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Patulin Production by *Penicillium glabrum* Isolated from *Coffea arabica* L. and the Activities of Some Natural Antifungal and Antimycotoxin Plants

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COFFEE bean seeds in developing countries are of the crops, which found to be contaminated with toxigenic fungi. Accordingly, reduction of mycotoxins in coffee bean seeds has been a major mission of many studies in such countries. Five of thirty coffee bean seed samples collected from Jeddah were contaminated with patulin (30-48 μgkg^{-1}). A toxigenic isolate of *Penicillium glabrum* were recovered from the contaminated samples. Factors affecting *P. glabrum* growth and patulin production included temperature and types of substrates were studied. The highest concentrations of patulin were produced at 20°C and in Coffee Dextrose (CD) medium. There was a significant correlation between ground coffee concentration in the liquid medium, *P. glabrum* growth and mycotoxin production. Increasing ground coffee concentrations in the CD medium increased fungal growth but decreased patulin production. Caffeine also decreased or inhibited mycotoxin production by *P. glabrum* when grown in liquid CD medium. Coffee in Saudi Arabia often is mixed with other plant materials or products. Addition of saffron or ginger to CD medium at 1g l^{-1} enhanced *Penicillium* growth and patulin production, addition of cardamom prevented mycotoxins production and allowed some fungal growth. On contrast, addition of cinnamon or cloves (1g l^{-1}) inhibited both fungal growth and mycotoxins production. In conclusion, coffee beans are exposed to contamination with toxigenic fungal isolates. Ground coffee, caffeine, cardamom, cinnamon and cloves may prevent mycotoxin production.

Keywords: Saudi coffee, *Coffea arabica*, Patulin, *Penicillium glabrum*, Additives, Mycotoxins, Caffeine, Cinnamon, Cloves, Cardamom, Natural antifungal, Antimycotoxin.

Patulin is a mycotoxin produced by some toxigenic species of *Aspergillus* and *Penicillium* including *A. terreus, A. clavatus*, *A. giganteus*, *P. urtica*, *P. cyclopium* and *P. glabrum* (Palmgren & Ciegle, 1983; Sibanda *et al.*, 2001 and Alves *et al*., 2006). It is water soluble material which is toxic to man, plants and animals in addition to prokaryotic cells (Gashlan, 2008). Patulin occurs naturally in many foods and feeds including corn, oilseeds, cereals, malting barley, wheat and coffee bean seeds (Lopez-diaz & Flannigan, 1997; Sweeney & Dobson, 1998 and Tapia *et al*., 2005). It is commonly produced in fruits and can be detected in apple and grape juices (Martins *et al.*, 2002).

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Patulin is carcinogenic and can cause acute and chronic hemorrhage in the stomach, lungs, brain and liver (McCallum *et al*., 2002 and Schumacher *et al.,* 2006) with LD50 for mice of 2 mg g^{-1} body weight (Pohland & Allen, 1970 and Tapia *et al.,* 2006). The most important environmental factors affecting toxin synthesis are media, especially carbon and nitrogen sources, pH, temperature, water activity (Abramson, 1998), caffeine concentration and the presence of plant metabolites (Bhatnagar *et al.,* 2003; Calvo *et al*., 2002 and Hector *et al*., 2008). Plant metabolites are considered sources of bioactive compounds and characterized by a broad spectrum of biological activities. They may affect toxin production and fungal development (Maraqa *et al.,* 2007). In previous works, plant components or their volatile extracts altered either fungus growth and/or aflatoxin production, *e.g.* volatile aldehydes (Clevestom *et al*., 2004), caffeine (Buchanan *et al*., 1983a, b) and unidentified compounds from neem leaf (Zeringue & Bhatnagar, 1996), cotton leaf (Zeringue & McCormick, 1990) and corn leaf (Wilson *et al.,* 1981). Anthocyanins and related flavonoids in some plant extracts also affect aflatoxin biosynthesis (Norton, 1999). In some cases, growth was not significantly affected by various metabolites but toxin biosynthesis and fungal development were decreased significantly (Juglal *et al*., 2002 and Basilico & Basilico, 1999). Addition of cloves or cinnamon inhibited fungal growth but *Allium cepa* or green tea leaves inhibited mycotoxin production through the length of time for which inhibition occurred varied by compound (Hitokoto *et al*., 1980 and Sinha *et al*., 1993). Since these compound could be used to prevent or decrease mycotoxin contamination in many foods and they could be used instead of synthetic antifungal products (Basílico & Basílico, 1999).

