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LIST OF ABBREVIATIONS

AP	:	Antitox plus
FAT	:	Fix-a-tox
BC	:	Black cumin
F. verticilloides	:	*Fusarium verticilloides*
A. flavus	:	*Aspergillus flavus*
SAS	:	Same time application sequence
PAS	:	Previous application sequence
SBAS	:	Subsequent application sequence
GDW	:	Grain dry weight
MPP	:	Mycotoxins-producing-pathogens
SCDW	:	Shelled cob dry weight
MIC	:	Minimal inhibitory concentration
USFA	:	Unsaturated fatty acids
SFA	:	saturated fatty acids

CHAPTER VI

SUMMARY

Corn grain rots incited by *Aspergillus flavus* and *Fusarium verticilloides* cause great losses in yield. Moreover, mycotoxins produced by these fungi are very dangerous to human and animal health. Treatment of grains with some imported synthetic mold inhibitors, i.e. fix-a-tox (FAT), antitox plus (AP) and butylated hydroxyanisole (BHA) antioxidant are recommended to suppress growth and mycotoxins production by these pathogens.

The present study was carried out to investigate the effect of these substances on growth and development of these pathogens and their capability to suppress mycotoxins production. Other indigenous herbal products were also studied as locally available natural mold inhibitors. Efficiency of synthetic and natural mold inhibitors were tested under laboratory, field and storage conditions. Moreover, the effect of treatment with synthetic and natural mold inhibitors on some growth and nutritional parameter was also studied.

The obtained results could be summarized in the following items:

I- Pathological studies:

(1) An isolate of *F. verticilloides*, previously isolated and tested by the author was used throughout this study. However, before using the isolate was recultured and purified whereas, identification was verified in specialized laboratories in France.
(2) An isolate of *A. flavus* was isolated from infected corn grains; however preliminary tests proved that it was incapable of producing aflatoxins. Therefore, an aflatoxin-producing isolate, No. NRBL 3352, was introduced from laboratory of mycotoxins, National Research Center to be applied throughout the present work.
(3) Pathogenicity of the applied isolates was tested in our laboratories and obtained symptoms similar to those of natural infection.
(4) Pathogenicity of the isolates was tested, using two concentrations of inocula. The high conc. of *A. flavus* inoculum significantly reduced % germination and plant length and increased % of infected grains. Inoculation with the low conc. of *F. verticilloides* inoculums resulted in significant increase in % of infected grains (72%). Differences in % of infected grains between low and high conc. inoculums were insignificant.
(5) Inoculation of healthy corn cobs with cultural filtrates of *A. flavus* resulted in symptoms, similar to those produced by inoculation with standard solutions of aflatoxins. Symptoms include yellowing, which turned gradually to brown and grains appeared burned.
(6) Similar inoculations with cultural filtrates of *F. verticilloides* resulted in symptoms similar to those produced by inoculation with standard solutions of

fumonisins. Symptoms include wilting, dryness, yellowing reddish discoloration on both sides of grain and on cob sheaths.

(7) Examination of the tested fungi, growing on thin agar films under ultraviolet at wavelengths 254 nm and 266 nm, indicated that the applied isolate of *A. flavus* is capable of producing aflatoxins (appearance of blue fluorescence) and that *F. verticilloides* is capable of producing fumonisins (appearance of violet fluorescence).

(8) Quantitative analysis showed that *A. flavus* produced aflatoxins at the rate of 7.6-8 ppb, whereas *F. verticilloides* produced fumonisins at the rate of 10.90-11.2 ppm.

II- Determination of some characteristics of the tested herbal medicinal plants.

(A) Active components:

(1) Thyme extract contained 32% carvacol and 67% thymol.

(2) Black cumin oil contained 4.36% carvacol and 41.25% thymol, whereas the ratios were 54.68% and 28.13, respectively in ground clove.

(3) Eugenol content was 78.41% in clove oil, 34% in ground clove and 27.16% in clove extract.

Vitamin E (Alpha-tocopherol)

Alpha-tocopherol content was 5 mg/kg in ground BC and 0.32 mg/kg in BC oil, however, no alpha-tocopherol was detected in BC extract.

(B) Fatty acids

(1) Unsaturated fatty acid content in thyme extract and ground BC were 1.2 to 2-fold as much as saturated fatty acids content, respectively. Moreover, unsaturated fatty acids content in BC oil (73.45%), was 5-fold as much as BC extract.

