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INHIBITION OF AFLATOXIN G_1 PRODUCTION IN GROUNDNUT BY HOMOEOPAHIC DRUGS

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ABSTRACT

Antiaflatoxic and antifungal properties of eight homoeodrugs each in seven potencies were tested against *Aspergillus parasiticus* under *in vitro* and *in vivo* conditions. Pre-inoculation treatments, Arsenicum album 6, 12, 30, Iodium 3, 12, 30, 1M,10M, Natrum phosphoricum 3, 6, 12, 30, 1M, 10M, Sulphur 3, 12, 30 and 10 M appeared as most effective preventives whereas Arsenicum album 200, Calcarea sulphurica 10M, Iodium 3, 6, 12, 200, 1M, 10M, Natrum phosphoricum all potencies, Sulphur 6 and 1M emerged as most curative treatments as their use could 100% control aflatoxin G_1 production on groundnuts . Hence, aflatoxin G_1 on groundnut could be controlled quite successfully by these homoeodrugs.

KEY WORDS: Aflatoxin, *Aspergillus parasiticus*, groundnut seeds, homoeopathic drugs

Aflatoxins B_1, B_2, G_1 and G_2 are fungal secondary toxic metabolites produced by certain *Aspergillus* species such as *Aspergillus parasiticus, A. flavus, A. nomius* and *A. niger*. Aflatoxins, the most important mycotoxins in the world's food supply, due to their potent toxic, carcinogenic, mutagenic, teratogenic and immunosuppressive properties, have become a major object of focus among toxicologists (Coulombe, 1991 and IARC, 1993). Recently, there has been a desperate search for alternatives that would provide satisfactory aflatoxin control with low impact on the environment and on human health (Holmes, 2008). As previously established by a few workers (Sinha and Singh, 1983 and Shrivastava and Atri 1998), homoeopathic drugs could fulfill the promise as they have been shown to possess antifungal properties. With this realisation, an attempt has been made in the present communication to control aflatoxin G_1 production in groundnut seeds through homoeopathic drugs.

MATERIALS AND METHODS

Aspergillus parasiticus, strain MTCC No. 411, the test pathogen in the present investigation was obtained from IMTECH, Chandigarh. *A. parasiticus* was grown on the malt salt agar medium at 28°C for seven days and stored at 4°C. For experimental purposes, eight homoeopathic drugs (Table-1) belonging to centesimal potencies marked as 3, 6, 12, 30, 200, 1M and 10M were used (customarily suffix c representing centesimal potency is dropped). They belonged to Medisynth Chemicals Private Limited, Navi Mumbai. In homoeopathy, concentration of drugs is inversely proportional to their potencies. Hence, drug concentration in 3, 6, 12, 30, 200, 1M and 10M potencies used in the present investigation were of the order of 10^{-6}, 10^{-12}, 10^{-24}, 10^{-60}, 10^{-400}, 10^{-2000} and 10^{-20000} dilutions respectively. From any standard these are ultramicrodilutions.

***In vitro* Studies**

Fungitoxicity of the drugs was evaluated in relation to their inhibitory effects on mycelial growth as well as aflatoxin production. For this purpose, 150 ml flasks were dispensed with 25 ml sterilized yeast extract sucrose broth containing 20g yeast extract, 200g sucrose and 1000 ml distilled water (Davis and Diener, 1966) and were provided with 0.1ml each of 3, 6, 12, 30, 200, 1M and 10M drug potencies. In control 0.1 ml 90% ethyl alcohol (drug medium) was used instead of the drug. Flasks were inoculated with the test pathogen *A. parasiticus* and incubated at 28 ± 1°C for 10 days. Thereafter, mycelial mats were removed and % inhibition of the mycelial growth over control was calculated.

Using the standard methods of Nabney and Nesbitt (1965), and Eppley (1968), the effects of homoeodrugs on aflatoxin G_1 production were determined by estimating the mycelial weights in different culture filtrates.

***In vivo* Effects**

Pre-inoculation treatment involved surface

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sterilization of 10.0g healthy groundnut seeds with 0.1% mercuric chloride solution, following which they were washed thoroughly with distilled water and dried. Then they were soaked in different drug solutions (1:25 V/V) of different potencies for 1 hour. Such treated seeds were inoculated with 1.0 ml aqueous spore suspension of the test pathogen and incubated at 28± 1°C for 10 days.

The post-inoculation treatment involved the same procedure as above, except for the difference that the treatment was done after inoculation. Seed lots soaked in ethylated water (1:25 V/V) served as controls. All treatments were triplicated. Subsequently, 10.0g seed samples from treated and control sets were processed for the quantitative estimation of aflatoxin G_1 as per the methods mentioned above.