The objectives of this study were to study coffee beans samples contaminated with different mycotoxins and their associated fungi, to evaluate the ability of *P.glabrum* to produce patulin under various environmental conditions and in the presence of traditional additives, *e.g.* saffron, ginger, cinnamon, cloves and cardamom, to coffee in Saudi Arabia.

Material and Methods

Fungal isolation

Thirteen of thirty coffee bean seeds samples collected in Jeddah were contaminated with one or more mycotoxins including aflatoxins, patulin, ochratoxin or sterigmatocystin. Samples number 2, 10, 18, 26 and 30 were highly contaminated with patulin (Bokhari & Aly, 2009). *Penicillium glabrum* which can produce patulin, was isolated on Sabouraud dextrose agar, identified (Watanabe, 1994) and preserved for further studies.

Extraction and detection of mycotoxins from coffee bean seeds samples

One hundred gram of each sample were homogenized with 100 ml chloroform using Waring blender (Waring International, New Hardfords, CT, USA) for 2 min, twice successive times. Mycotoxins extraction and detection was carried out as previously described (Roberts & Patterson, 1998 and El-Shanwany *et al*., 2005).

Cultivation of P. grabrum in liquid media

P. glabrum was cultured in liquid media in the dark and using static conditions to evaluate factors affecting growth and patulin production. Determinations were made in triplicate. Inoculums was prepared by growing *P. glabrum* on Sabouraud dextrose agar containing rose bengal and chloramphenicol (25 µg ml^{-1}) in Petri dishes at 28°C for 10 days (Bokhari, 1993). From this culture 2 ml containing $2x10^4$ spore/ml were used to inoculate 250 ml Erlenmeyer flasks containing 48ml of growth medium. The flasks were incubated for 10 days. After fungal growth, the mycelia were harvested by centrifugation at 3000 rpm for 15 min. The mycelial cakes were collected, washed, dried at 60°C for 2 days and weighted to determine the fungal growth (mg l^{-1}).

Extraction of patulin

After incubation for ten days, the contents of each flask (medium + mycelium) were homogenized for 2 min in a high-speed blender (Ultra-turrax™, Janke and Kunkel, Staufen, Germany) 170 W, 20000 rev. min^{-1} with 100 ml of a mixture of chloroform: water (90:10 v/v) followed by vigorous shaking by rotary shaker (200 rpm) overnight. The extract was sequentially filtered through anhydrous sodium sulfate. The chloroform extracts were washed, dried, filtered, and concentrated to dryness, redissolved in a known quantity of chloroform and analyzed by TLC (Dos Santos *et al*., 2003) to detect patulin. Patulin was observed as a fluorescent orange spot under the UV light (366 nm). Quantification of patulin was carried out using TLC as described by Scott (1974).

Effect of media on fungal growth and patulin production

P. glabrum was cultured in Erlenmeyer flasks (250 ml) containing 48 ml of different media and inoculated with 2 ml of fungal suspension ($2x10^4$ spore/ml, O.D = 0.65) to identify the best medium for growth and/or patulin accumulation. The media used were coffee dextrose (Gaime-Perraud *et al.*, 1993), Difco™ potato dextrose, Difco™ malt extract, Difco™ Sabouraud dextrose or Czapex's-glucose (Difco Laboratories, Detroit, Mich.) supplemented with 0.2% yeast extract and 0.8% glucose. After incubation at 28°C for 10 days, the fungal growth and patulin production were measured as described above.

Effect of temperature on fungal growth and patulin production

Erlenmeyer flasks (250 ml) containing 50 ml of sterile coffee dextrose medium were inoculated with approximately 2 ml of a spore suspension ($2x10^4$ spore/ml). Flasks were incubated at different temperatures (4,10, 16, 20, 28, 30 and 34°C) in a static condition for 10 days and then analyzed for the presence of patulin. After 10 days, the contents of each flask were mixed with 100 ml of chloroform: water (100:10 v/v). The chloroform extract was dried using rotary evaporator at 40°C and patulin was measured as µg l^{-1}.

