (2008) and El Wakil *et al*. (2009). The number of colonies growing on each plate was counted for each identified fungus, then their frequency percentages were calculated according to the formula, recommended by Pitt and Hocking (1997).

$$FP\% = \frac{\text{No.of seeds on which fungus appear X100}}{\text{Total No.of seeds}} X100$$

Mycotoxin Production Ability of The Isolated Fungi

The ability of the isolated fungi for the production of mycotoxins was carried out using a qualitative method (Agar plug), recommended by Frisvad and thrane (1995) with minor modifications according to Mossini and Kemmelmeir (2008). The resulted fluorescence color was observed and registered.

Detection of Alternariol And Citrinin In Fungal Solid Cultures (confirmatory test):

Alterniol (alternariol) and citrinin were quantitatively detected in their solid cultures using HPLC-UV technique according to Azcarat *et al.* (2008) and Brzonkalik *et al*. (2011) at The Central Lab, Faculty of Pharmacy, Alexandria University. The obtained citrinin and alternariol standards were brought from Sigma, Aldrich, Cairo, Egypt.

Effect of the Tested Treatments on Fungal Radial Growth and Mycotoxin Reduction:

The effect of benzoic and sorbic acids tested treatments added to PDA culture, each on fungal growth was carried out using the colony growth measurement technique (Pitt and Hocking, 1997) and mycotoxin inhibition. Different concentrations of benzoic and sorbic acids (5, 10, 15 and 25 ppm) were used and compared its effectiveness with ridomyl and metalaxyl at their recommended dose (X) and, or (X) ± ½ X. The MIC values were determined and registered for each treatment according to Krisch *et al*. (2011).

Estimation of Citrinin And Alternariol In Infected Broad Bean Grains Treated with Sorbic And Benzoic Acids:

20 gm of infected broad bean grains were soaked for 12 hours in 100 ml deionized distilled water plus treatment at different concentrations as mentioned above and deionized distilled water as a control. At the end of the soaking process time, broad bean seed samples were re-soaked in 100 ml deionized distilled water for 4 hours to get rid of any acid traces, then grains were taken for alternariol and citrinin estimation after the accomplishment of the sensorial test according to Kütt *et al.* (2010), Asam *et al*. (2011) and Brzonkalik *et al*. (2011).

Sensorial Test:

Aspect and texture of the treated infected seeds were examined after each treatment to test the emergence of any adverse effect of the treatment on the morphology of the treated infected grains in comparison to healthy ones.

RESULTS AND DISCUSSION

Identification and Occurrence Frequencies of Fungi Associated with Infected Broad Bean Grains:

Fungi associated with infected grains were isolated, purified and identified as *Alternaria alternata*, *Penicillium citrinum* and *Aspergillus flavus*. Their occurrence frequencies were shown in Table1. *Alternaria alternata* showed the highest occurrence frequency (75.76%), followed by *Penicillium citrinum* (15.16%), whereas *Aspergillus flavus* was the least detected frequency (9.08%).

Myctoxins Production Potential:

Toxins production potentials of the isolated fungi were tested using a qualitative Agar plug method. Colony fluorescence color was yellow for citrinin and bluish green for alternariol. These results were in agreement with Frisvad *et al*. (2007); Mossini & Kemmelmeir (2008) and Sofia Agriopoulou *et al.*, (2020). No fluorescence appeared at aretention time of aflatoxin standard which indicates the inability of the isolated *Aspergillus flavus* strain to produce aflatoxins.

Alternariol and citrinin were quantitatively detected using HPLC-UV technique according to Azcarat *et al.* (2008) and Brzonkalik *et al*. (2011). Results are presented in table (1). According to the obtained data, the ability of *Penicillium citrinum* isolates to produce citrinin (CTN) (337 µg/g) was higher than that of *Alternaria alternata* isolate to produce alternariol (AOH) (237 µg/g).

