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Research Article

INHIBITORY EFFECT OF HOMOEOPATHIC DRUGS ON THE PRODUCTION OF AFLATOXIN B_1 IN GROUNDNUTS

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ABSTRACT

Presently chemical methods dominate over others in the control of the aflatoxins. No doubt some of them are effective but they their prolonged used is abscessed with untoward side effects. It implies that their is need to revised document approach. This as alarmed the researchers to explore ecofriendly alternatives. Hence the use of homoeopathic drugs. Effect of six homoeodrugs each in six potencies were tested against aflatoxin B_1 production and mycelial growth under '*in vitro*' and '*in vivo*' conditions. Preventives treatments such as Bryonia 3, Coffea cruda 3, Spongia 3, Thuja occidentalis 6 and 200 appeared as most effective whereas Carbo vegetabilis 3, 6, Coffea cruda 200, 1M, Opium 3, Spongia 6 and 30 emerged as most curative treatments as their employments could curtail aflatoxin production on groundnuts to an appreciable extent. Thus, aflatoxin on groundnut could be dealt with quite successfully by these homoeodrugs.

Keywords: Aflatoxin, *Aspergillus parasiticus*, Groundnut seeds, Homoeopathic drugs.

INTRODUCTION

Aflatoxins are naturally occurring mycotoxins that are produced by many species of *Aspergillus*. Aflatoxins can contaminate food products during several stages: processing, storage, or transport under conditions favorable for mold growth. Aflatoxins are carcinogenic, mutagenic teratogenic and immunosuppressive agents[1,2]. Chemicals methods have been suggested as treatment for aflatoxins. Though they are effective to a large extent, they are not satisfactory. As their exposure is known to have introduced certain undesirable side problems related to solvent residues, flavor changes and nutritional status leading to health hazards. There has recently been an intensive fumbling search for ecofriendly alternatives that would provide satisfactory aflatoxin control with low impact on human health[3]. Antifungal and antiaflatoxic action of homoeopathic drugs has great potential as they are easy to prepare and apply. Furthermore, they are cheap and devoid of residual effects. A few workers[4,5,6] have already reported homoeodrugs possessing antifungal and antiaflatoxic properties. But to make it sure that the drugs do not contain undesirable adulterants such as toxic metals, pesticide residues, aflatoxin etc. Their sources should be analyzed for pharmacognostic standardization as also been stressed by a few workers in case of *Glycyrrhiza glabra*[7] and *Pulsatilla nigricans*[8] respectively.

MATERIAL AND METHODS

Aspergillus parasiticus, strain MTCC No. 411, the test organism in the present endeavour was acquired from IMTECH, Chandigarh. It was grown on the malt salt agar medium (20g malt extract, 20g sodium chloride, 20g dextrose, 1.0g peptone, and 1000 ml distilled water) at 28°C for seven days and stored at 4°C. For experimental design, six homoeopathic drugs (Table 1) belonging to centesimal potencies labeled 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 employed in the present venture were of the order of 10^{-6}, 10^{-12}, 10^{-24},10^{-60}, 10^{-400}, $10^{-2000}$and 10^{-20000} dilutions respectively. From any angle these are ultramicrodilutions. Drugs were randomly picked up from materia medica devoted for human ailments as no materia medica is available for the treatment of plant sufferings.

In vitro studies

Antifungal profile 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[9] 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 organism *A. parasiticus* and incubated at 28 ± 1°C for 10 days. Thereafter, mycelial mats were separated and % inhibition of the mycelial growth over control was calculated.

Effects of homoeodrugs on aflatoxin B_1 production were measured by estimating the mycelial weights in different culture filtrates following the standard methods[10,11].

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 organism and incubated at 28± 1°C for 10 days.

In post-inoculation treatments, seeds received homoeodrug treatment after inoculation with the test organism, rest of the protocol 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 determination of aflatoxin B_1 as per the methods mentioned above.

RESULTS AND DISCUSSION

In vitro effects

Responses towards mycelial growth and aflatoxin B_1 production brought about by homoeopathic drugs could be placed into certain specific slots (Table 1). A few cases were recorded where drugs mitigated both fungal growth and aflatoxin B_1 production to a remarkable extent. For example Bryonia 3, Carbo vegetabilis 6, Thuja occidentalis 30 and 10M. Next, there were several cases where drugs caused least effect on fungal growth, though they curtailed aflatoxin B_1 production to a remarkable extent. These were Bryonia 6, 1M, 10M, Carbo vegetabilis 3, 1M, 10M, Coffea cruda 200, Spongia and Thuja occidentalis, all potencies. There were only two drugs, Carbo vegetabilis 12 and Coffea cruda 3 which were strong fungitoxicants but poor against aflatoxin B_1 production. Interestingly several potencies were found to stimulate aflatoxin B_1 production such as Bryonia 12, Carbo vegetabilis 30, Coffea cruda 3, 12 and 1M.