RESULTS AND DISCUSSION

In vitro Effects

Effects of homoeodrugs expressed as responses towards mycelial growth and aflatoxin G_1 production could be placed into certain specific categories (Table,1). A few cases were recorded where drugs curtailed both fungal growth and aflatoxin G_1 production to a remarkable extent. For example Arsenicum album 3, 10M, Natrum phosphoricum 6, 1M and Sulphur 10M. Next, there were several cases where drugs were recorded as poor fungitoxicants, though they reduced aflatoxin G_1 production to a remarkable extent. These were Arsenicum album 6, 12, 200, 1M, Calcarea sulphurica 30, 200, 1M, 10M, Graphites 3, 12, 1M, Hepar sulphuris 3, 30, 200, 1M, 10M, Iodium 3, 1M, 10M, Natrum phosphoricum 3, 12, 30, 200, Sulphur 3, 30, 200 and 1M. There was only one drug, Natrum phosphoricum 10M which was strong fungitoxicant but poor against aflatoxin production. Interestingly one potency was found to stimulate aflatoxin G_1 production such as Natrum phosphoricum 10M. These stimulatory effects could be due to the presence of certain polyunsaturated fatty acids which are abundant in groundnut. These drugs might have activated lipoperoxidation that might have induced aflatoxin G_1 production considerably. Such a boost in aflatoxin B_1 production was also observed in *A. parasiticus* and *A. flavus* cultures treated with synthetic lipoperoxides (Passi et al., 1984).

As already mentioned, the lack of correlation between fungal growth and aflatoxin B_1 production in *A. parasiticus* has also been recorded by Sinha and Singh, (1983) and Shrivastava and Atri ,(1998).

In vivo Effects

The antiaflatoxic responses of the groundnut seeds have differed with respect to mode of drug treatment, as is evident from the observations (Table,2). Some drug potencies worked better as preventives; for examples Arsenicum album 6, 12, 30, Iodium 3, 12, 30, 1M, 10M, Sulphur 3, 12, 30 and 10M. These curtailed aflatoxin production in a range of 100%. Iodium 3, 12, 1M, 10M, Natrum phosphoricum 3, 6, 12, 30, 1M and 10M were also found to work well as curatives bringing about a good deal of reduction in aflatoxin production by 100% respectively. A number of drug potencies have proved better as curatives when used in post inoculation treatments, as these brought about more than 90% reduction in aflatoxin production. These were Arsenicum album 1M, Calcarea sulphurica 12, 30, Graphites 6, 12, 200, Hepar sulphuris 3, 6, 30, 200, Kali phosphoricum 10M, Sulphur 30 and 10M. However, some of these drugs, e.g., Calcarea sulphurica 10M, Graphites 6, 200, 1M, Iodium 6 and Natrum phosphoricum 200 have shown poor antiaflatoxic potentials as preventives.

Besides, the *in vitro* performances of certain homoeopathic drugs were found to be more or less altered on host front. For example, efficacies of Calcarea sulphurica 10M, Graphites 3, 6, 1M and Iodium 6 were rendered weaker and those of Arsenicum album 6, 12, 1M, Calcarea sulphurica 3, 12, 30, 200, 1M, 10M, Graphites 3, 6, 12, 1M, 10M, Iodium 3, 6, 30, 200, 10M, Natrum phosphoricum 3, 12, 30, 200, Sulphur 3, 12, 30, 200 and 1M were made stronger as preventives. Similar irregularities were also recorded with respect to curative treatments. Some host factors of unknown nature might be responsible for such modifications (Chandra and Khanna 1981; Khare and Atri 1995).

A study of data (Table,1 and 2) would also exhibit certain unconventional features of homoeodrug action. A large number of drug potencies were used, and though

Table1: Effect of homoeodrugs on mycelial growth and aflatoxin G_1 production by *Aspergillus parasiticus*