Table 1: Frequency of identified fungi associated with infected broad bean seeds and their ability to produce mycotoxins.

Identified fungi	Frequency %	Mycotoxins production ability (µg/g)		
		AOH	CIT	AFIB
Alternaria alternata	75.76	231.00	-	-
Penicillium citrinum	15.16	-	337.00	-
Aspergillus flavus	9.08	-	-	0.0
Total Frequency	100			

Where: AOH = Alternariol; CIT = citrinin; and AFLB = Aflatoxin B.

Results in Table (2) showed the effect of benzoic and sorbic acids at concentrations. 5, 10, 15 and 25 ppm comparing with metalaxyl and ridomyl fungicides at their recommended dose (X) and, or (X) ± ½ X on radial growth and mycotoxins inhibition. MinimumInhibitory concentration (MIC) values were calculated and presented in table (3).

Effect of the Tested Treatments on Fungal Radial Growth and Mycotoxins Inhibition:

A. *Alternaria alternata*:

All the tested treatments have relatively similar efficacy in reducing the growth of *alternaria*, but benzoic acid was rather best, it realized the highest efficacy ratio (99.86%). These findings were, relatively, in agreement with those of Erich (1980) and Combina *et al*. (1999), who reported that levels above 10 mg/kg of sodium benzoate and potassium sorbate produced a total inhibition of fungal development and toxin biosynthesis. Our results were also in line with those reported by Embaby *et al.* (2013), Javaid *et al.* (2006), and Sitara and Hassan (2011), who concluded that ridomyl was effective against *Alternaria alternata* at concentrations 0.15 and 0.25%.

An increase in treatment's concentration in the present study was not necessarily followed by an increase in the fungal growth inhibition. However, the relationship between the fungicide's concentration and fungal growth inhibition was directly proportional. Benzoic acid (25ppm) and ridomyl 1 ½ X completely inhibited AOH production; however, fungal behaviour in responding against benzoic acid and ridomyl has differed. Our results are relatively in agreement with those of Uraih and Chipley (1976) and Uraih and Offonery (1981), who found that benzoic acid completely inhibited *Aspergillus flavus* growth and aflatoxin production at 10 mg/g.

Inhibition of Alternaria growth throughout our work was in line with that reported by Sofos and Busta (1983 and 1993) & (Gerez *et al.*, 2016). They concluded that the antimicrobial effect of sorbic acid is due to its inhibitory influence on various enzymes in the microbial cell.

B. *Penicillium citrinum*:

All the tested concentrations of benzoic and sorbic acids and ridomyl at concentration 1 ½ X (350 ml/10 L) were similar in their efficiency in reducing fungal growth. The least *Penicillium* growth reduction rates were attained by Metalaxyl. These findings coincided with those of Cohen (1981), who found that metalaxyl enhanced *Penicillium* growth. This contradiction may due to variations in the tested isolates of *Penicillium citrinum*. The highest growth reduction rates were observed in ridomyl treatments. This was in harmony with those of Javaid *et al.* (2006), who found that mancozeb (ridomyl) was the most effective fungicide in reducing fungal growth in *Penicillium*.

C. MIC of the Tested Treatments:

Data in Table (3) indicated that metalaxyl at the tested concentrations failed in reaching MIC >90% growth inhibition. Fungal growth inhibition in general was more sensitive to treatments than mycotoxin inhibition. Both of the tested fungi have similar behavior toward both of the two tested acids; however, they differed in their response to the effect of the tested fungicides. *Penicillium citrinum* was more tolerant to both fungicides than *Alternaria alternata.* Similar results were obtained by Abd-El-Ghany and Tayel (2009), who reported that *Penicillium citrinum* was more tolerant to metalaxyl at high concentrations (400-800 ppm).