Effect of caffeine or ground coffee concentration on growth and patulin production

Different concentrations of caffeine or ground coffee were added to 250 ml Erlenmeyer flasks containing 48 ml of sterile coffee dextrose broth and inoculated with 2 ml of fungal suspension ($2x10^4$ spore/ml). The caffeine concentrations ranged from 0 to 1 g l^{-1} and the coffee concentrations were 0, 20,

40 and 60 g l^{-1}. Fungal growth and patulin production were measured after ten days of incubation at 20°C in static conditions and compared with the results of control culture (containing no exogenous caffeine or ground coffee).

Effect of antifungal plant products on fungal growth and patulin production

The growth of *P. glabrum* and patulin production were evaluated in coffee dextrose broth (pH 5.5), containing cardamom (seeds), cinnamon (stem), cloves (flowers), saffron (flowers), and ginger (rhizome). These plants were collected from Jeddah, washed, cut into pieces, dried at 60°C, ground to powder using coffee grinder (Antique Enterprise Coffee Grinder Model 512) and added to the fungal medium at concentration of 1g 1^{-1} as described by Sinha *et al.* (1993). After 10 days of incubation at 20°C, the fungal growth and patulin were measured.

Statistical analysis

Three determinations for each experiment were conducted. Variable means and standard deviations were calculated and a student's t test was carried out to detect significant differences in patulin concentrations between the treatments and controls.

Results

Five of 30 coffee bean seed samples, collected from Jeddah, were contaminated with toxigenic fungi, especially with members of genus *Penicillium.* Different mycotoxins (aflatoxin B_1, B_2, G_1, G_2, ochratoxin A and patulin) were detected by TLC (Table 1). The five contaminated coffee bean samples contained at least two mycotoxins except samples 18 and 26 contained four mycotoxins. The five samples were contaminated with both patulin and *Penicillium* sp. which may responsible mainly about patulin production. *Penicillium* sp. was isolated on Sabauroud dextrose agar from sample 10 of the contaminated coffee bean seeds which contained the highest level of patulin and the pure culture was maintained on potato dextrose agar (PDA). It was identified as *Penicillium glabrum* (Watanabe, 1994) and was a producer of patulin. In this survey, patulin in coffee bean seeds was produced primary by the members of the genus *Penicillium*. Its concentrations in the contaminated samples ranged from 30-48 µg kg^{-1} (Table 1).

Penicillium glabrum was grown in different broth media at 28°C for ten days in 250 ml conical flasks and patulin was extracted, purified, identified and quantified on TLC plates against a standard of patulin. Growth and patulin production were found in many media. The highest fungal growth and patulin accumulation were recorded in coffee dextrose, followed by Sabauroud dextrose, Czapek's-glucose and potato dextrose and finally malt extract media (Table 2). Fungal growth occurred from 4-38°C and patulin production from 16-30°C. A temperature of 20°C was optimal for both fungal growth and patulin production (Table 3).

TABLE 1. Sample numbers, source, toxigenic fungi recovered, mycotoxin produced and quantities of patulin produced (µg kg^{-1} dry weight).

Sample number	Source	Toxigenic isolates	Toxins detected	Quantity of patulin detected (µg kg^{-1})
2	Solimania	*A. fumigatus* *P. glabrum*	Afl. G_1, patulin	39 ± 0.4
10	El-Riewda	*A. fumigatus, A. terreus, P. glabrum*	Afl B_1, Och, patulin	48 ± 0.6
18	El-Gamiaa	*A. flavus, A. fumigatus, P. glabrum*	Afl B_1, B_2, G_2, patulin	30 ± 0.3
26	El-Balad	*A. ochraceus, P. glabrum, P. chrysogenum*	Afl B_1, B_2, Och.A, patulin	40 ± 0.5
30	El-Balad	*A. flavus, A. fumigatus, P. glabrum*	Afl B_1, B_2, patulin	45 ± 0.5

A.: Aspergillus, P.: Penicillium. Afl : Aflatoxin, Och. A : Ochratoxin A

TABLE 2. Effect of different media on growth and patulin production by *Penicillium glabrum* grown for 10 days at 28°C.

Medium used	Fungal growth (mg l^{-1})	Quantity of patulin produced (µg l^{-1})
Malt extract (control)	100 + 8.3	12 + 1.3
Potato dextrose	120 ± 4.1	13 ± 0 .9*
Coffee dextrose	127.3 ± 2.3	17.6 ± 1.31*
Sabouraud dextrose	125 ± 12.0	17 ± 0.3*
Czapek's-Glucos	120 ± 11.0	16 ± 0.9*

*: Significant difference from the control at $p<0.05$

TABLE 3. The effect of different temperature on growth and patulin production by *Penicillium glabrum*, grown in Coffee dextrose for 10 days.