(2) Linolenic acid was the most predominant fatty acid in BC oil and extract (42.87% and 35.14%, respectively). Moreover, behimic and arashidic fatty acids were detected only in BC oil (10.25 and 2.33%, respectively).

(3) Stearic acid was the main fatty acid in clove oil and extract (58.43 and 59.59%, respectively).

(4) Oleic acid was the prevailing fatty acid in thyme extract (34.11%).

(C) Occurrence of mycotoxins

(1) Using fluorometric techniques, all the tested herbal products proved to be free of aflatoxins or fumonosins.

III- Effect of the tested substances on growth of *A. flavus* and *F. verticilloides* under laboratory conditions

(A) Hole-plate diffusion method

Antifungal activity assay indicated that clove oil and BHA antioxidant completely inhibited growth of both tested fungi. On the other hand, clove extract completely inhibited growth of *A. flavus*, but partially inhibited growth of *F. verticilloides* (73.5%). On the contrary, the both tested synthetic treatments did not affect growth of the the tested fungi in this technique.

(B) Linear growth technique

Clove oil and extract and BHA completely suppressed growth of both fungal isolates, however, ratios of inhibition were less in BC extract and thyme extract. Their effect was more pronounced on *F. verticilloides* than *A. flavus.* On the other hand, antitox-plus (AP) had no affect on growth of both isolates.

(C) Minimal inhibitory concentration (MIC)

MIC values were 0.1% for clove oil and extract and 200mg/kg for BHA. *A. flavus* proved to be more sensitive to FAT and AP than *F. verticilloides*, which was more sensitive to BC oil. Both isolates were similar in their sensitivity to thyme extract.

(D) Floating on liquid medium

Microscopic observation of *F. verticilloides* cellophane films showed that treatment with FAT, AP and BHA resulted in characteristic agglomeration of macroconidia, formation of chlamidospore-like structures, lyses and disintegration of cell walls, leakage of cell components And deformation of macroconidia. BC oil and clove extract induced similar symptoms in their high concentrations.

Microscopic observation of *A. flavus* cellophane films indicated that treatment with synthetic substances resulted in segregation of head parts, breakdown of heads, changes in cell wall thickness, coarceness and deformation of conidiophores and heads. Treatment with clove extract gave similar effects at high concentrations.

(E) Mycotoxin production

All the tested substances, successfully suppressed the production of aflatoxin by *A. flavus* under laboratory conditions. Moreover, significant reduction of fumonisins production was realized by these substances. Highest reductions in fumonisins production by *F. verticilloides* was incited by clove oil (92.52%), whereas, BC extract induced the lowest reduction rates (17.33%).

IV- Effect of the tested substances on *A. flavus and F. verticilloides* under field conditions.

Preliminay determination of soil EC (3.4 moh/g) and pH (7.5) were carried out before planting. Moreover, effect of application sequences of treatment with mold inhibitors and inoculation (previous-PAS, at same time-SAS and subsequent-SBAS) were also included throughout field experiments.

(A) Effect on germination%

All treatments led to significant reduction in germination % of maize grains, compared with untreated control. The highest percentages of germination was obtained in FAT treatment (78% and 50%, in laboratory (in vitro) and in field (in vivo), respectively), followed by ground clove (55.33% and 40.66%, in laboratory and in field, respectively).

(B) Effect on stalk length

At same time, treatments gave the highest values of stalk lengths. This was true for all the tested substances and the applied pathogens. Moreover, ground clove with *F. verticilloides* and ground BC with *A. flavus* gave the highest stalk length values (110.43 cm and 142.43 cm, respectively).

(C) Effect on shelled cob dry weight

The highest values of shelled cob dry weight were realized by ground BC and FAT with *F. verticilloides*, followed by ground clove (94.45 %, 94.45 % and 83.39 %,respectively), compared with untreated non-inoculated control. Moreover, same time treatment gave the best results. In *A. flavus* treatments, the highest shelled cob dry weight values were realized by clove oil and antitoxplus (AP) (74.71% and 72.11%, respectively of untreated non inoculated control). This was true for all sequences.

(D) Effect on grain dry weight

Same time treatment gave the highest values of grain dry weight, compared with other tested sequences in both *A. flavus* and *F. verticilloides*. Moreover, highest values were obtained by ground BC in *F. verticilloides* treatment (12.86gm) and FAT in *A. flavus* treatment (8.51 gm).