Table 2: Effects of the tested weak acids and fungicides on fungal growth and mycotoxins production

Treatments		Fungal radial growth/mm three replicates each		Efficacy ratio (ER) of treatments on radial growth%		mycotoxins production /µg/g		ER %on AOH and CIT reduction %	
		A. alternata	*P. citrinum*	*A. alternata*	*P. citrinum*	AOH µg/g	CIT µg/g	AOH ER%	CIT ER%
Control		76.38 a	64.35 a	-------	------	231.00a	337.00a	------	------
Benzoic acid	**5.0**	0.285 ef	0.425 f	99.627	99.339	68.676 c	63.203 e	70.270	81.245
	10 ppm	0.105 def	0.105 f	99.862	99.837	29.400 fg	60.605 e	87.273	82.016
	15 ppm	0.012 f	0.090 f	99.984	99.860	25.200 g	11.234j	89.091	96.666
	25 ppm	0.525 def	0.120 f	99.313	99.813	0.00 j	0.000 l	100.0	100.00
Sorbic acid	**5.0**	0.940 bcd	0.397 f	98.769	99.383	83.76 b	67.400 d	63.740	81.086
	10 ppm	0.400 def	0.305 f	99.476	99.526	66.00 c	53.358 fg	71.428	84.167
	15ppm	0.375 def	0.105 f	99.509	99.837	25.67 g	5.6144 k	88.887	98.334
	25ppm	0.935 bcd	0.134 f	98.776	99.792	15.40 h	0.000 l	100.0	100.00
Metalaxyl	**½ X**	1.04 bc	43.40 c	98.638	32.562	49.611 d	112.50b	78.523	66.617
	1.0 X	0.19 f	28.85 d	99.751	55.171	85.268 b	106.25c	63.087	68.472
	1 ½ X	0.11 ef	62.80 b	99.856	2.416	5.168 i	31.250h	97.763	90.727
Ridomyl	**½ X**	1.48 b	10.2 5e	98.062	84.073	30.697 f	52.114 g	86.711	84.536
	1X	0.835 cde	9.60 e	98.906	85.083	40.308 e	56.25 f	75.649	83.309
	1 ½ X	0.24 ef	0.7 f	99.686	98.912	0.00 j	20.83 i	100.0	93.818
L.S.D 0.5		0.50966	1.49717			4.50635	3.8749		

Table 3: Minimum inhibitory concentration (MIC) of the tested treatments.

Treatments	**MIC**			
	A. alternata	***P. citrinum***	**Alternariol**	**Citrinin**
Benzoic acid	**5 ppm**	**5ppm**	**25ppm**	**15ppm**
Sorbic acid	**5ppm**	**5ppm**	**25ppm**	**15ppm**
Metalaxyl	**½ X**	**-(*)**	**1 ½ X**	**1½ X**
Ridomyl	**½ X**	**1½X**	**1½ X**	**1½ X**

Effect of water soaking process on mycotoxins production (Sensorial test):

Data in Table (4) indicated that the soaking process without the tested treatments did not significantly affect mycotoxin inhibition. Our results may appear incompatible and ambiguous with other findings concerning the washing grains process with tap water under pressure which significantly reduces the mycotoxin content in mould-contaminated grains by reducing fungal inocula (Wilson *et al*., 2004; Fandohan *et al*., 2005 and Jouany 2007). This contradiction may due to differences in the soaking process. Throughout the present experiment water soaking of infected seeds contaminated with mycotoxin was carried out without any water pressure. Washing infected seeds under water pressure may lead to the removal of the fungal spores and consequently reduced the produced mycotoxin.

The efficiency of benzoic and sorbic acids in reducing AOH increased during the soaking process. All the tested concentrations except for 25 ppmtreatment gave higher reduction rates during the soaking process. Our results are partially in agreement with Darman (2013). On the other hand, during the soaking process, all the tested concentrations of both acids significantly inhibited CTN production.

Generally, our findings fairly consistent with those of Ragab *et al*., (2007), who reported that modifying the traditional process of 'balila' preparation by using Na_2CO_3 solution during the water soaking process may be useful to reduce the risk of mycotoxin exposure via 'balila'. The soaking process ameliorate the efficiency of the tested weak acids.