Temperature	Fungal growth (mg l^{-1})	Quantity of patulin produced (µg l^{-1})
4°C	22 ± 1.3*	Nil
10°C	45 ± 2.4*	Nil
16°C	55 ± 1.0*	11.5*
20°C	120 ± 0.8*	19.0*
25°C	100 ± 1.3	17.0
28°C (control)	110 ± 1.0	17.0
30°C	108 ± 2.4	12.0*
35°C	81 ± 1.0*	Nil
38°C	70 ± 0.6*	Nil

*: Significant difference from the control at $p<0.05$

The effects of different concentrations of caffeine on the growth and patulin production with *P. glabrum* are presented in Table 4. Both fungal growth and patulin production were affected by the presence of caffeine in the growth medium. Increasing caffeine concentration up to 1 g/l decreased both growth and patulin production by 64% and 11%, respectively. Addition of ground coffee to the medium also reduced fungal growth and patulin production (Table 5). Complete inhibition of patulin production occurred in flasks containing 6% (w/v) ground coffee, while 2% and 4% (w/v) ground coffee inhibited patulin production by 30 and 50%, respectively.

TABLE 4. The effect of different concentration of caffiene on patulin production by *Penicillium glabrum* grown in Coffee dextrose for 10 days.

Caffeine concentration g/l	Fungal growth (mg l^{-1})	Quantity of patulin produced (♀µg l^{-1})
0.0 (control)	122 ± 4.3	20.5 ± 2.3
0.2	111 ± 7.3	17.9 ± 1.3*
0.4	101 ± 4.3	14.0 ± 4.0*
0.6	118 ± 11.9	12.8 ± 2.3*
0.8	98 ± 11. 9	8.0 ± 1.3*
1.0	78 ± 5.6	Nil

*: Significant difference from the control at $p<0.05$

TABLE 5. The effect of different concentrations of coffee on growth and patulin production by *Penicillium glabrum* grown in Coffee dextrose for 10 days.

Coffee concentration % (w/v)	Fungal growth (mg l^{-1})	Quantity of patulin produced (♀µg l^{-1})
0.0 (control)	121 ± 2.3	19 ± 1.8
1.0	133 ± 1.3	17 ± 1.3*
2.0	139 ± 0.3	14 ± 2.0*
4.0	142 ± 0.9	10 ± 1.3*
6.0	145 ± 1.1	Nil

*: Significant difference from the control at $p<0.05$

Some plant materials, Cinnamon, cloves, cardamom, saffron and ginger, usually added during Arabic coffee preparation, were collected and grounded. Addition of these materials individually or in combinations (1 g l^{-1}) to liquid broth medium may alter both *Penicillium* growth and patulin production. Cinnamon and/or cloves completely inhibited growth and patulin production for up to 10 days at 20°C (Table 6). In the presence of either saffron or ginger, fungal growth was not significantly altered and patulin production increased significantly. Cardamom did not affect fungal growth but completely inhibited patulin production.

TABLE 6. The effect of different addition of medicinal plants or spices on growth patulin production by *Penicillium glabrum*grown in Coffee dextrose for 10 days.

Type of spices (gl^{-1})	Fungal growth ($mg\ l^{-1}$)	Quantity of patulin produced ($♀\mu g\ l^{-1}$)
Control	122 ± 1.0	18.0 ± 2.0
Saffron	124 ± 2.5	28.7 ± 1.2*
Ginger	123 ± 2.3	24.0 ± 1.4*
Cinnamon	Nil	Nil
Cloves	Nil	Nil
Cardamom	100 ± 1.3	Nil
Cloves+ Cinnamon	Nil	Nil