(E) Effect on mycotoxins content in grains

In *F. verticilloides* treatment, mycotoxin content in grains significantly varied according to the tested application sequence and the applied substances. All of the tested substances completely suppressed fumonisin production if applied one week previous inoculation (PAS). Subsequent application (SBAS) of clove oil and clove extract were the most efficient in suppressing mycotoxin production in grains. At SAS treatments, BC oil and BC extract realized the highest suppressive rates, compared with the other tested substances. Moreover, AP induced the highest mycotoxin suppression rates, regardless of the application sequence tested.

(9) In *A. flavus* treatments, the highest suppressive rates were realized in SAS. Moreover, AP was the most effective among the tested substances in suppressing mycotoxin production.

V. Effect of the tested substances on *A. flavus* and *F. verticilloides* under storage conditions.

(A) In Immature grains

Fumonisins content in inoculated untreated stored immature grains was 7.5 ppm. The highest toxin reduction rates were realized by BHA antioxidant (76.33%, compared with untreated control). On the other hand, aflatoxin content in untreated stored grain inoculated with *A. flavus* was 11 ppb. FAT was the most effective among the other tested substances in reducing aflatoxin production in grains (reduction rate was 90.88% of control).

(B) In mature grains

Fumonisin content in stored untreated mature grains inoculated with *F. verticilloides* was 8.8 ppm. All the tested substances significantly reduced fumonisins production (reduction rates were 67.59-88.86% of control). In *A. flavus* treatments, aflatoxins content in stored untreated mature grains were 462.9 ppb. Treatment with clove oil, clove extract, BC oil and BC extract completely suppressed aflatoxins production during storage. In addition, treatment with synthetic mold inhibitors FAT and AP resulted in considerable reduction in aflatoxins production (84.58-90.88% of untreated control).

VII- Effect of treatment with the tested substances on some nutritional components of maize grain under storage conditions

(A) Proteins and aminoacids

Protein content in stored grains inoculated with *F. verticilloides* showed insignificant changes by treatment with FAT or ground clove. However, treatment with BHA significantly increased protein content (8.5% more than untreated control). In contrast , the ground clove was the only tested substance that increased protein content in grains inoculated with *A. flavus* (14.63% of untreated control).

In *F. verticilloides* trials, treatment with FAT resulted in significant reduction in the content of many amino-acids, i.e. proline (19.54 %), aspartic (12.69 %), cysteine (60.71), valine (35.45 %), isoleucine (53.25 %) and leucine (35.53%). Moreover, treatment with BHA significantly reduced the content of threonine, serine, glutamic, glycine, alanine, phenylalanine and tyrosine. Amino-acid content showed insignificant changes in ground clove treatment.

In *A. flavus* treatments, FAT significantly increased the content of methionine and threonine (67.4 and 10.17 % of untreated control, respectively), whereas, aspartic acid showed significant decrease (74%). On the other hand, BHA caused pronounced reduction in threonine, isoleucine and leucine content (46.5 %, 49.93 % and 41.30 %, respectively).

(B) Fibers

Fiber content in grains inoculated with *F. verticilloides* and treated with BHA showed significant increase (1.34 to 2.05-fold as much as control). Similar increase was obtained in *A. flavus* treatments (1.35-fold as much as untrated control).

(C) Moisture

Treatment of stored grains with FAT and BHA resulted in pronounced reduction in moisture content both in *F. verticilloides* treatments (82.16 % and 68.94 %, respectively) and *A. flavus* (91.95% and 95.94%, respectively). In contrast,

treatment with ground clove increased moisture content in *A. flavus* treatment (1.3 fold as much as control).

(D) Ash

Treatment with FAT led to significant increase in ash content in both *A. flavus* and *F. verticilloides* treatments (1.4-fold as much as control). On the contrary, the other tested treatments significantly reduced ash content in *F. verticilloides* treatments, however ash content showed insignificant changes in *A. flavus* treatments , compared with control.

(E) Fats

Treatment with the tested synthetic mold inhibitors resulted in significant increase in fat content in both *A. flavus* and *F. verticilloides* treatments (19% and 29%, respectively). On the other hand, treatment with ground clove significantly reduced fat content in *A. flavus* treatment (34.15%), however, no changes were detected in fat content in *F. verticilloide*s treatments.

(F) Carbohydrates

Treatment with the tested substances, significantly reduced carbohydrate content in both inoculated treatments in both inoculation treatments. Reduction rates were higher in *A. flavus* treatments treated with ground clove (2.5% compared with control) and in *F. verticilloides* treatments treated with FAT and BHA (5.64% and 5.22%, respectively.