Table 4: Effect of seed re-water soaking process on mycotoxins production throughout the tested treatments**.**

Treatments	**Av. of AOH µg/kg**	**E.R.% of Soak.P**	**Av. of CTN µg/kg**	**E.R. %of Soak.P**
Soak. control	229.656a	-	334.104a	0.4458*
Non Soak control	230.786a	0.4896	335.600a	---------
Soak + Benzoic 5ppm	16. 587cd	92.777	49.579c	85.161
Soak + Benzoic 10ppm	14.925cde	93.501	24.789d	92.570
Soak + Benzoic 15ppm	10.837 de	95.281	0.000e	100.0
Soak + Benzoic 25ppm	7.9420e	96.542	0.000e	100.0
Soak + Sorbic 5ppm	23.794b	89.639	67.635b	79.756
Soak + Sorbic 10ppm	19.589bc	91.470	27.805d	91.678
Soak + Sorbic 15ppm	16.455cd	92.835	0.000e	100.0
Soak + Sorbic 25ppm	13.123cde	94.286	0.000e	100.0
L.S.D $_{0.05}$	5.27712		4.27495	

Where: SOAK = saoking broad bean seeds in distilled water; ER% of Soak.P = Efficacy ratio of soaking process = (soaked seeds - soaked seeds + treatment/soaked seeds) x100; AOH = Alternariol; CTN = Citrinin; and * = (Non soaked seeds- soaked seeds/ Non soaked seeds) x100.

The appearance, texture and overall shape of the treated infected seeds were similar to those of the natural seeds. However, water-soaked seeds appeared steel blackish, which may be due to changes in the chemical structure of water during the soaking process which allows seeds to get rid of pigment. Koffi-Nevry *et al.* (2013) found that water soaking reduces some nutritive components involved in the activation of enzymes responsible for mycotoxin production. These findings were explained by Calvo *et al*. (2002), who found that in *Alternaria alternata*, melanin pigment deposition is also involved in spore development. Disruption of the *A. alternata* melanin biosynthetic gene *brm2* dramatically decreases melanin production in this fungus (Kumura and Tsuge 1993), and Kawamura *et al*. (1999). In our study, this disruption of melanin biosynthesis may occur as a result of denaturation of gene *brm2* encoding enzymes responsible for melanin production enhanced by treatment with benzoic and sorbic acids during water soaking. This finding was highly in agreement with those of Shad *et al*. (2012), who reported that sorbic acid works on the active sites of enzymes and interacted with the substrate to inactivate enzymes. This enzyme's inactivation was positively proportional to sorbic acid concentration.

Inhibition of alternariol and citrinin by benzoic and sorbic acids throughout the present study may due to the inhibition of the accumulation of Acetyl CoA, the precursor of mycotoxins biosynthesis (Uraih *et al*., 1977; Hajjaj *et al*., 1999; Scott, 2001; Elizabeth, 2008 and Saha *et al*., 2012) and preventing the condensation of acetyl CoA with malonyl (Eeckhout *et al*., 2013), which finally resulted in complete blocking of mycotoxins biosynthesis.

CONCLUSION

According to the obtained results, we can remark that benzoic and sorbic acids are highly effective in inhibiting *A. alternata*, *P. citrinum* growth and alternariol and citrinin production by these fungi.

The problem of non-safe use of these food preservatives does not exist in our study because the tested concentrations of these acids were less than recommended doses (Codex Alimentarius, 1991). In addition to that broad bean seeds greatly differed in structure, compared with acidic foods and beverages. Furthermore, For humans, the World Health Organization's International Programme on Chemical Safety (IPCS, 1999&2004, and JECFA, 2005) suggests a provisional tolerable intake for infants would be 5 mg/kg body weight per day for benzoic acid and its salts (Takao and Takeda, 2003 and WHO, 2009).