*: Significant difference from the control at $p<0.05$

Discussion

Contamination of foods and feeds with toxigenic fungi is an increasing problem (Roberts & Patterson, 1998). Since the death of Pekin ducklings that were feed maize meal contaminated with patulin and forty-six toxigenic strains of *Penicillium sp*. (Scott, 1964), many studies of patulin contamination have been carried out. Patulin often was investigated in many fruity foodstuffs samples commercially used (Piemontese *et al.,* 2005 and Spadaro *et al.,* 2008). It is often found in damaged apples, apple juice, apple cider and sometimes in other fruit juices and feed (Piemontese *et al.,* 2005 and Murillo-Arbizu *et al.,* 2008). Patulin was produced by toxigenic fungal isolates belonging to *Aspergillus* and *Penicillium* genera which grow commonly on/in coffee bean seeds, thus seeds do not appear to be heavily infected could still become contaminated with high levels of patulin. Contamination of coffee bean seed samples collected from Saudi Arabia with toxigenic isolates of the genus *Penicillium* was 17% and patulin was also detected in 17% of the examined samples with concentration of 30-48 µg kg^{-1}. No record was found concerning coffee bean seeds contamination with patulin or by *Penicillium* sp. in Saudi Arabia. Contamination % of coffee bean seed samples by *Penicillium* species was 18% in samples collected from Mexico city and was 44 % in samples collected from Brazil (Batista *et al.,* 2003). Vega *et al.* (2006) isolated 11 species of the genus *Penicillium* from coffee plants collected from Hawaii and Colombia, four of them were toxigenic isolates. Patulin which considered the most dangerous mycotoxin in fruit juices is not common in coffee bean seeds. Its highly reactive double bonds readily react with sulfhydryl groups in foods, so patulin is not very stable in foods containing these groups. The concentrations of patulin detected by many authors for different food materials were 3-11 µg kg^{-1} and with maximum levels at 16 and 44.5 µg kg^{-1} (Piemontese *et al.,* 2005). The highest mean of patulin content detected by Martins *et al.* (2002) was 80.50 µg kg^{-1} . The European Union limit (Food Standards Agency, Saturday 18 April 2009) was of 50 µg kg^{-1} for this mycotoxin. Their previous agreed limits appeared in 2003 for patulin were 25 µg kg^{-1} in solid food products and 10 µg kg^{-1} in products for infants and young children. This

means that the range of coffee contamination with patulin in Saudi Arabia is acceptable.

Coffee dextrose medium was the most favorite medium for Penicillium growth and patulin production (19 µg l^{-1}) at 20°C. Many studies reported production of patulin by *Penicillium* spp. on defined or semi-artificial media. Media used successfully include yeast extract sucrose medium, potato dextrose medium, Sabouraud dextrose medium and Czapex 's dextrose medium at temperatures between 20-30°C (Jimenez *et al.*, 1991; Tapia *et al.*, 2005 and El-Shanwany *et al.*, 2005). Neither growth nor patulin production is directly comparable across studies due to differences in experimental protocols. Incubated at room temperature (25ºC) or at refrigerator at 4°C also support *Penicillium* growth and patulin production (McCallum *et al.*, 2002). Welke *et al.* (2008) reported that patulin content in juice or drinks lower than the limit of 50 µg l^{-1} considered acceptable by the Codex Alimentations Commission but not by the Commission of the European Communities which accept 10-25 µg l^{-1}. The maximum level of patulin detected at in the liquid medium was not exceed 19 µg l^{-1} which was through the acceptable limit and no risk might be faced through drinking contaminated coffee with patulin.

Addition of caffeine to the growth medium decreased both growth and patulin production. Buchanan *et al*. (1982, 1984) reported that caffeine inhibited the growth of three fungal species and decreased the amount of mycotoxin produced especially of sterigmatocystin, citrinin and patulin. It is an effective inhibitor of aflatoxin production and fungal growth (Nunes *et al*., 2001 and Bokhari & Aly, 2009). This inhibition could help to explain why aflatoxin does not accumulate in cocoa beans under natural storage conditions (Lenovich, 2006). A preliminary evolution of mycotoxin production by *P. urticae* suggested that caffeine's anti-mycotoxigenic activity does not involve a generalized inhibition of lipid synthesis (Buchanan *et al*., 1982). The addition of ground coffee often is more effective than adding of caffeine (Bokhari & Aly, 2009). Although exogenous caffeine may block the synthesis of a toxin, the addition of ground coffee may inhibit the production of several toxins produced by a single strain (Maraqa *et al.*, 2007). The higher activity of ground coffee relative to caffeine could indicate that other active ingredients in the coffee beans have either additive or synergistic properties with caffeine.