Drugs	POTENCY													
	3		6		12		30		200		1M		10M	
	Percent Inhibition or Stimulation (-)													
	MG	AP	MG	AP	MG	AP	MG	AP	MG	AP	MG	AP	MG	AP
Arsenicum album	61. 64	98. 05	24. 60	99. 10	22. 29	100. 00	67. 67	51. 59	30. 31	98. 93	17. 20	97. 30	66. 57	84. 40
Calcarea sulphurica	11. 48	97. 24	14. 19	75. 68	15. 00	93. 86	14. 58	98. 25	13. 26	92. 26	7. 23	97. 59	-7. 43	98. 28
Graphites	23. 83	92. 17	29. 98	96. 80	27. 90	92. 76	36. 56	94. 12	33. 80	85. 36	27. 16	95. 90	26. 76	97. 96
Hepar sulphuris	11. 12	96. 64	5. 60	99. 21	15. 55	96. 47	2. 18	93. 90	6. 82	100. 00	14. 30	94. 78	16. 63	92. 85
Iodium	13. 62	97. 41	19. 12	99. 07	40. 92	96. 21	4. 42	92. 98	28. 64	97. 91	25. 63	74. 95	20. 97	95. 28
Kali phosphoricum	37.32	60.25	31.31	85.89	40.57	96.23	33.72	84.26	25.18	97.01	37.38	83.70	37.19	44.24
Natrum phosphoricum	17. 70	92. 73	67. 67	100. 00	22. 51	100. 00	9. 54	95. 05	11. 27	94. 12	67. 67	83. 86	70. 41	-46. 06
Sulphur	7. 72	99. 19	69. 86	67. 85	16. 36	100. 00	18. 20	100. 00	7. 25	100. 00	10. 31	100. 00	60. 00	100. 00
Control	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00

MG=Mycelial Growth, AP=Aflatoxin Production

Table -2: *In vivo* effect of homoeodrugs on aflatoxin G_1 production on groundnut seeds by *Aspergillus parasiticus*

Drugs	POTENCY													
	3		6		12		30		200		1M		10M	
	Percent Inhibition or Stimulation (-)													
	PR	PO	PR	PO	PR	PO	PR	PO	PR	PO	PR	PO	PR	PO
Arsenicum album	62. 85	31. 48	100.00	00.00	100. 00	79. 99	100. 00	65. 71	71. 45	100. 00	71. 45	88. 57	94. 28	25. 71
Calcarea sulphurica	59. 99	57. 14	85. 11	79. 99	85. 11	88.57	94. 28	97. 14	82. 85	62. 85	97. 14	68. 67	25. 71	100. 00
Graphites	42. 90	37. 19	8. 40	94. 28	65. 71	91. 42	74. 28	79. 99	22. 85	97. 14	37. 19	8. 48	94. 28	65. 71
Hepar sulphuris	65. 71	91. 42	71. 45	88. 57	88. 57	00. 00	94. 28	88. 57	85. 11	94. 28	97. 14	-14. 35	85. 11	-83. 03
Iodium	100. 00	100. 00	-37. 19	100. 00	100. 00	100. 00	100. 00	65. 71	91. 42	100. 00	100. 00	100. 00	100. 00	100. 00
Kali phosphoricum	91.42	77. 14	82.85	82.85	71.45	54.32	88.57	77.14	91.42	77.14	85.71	77.14	88.57	94.28
Natrum phosphoricum	100. 00	100. 00	100. 00	100. 00	100. 00	100. 00	100. 00	100. 00	62. 85	100. 00	100. 00	100. 00	100. 00	100. 00
Sulphur	100. 00	77. 00	71. 45	100. 00	100. 00	71. 45	100. 00	88. 57	77. 14	71. 45	77. 14	100. 00	100. 00	94. 28
Control	00. 00	00. 00	00.00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00	00. 00

PR=Pre-inoculation Treatment
PO= Post-inoculation Treatment

many of them emerged as strong fungicides, none of them could totally suppress mycelial growth. Earlier workers using homoeopathy (Khare and Atri 1995; Shrivastava and Atri 1998 and Dua and Atri 1986-87) also arrived at similar observations. Reasons for such happenings are not clear. Presumably homoeodrugs do not act against the pathogens

in vitro as effectively as they do against them *in vivo*. Homoeopathy, unlike allopathy, considers host as the main site of action where basic contradictions of health and disease operate, wherefrom the drugs gather their powers to fight against the pathogen, the latter being considered as playing the second fiddle in producing the disease (Khare and Atri 1995; Shrivastava and Atri 1998 and Dua and Atri 1986-87).

Unlike conventional substances, where drug responses are commonly concentration dependent, a majority of cases displayed a striking feature that several drug responses were not proportional to the concentration of the drug. The mode of drug preparation which uniquely involves potentization might account for this feature . The process of potentization probably produces different physical forms of the drug molecules, each form endowed with a distinct medicinal property, suggestive of multiple site action of homoeopathic drugs. Hence sinusoidal responses over a range of drug potencies. Earlier workers (Goswami and Das 1980; Khare and Atri 1995) have observed the same. In such a case it would not be possible for the pathogen to develop resistance against homoeodrugs through alternative pathways. Conventional substances are site specific selective fungicides and do not demonstrate this property. This could possibly be the reason why pathogens evolve resistance against conventional substances (Georgopulos and Owens 1969).

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