There are currently no statutory or guideline limits set for *Alternaria* mycotoxins because surveys to date have shown that their natural occurrence in foods is very low and the possibility for human exposure is very limited. The need for regulation is kept under review as new information becomes available.

The re-water soaking process proves that benzoic and sorbic acids are not acting as fungistatic but fungicides. So, we can consider these tested weak acids as fungicides alternatives to metalaxyl and ridomyl synthetic fungicides.

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ARABIC SUMMARY

تواجد ومقاومة السموم الفطرية للالترناريا الترناتا, بنيسيليوم سترينيم وأسبرجلس فلافس على بذور الفول بوسطة أحماض البنزويك والسوربيك

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إستهدفت الدراسة التعرف على الفطريات المصاحبة للبذور وتنقيتها وتعريفها, وإختبارمدى قدرة السلالات الفطرية المعزولة على إنتاج السموم الفطرية وتعريف كمى ونوعى لهذه السموم, وإستحداث أسلوب عملى بسيط لمعالجة البذور قبل الطهى والأكل للتخلص وتقليل محتوى البذور من السموم الفطريةوذلك باستخدام بدائل أكثر امانا على صحة كل من الانسان والحيوان مثل حمضى السوربيك والبنزويكفى حدودهما الامنة ومقارنة مدى كفاءة كل من المبيدات الفطرية والاحماض المذكورة على النمو الفطرى وتثبيط انتاج السموم الفطرية.
ولقد توصل البحث إلى عزل سلالات من فطرألترناريا ألترناتا, بنيسيليوم بنيسيليوم سترينيموأسبرجلس فلافس بنسب تواجد بلغت 75,76%, 15,16% و9,08%, على التوالى. ولقد دلت الإختبارات على قدرة سلالة الألترناريا على إفراز الألترناريول بنسبة 23100 ng/g وقدرة سلالة البنيسيليم على إفراز السترينين بنسبة 33700 ng/g, ولم تتمكن سلالة الأسبرجلس من إفراز الأفلاتوكسين, لذا تم إستبعاد الأخير من الدراسة. وبمعاملة البذور بكل من الحمضين البنزويك والسوربيك والمبيدين الفطريين ميتالاكسيل وريدوميل, تبين تفوق فاعلية الأحماض المختبرة على المبيدات الفطرية فى قدرتها على خفض النمو الفطرى للفطرين محل الاختبار. وكان الفطر الترناريا الترناتا اقل حساسية لحمض السوربيك بالمقارنة بحمض البنزويك. وتقاربت نتائج نتائج تثبيط النمو الفطر فى جميع معاملات الألترنريا مع تفوق طفيف لحمض البنزويك. كماكانجميع معاملات البنيسيليوم عالية الكفاءة, عدا الميتالاكسيل. كما أدت جميع المعاملات, عدا الميتالاكسيل, إلى خفض إنتاج السموم المختبرة, بل تعدى الأمر إلى إحداث تثبيط تام لإنتاجها. أدت عملية النقع للبذور الغير معاملة بالماء لمدة 12 ساعة إلى تأثيرات غير جوهرية على المحتوى من الألتناريول والسترينين, بينما أدت عملية النقع فى وجود البنزويك والسوربيك إنحفاض معنوى فى إفراز السموم, كما حدث تثبيطا تاما لإفرازالسيترينين على تركيزى 15 و 25 جزء فى المليون لحمضى البنزويك والسوربيك, على التوالى. ولقد أظهرت النتائج الحسية التى تمت بعد عملية النقع اختفاء صبغة الميلانين السوداء والمميزة للاصابة بفطر ألترناريا وذلك فى البذور المنقوعة فى الماء والمعاملة فقط, حيث استعادت البذور المصابة لونها الطبيعى واصبحت البذور المصابة تماثل البذور السليمة فى شكلها الظاهرى من حيث لونها ونسيج البذورنفسها.