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CONTROL OF AFLATOXIN G_1 PRODUCTION IN GROUNDNUTS BY HOMOEOPATHIC DRUGS

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ABSTRACT

Antiaflatoxic and antifungal properties of seven homoeodrugs each in seven potencies were tested against *Aspergillus parasiticus* under '*in vitro*' and '*in vivo*' conditions. Pre inoculation treatments, Belldona 3, 6, 12, Caladium 3, 1M, 10M, Drosera 6, 12, 30, 200, 1M, 10M, Hypericum 3, 30, 200, 1M, 10M, Lachesis 3, 6, 12 and 1M appeared as most effective preventives whereas Belldona 12, 30, 200, Drosera 3, 12, 30, 1M, Hypericum 3, 6, 12, 1M, 10M, Lachesis 3, 6, 12, 30 and 10M emerged as most curative treatments as their use could control 100% aflatoxin G_1 production on groundnut seeds. Hence, aflatoxin on groundnut could be controlled quite successfully by these homoeodrugs.

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

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INTRODUCTION

Groundnuts are vulnerable as are many agricultural commodities, to attacks by a group of fungi commonly known as *Aspergilli*. Apart from reducing the nutritive value and taste of the edible commodity, they produce harmful by products of mould growth called mycotoxins. Aflatoxins are produced by *Aspergillus parasiticus, A. flavus* and *A. nomius*[1]. Aflatoxin contamination of foods and feeds is a serious long standing inextricable worldwide problem resulting from either improper storage of commodities or preharvest contamination, especially during drought conditions. Aflatoxins are potent toxic, carcinogenic, mutagenic, teratogenic and immunosuppressive substances that have become a major object of focus among toxicologists[2,3]. Over the last few decades, several physical, biological and chemical strategies have been offered to cope with them of which chemical ones have obviously dominated. A range of chemicals have been employed. They have undoubtedly produced encouraging results but majority of them appear to be unsatisfactory as they themselves may pose problems related to toxicity, mutagenicity, carcinogenicity etc. on several biological systems besides being environmental pollutants and costly. There has recently been an extensive search for alternatives that would provide satisfactory aflatoxin control with low impact on the environment and on human health[4]. In view of a few workers [5,6] homoeopathic drugs could fulfill the promise as they have been shown to possess antifungal properties. Conceding the facts, 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^{o}C for seven days and stored at 4^{o}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. Drugs were randomly picked up from materia medica devoted for human sufferings. In fact, a parallel materia medica should be developed for the treatment of plant sufferings. Homoeopathic law of similars needs be extended to plant world as well using many plant-pathogen-drug systems. And depending upon the requirement, additional drugs should be incorporated from the products of the living world including even secondary metabolites.

2.1 *IN VITRO* STUDIES

Fungitoxicity of the drugs was examined 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[7] 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^{o}C for 10 days. Thereafter, mycelial mats were removed and % inhibition of the mycelial growth over control was calculated.

Effects of homoeodrugs on aflatoxin G_1 production were determined by estimating the mycelial weights in different culture filtrates following the standard methods of Nebney and Nesbitt, (1965) and Eppley, (1968)[8,9].

2.2 *IN VIVO* EFFECTS

For pre-inoculation treatments, 10.0g healthy groundnut seeds were surface sterilized with 0.1% mercuric chloride solution, 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^{o}C for 10 days.

In post-inoculation treatments, seeds received homoeodrug treatment after inoculation with the test pathogen, rest of the procedure remaining the same. 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

3.1 *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,Drosera 30 and 1M. Next, there were several cases where drugs were recorded as poor fungitoxicants with respect to fungal growth, though they reduced aflatoxin G_1 production to a remarkable extent. These were Arnica montana all potencies, Belladona 3, 6, 1M, 10M, Caladium all potencies, Drosera 3, 6, 12, 200, 10M, Euphrasia 3, 6, 12, 30, 200, 10M, Hypericum 3, 6, 30, 1M, Lachesis 3, 6, 12, 1M and 10M. Only one drug,Belladona 30 was strong fungitoxicant though poor against aflatoxin G_1 production.

The lack of correlation between fungal growth and aflatoxin B_1 production in *A. parasiticus* as already mentioned, has also been recorded by Sinha and Singh, (1983) and Shrivastava and Atri, (1998)[5,6].

3.2 *IN VIVO* EFFECTS:

As is evident from the observations (Table 2) the antiaflatoxic responses have differed with respect to mode of drug treatment. Some drug potencies worked better as preventives; for examples Belladona 3, 6, 12, Caladium 3, 1M, 10M, Drosera 6, 12, 30, 200, 1M, 10M, Hypericum 3, 30, 200, 1M, 10M, Lachesis 3, 6, 12 and 1M. These curtailed aflatoxin production in a range of 100%. Belladona 12, Drosera 12, 30, 1M, Hypericum 3, 1M, 10M, Lachesis 3, 6 and 12 were also found to work well as curatives bringing about a good deal of reduction in aflatoxin production G_1 by 100%. A range of drug potencies have proved better as curatives when used in post inoculation treatments, as these brought about 100% reduction in aflatoxin production G_1. These were Belladona 12, 30, 200, Drosera 3, 12, 30, 1M, Hypericum 3, 6, 12, 1M, 10M, Lachesis 3, 6, 12, 30 and 10M.