Plant materials, including extracts of spices and herbs, have been used for thousands of years to enhance the flavor and aroma of foods. Early cultures also recognized the value of these plant materials in food preservation and medicine . The antimicrobial and antitoxin properties of some spices, herbs, and their components have been documented since the late 19 th century (Snyder, 1997). Addition of some spices, medicinal plants or their extracts may inhibite or enhance fungal growth and toxin production in liquid media. Although ginger or saffron increased fungal growth and mycotoxin production, addition of cardamom, neem extract reduced fungal growth and decreased or completely inhibited mycotoxins production (Bokhari & Aly, 2009; Mossini *et al*., 2003; Zeringue & Bhatnagar, 1996 and Sinha *et al*., 1993). Cloves and cinnamon

caused complete inhibition of the growth and ochratoxin A production by the toxigenic *Aspergillus* strains (Hitokoto *et al*., 1980). Likewise, inhibitions in mycotoxins biosynthesis were noticed by Juglal *et al*. (2002) using clove and by Bokhari (2007a and b) using cloves, black pepper, peppermint, cardamom, cumin and marjoram. She attributed the inhibition of fungal growth and mycotoxin production to the presence of the antitoxic eugenol, found in both cinnamon and cloves (Bokhari, 2007b) but Maraqa *et al.* (2007) attributed this inhibition to the presence of active constituents of N-thymoquinone, dithymoquinone, thymohydroquinone and thymol.

In short, it could be concluded that coffee bean seeds can be contaminated by toxigenic fungi including *P. glabrum* which was a producer of patulin. The range of coffee seeds contamination in Saudi Arabia was still acceptable. The ability *P. glabrum* to produce patulin in the liquid medium was tested and the quantities of patulin were much lower compared with that detected for apple juice. Thus, there is no series risk of drinking patulin contaminated coffee. Storing ground coffee must be below 10°C or above 35°C where no patulin was detected. Adding of caffeine, cinnamon, cloves or both to ground coffee could prevent fungal contamination and mycotoxin production. It was not recommended to drink decaffeinated coffee because presence of caffeine prevents mycotoxins accumulations.

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إنتاج الباتيولين بواسطة *فطر بنسيليوم جليبريم* معزول من بذور البن العربى وتأثير بعض النباتات كمبيدات فطرية ومضادات سموم

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البن العربى هو أحد المحاصيل المنتجة فى الدول النامية التى تتعرض للتلوث بالفطريات القادرة على إنتاج السموم. وعليه، فأن كثير من الدراسات والأبحاث تناولت طرق مختلفة للتقليل من كمية التلوث بالسموم الفطرية، وفى هذا البحث خمس عينات من مجموع ثلاثين عينة جمعت من أماكن مختلفة من جدة، المملكة العربية السعودية، وجدت ملوثة بسم الباتيولين وتراوح من (٣٠ – ٤٨ ميكروجرام/ كجرام)، كما عزلت سلالات من *فطر بنسيليوم جليبريم* المنتج للباتيولين من هذه العينات وتم دراسة العوامل التى ساعدت على نمو هذا الفطر وإنتاجه للتوكسين مثل درجات الحرارة والبيئة الغذائية المناسبة له ، حيث وجد أن أعلى إنتاج للباتيولين كان عند درجة حرارة ٢٠ °م على بيئة دكستروز البن ، وهناك علاقة ايجابية بين تركيز البن المطحون فى البيئة السائلة وبين نمو *فطر البنسيليوم جليبريم* وإنتاج التوكسين ، فعند زيادة تركيز البن المطحون فى بيئة دكستروز البن يزيد من النمو الفطرى ولكن يقلل إنتاج الباتيولين وعند مقارنة هذه النتيجة بقدرة إنتاج الفطر للباتيولين فى بيئة سائلة مضاف إليها الكافيين النقى وجد أنه يقلل إنتاج السم الفطرى أيضَاَ . وحيث أن القهوة العربية تحضر بإضافة بعض البذور النباتية المطحونة عليها لتحسين الطعم والنكهة، وجد فى هذا البحث أن إضافة الزعفران والجنزبيل للبيئة بنسبة ١ جرام/لتر يحفز الفطر للنمو وإنتاج التوكسين بينما إضافة الهيل ينمو الفطر ولكن يعيق إنتاج سم الباتيولين. وبالعكس عند إضافة القرفة والقرنفل ثبط نمو الفطر وإنتاج التوكسين معا، ويتضح من هذا البحث أن بذور البن العربى ملوثة بالفطريات السامة وأن البن مادة غذائية يستطيع الفطر النمو عليها وإنتاج التوكسين وأن إضافة بعض البذور النباتية المطحونة للقهوة العربية يعمل على أما إيقاف نمو الفطر أو إيقاف الفطر وإنتاج التوكسين .