Moreover '*in vitro*' performances of certain homoeopathic drugs were found to be more or less altered on host front. For example efficacies of Arnica montana 3, Caladium 6, Drosera 3, Euphrasia 200, 1M, Hypericum 12 and Lachesis 10M were rendered weaker and those of Arnica montana 12, 200, Caladium 3, 30, 1M, 10M, Drosera 6, 12, 200, 10M, Euphrasia 3, 30, Hypericum 3, 30, 200, 1M, 10M, Lachesis 3, 6, 12, 30 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 [10].

DISCUSSION

A study of data (Table 1 and 2) would also exhibit certain unconventional features of homoeodrug action. Among the large number of drug potencies used, though many emerged as strong fungicides, yet none could suppress mycelial growth totally. Such observations have also been made by earlier workers using homoeopathy [6,10,11,12]. 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*. Unlike allopathy, homoeopathy considerers host as the primary site of action where basic contradictions of

health and disease operate, wherefrom the drugs amass their powers to fight against the pathogen, the latter being considered as playing the second fiddle in producing the disease [6,10,11,12].

Another feature striking in majority of cases was that several drug responses were not proportional to the concentration of the drug. This is unlike conventional substances were drug responses are commonly concentration dependent. The mode of drug preparation which uniquely involves potentization might account for this feature [13, 14, 15]. The process of potentization presumably produces different forms (physical state) of the drug molecules, each form endowed with a distinct property (medicinal value), suggestive of multiple site action of homoeopathic drugs[13, 14, 15]; hence sinusoidal responses over a range of drug potencies. Such observations have also been made earlier [10, 11]. If such is the case then it would not be possible for the pathogen to develop resistance against homoeodrugs through alternative pathways. This is not demonstrated with conventional substances which are site specific selective fungicides. Probably this could be the reason why pathogens evolve resistance against conventional substances[16, 17, 18].

Table 1

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
Arnica montana	17. 68	100. 00	14. 73	99. 12	13. 23	93. 98	13. 18	94. 85	5. 20	98. 42	4. 26	97. 66	3. 84	98.45
Belladona	30. 22	100. 00	56. 05	100. 00	23. 08	95. 15	66. 98	32. 30	66. 57	66. 55	27. 61	100. 00	32. 81	91. 12
Caladium	28. 65	100. 00	35. 05	95. 41	29. 62	100. 00	28. 50	100. 00	30. 85	92. 45	28. 29	100. 00	20. 65	100. 00
Drosera	34. 12	96. 60	27. 41	100. 00	25. 82	100. 00	67. 67	100. 00	22. 95	100. 00	74. 79	92. 62	30. 70	96. 77
Euphrasia	38. 64	93. 92	29. 86	61. 75	35. 76	100. 00	28. 87	98. 95	29. 70	94. 68	37. 12	86. 96	32. 12	97. 74
Hypericum	-0. 68	95. 56	8. 76	98. 36	21. 31	89. 57	9. 19	95. 89	11. 06	84. 92	14. 15	99. 13	43. 23	96. 06
Lachesis	17. 08	95. 50	-3. 08	97. 10	1. 19	96. 22	-8. 61	45. 48	21. 24	83. 91	17. 70	96. 37	-8. 61	96. 83
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' effects 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
Arnica montana	25. 71	25. 71	79. 99	79. 99	91. 42	62. 85	65. 71	85. 11	94. 28	88. 57	94. 28	79. 99	65. 71	25. 71
Belladona	100. 00	88. 57	100. 00	71. 45	100. 00	100. 00	85. 11	100. 00	91. 42	100. 00	91. 42	88. 57	94. 22	88. 57
Caladium	100. 00	85. 71	5. 71	94. 28	74. 28	48. 57	94. 28	85. 71	77. 14	22. 85	100. 00	88. 57	100. 00	65. 71
Drosera	22. 85	100. 00	100. 00	85. 11	100. 00	100. 00	100. 00	100. 00	100. 00	79. 99	100. 00	100. 00	100. 00	74. 28
Euphrasia	91. 42	94. 28	79. 99	85. 71	-28. 71	57. 14	82. 85	-43. 06	-40. 13	85. 71	28. 54	97. 14	19. 90	85. 71
Hypericum	100. 00	100. 00	79. 99	100. 00	31. 48	100. 00	100. 00	94. 28	100. 00	17. 03	100. 00	100. 00	100. 00	100. 00
Lachesis	100. 00	100. 00	100. 00	100. 00	100. 00	100. 00	97. 14	100. 00	68. 67	85. 11	100. 00	88. 57	-5. 70	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

PR=Pre-inoculation Treatment
PO=Post-inoculationTreatmen

CONCLUSIONS

In pre inoculation treatments, a number of homoeo substances have brought about total inhibition in the synthesis of aflatoxin G_1 production on groundnut seeds. These were Belldona 3, 6, 12, Caladium 3, 1M, 10M, Drosera 6, 12, 30, 200, 1M, 10M, Hypericum 3, 30, 200, 1M, 10M, Lachesis 3, 6, 12 and 1M. In post inoculation treatments, 100% inhibition of aflatoxin G1 could also be scored by a number of drugs e.g., Belldona 12, 30, 200, Drosera 3, 12, 30, 1M, Hypericum 3, 6, 12, 1M, 10M, Lachesis 3, 6, 12, 30 and 10M. Thus, we can infer that homoeodrugs may fulfill all the prerequisites of a promising fungicide. Being cheap, posing no health hazard or pollution problem, they may be used without risk as protectant or therapeutant in controlling aflatoxin G_1 contamination.

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