These stimulatory effects could be on account of the presence of certain polyunsaturated fatty acids which are abundant in groundnut. These drugs might have activated lipoperoxidation that might have induced aflatoxin B_1 production considerably. Such a rise in aflatoxin B_1 production was also observed in *A. parasiticus* and *A. flavus* cultures treated with synthetic lipoperoxides[12].

The lack of mutual relationship between fungal growth and aflatoxin B_1 production as observed in present study has also been mentioned by other workers[4,5,6].

***In vivo* effects**

As is obvious from the data (Table 2) the antiaflatoxic responses have differed with respect to mode of drug treatment. Some drug potencies worked better as prophylactives or preventives; for examples Coffea cruda 3, Spongia 3, Thuja occidentalis 6, 200 and Bryonia 3. These curtailed aflatoxin production in a range of 85-97%. Coffea cruda 3 and Spongia 3 were also found to work well as therapeutics or curatives bringing about a good deal of reduction in aflatoxin production by 81.08 and 89.19 respectively. However, Thuja occidentalis 6 and 200 failed as curatives; instead of curtailing they boosted aflatoxins production. A range of drug potencies have proved better as curatives when used in post inoculation treatments, as these brought about more than 90% suppression in aflatoxin production. These were Carbo vegetabilis 3, 6, Coffea cruda 200, 1M, 10M, Spongia 3, 6, 30, Opium 3. However, some of these drugs, e.g., Coffea cruda 200, Spongia 6, 30 and Opium 3 have shown extremely poor antiaflatoxic properties as preventives.

Furthermore '*in vitro*' execution of certain homoeopathic drugs were found to be more or less changed on host front. For example effectiveness of Bryonia 6, 1M, 10M, Carbo vegetabilis 10M, Opium all potencies, Spongia 200, 1M,Thuja occidentalis 6 and 200 were made weaker and those of Carbo vegetabilis 3, 30, 1M, Coffea cruda 30, 10M, Opium 6, Spongia 3, 1M, Thuja occidentalis 3, 6 and 200 were rendered stronger as preventives. Similar discordant observations were also recorded with respect to curative treatments. Some host factors of unknown nature might be responsible for such modulations[12].

Besides, a study of data (Table1 and 2) also reveals certain unconventional features of homoeodrug action. Among the large number of drug potencies used, though many acted as strong fungicides, yet none could curtail mycelial growth completely. Such observations have also been made by earlier workers using homoeopathic drugs[5][13,14,15]. Reasons for such mishaps 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 conflicts of health and disease operate, wherefrom the drugs derive their powers to fight against the pathogen, the latter being considered as playing the auxiliary role in producing the disease[5,13,14,15].

Table 1: Effect of homoeodrugs on mycelial growth and aflatoxin B_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
Bryonia	63. 84	72. 10	24. 13	94. 44	27. 30	-47. 51	-3.76	24. 52	48. 26	96. 60	28. 97	96. 60	32. 01	99. 08
Carbo vegetabilis	34. 91	82. 44	63. 29	99. 9	70. 41	38. 91	37. 74	-78. 05	32. 01	44. 17	36. 59	83. 84	27. 06	90. 91
Coffea cruda	87. 63	-513. 72	30. 65	20. 94	34. 23	-30. 07	20. 72	20. 96	33. 74	93. 63	37. 44	-65. 64	30. 55	14. 12
Opium	15. 44	97. 86	16. 27	94. 24	15. 28	95. 73	20. 41	87. 97	13. 76	95. 80	19. 93	94. 12	13. 78	88. 82
Spongia	10. 77	67. 59	13. 20	70. 85	31. 04	69. 42	22. 41	52. 63	18. 98	85. 12	15. 44	99. 28	8. 20	66. 53
Thuja occidentalis	-6. 13	94. 32	13. 30	95. 83	-3. 38	95. 92	68. 77	94. 21	10. 09	83. 91	6. 04	73. 07	64. 38	92. 99
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 B_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
Bryonia	86. 48	64. 86	56. 75	83. 78	59. 45	37. 83	64. 86	56. 75	8. 10	70. 27	64. 86	32. 43	70. 27	29. 72
Carbo vegetabilis	70. 27	97. 29	59. 45	94. 59	78. 37	56. 75	81. 08	10. 81	67. 56	72. 97	72. 97	37. 83	59. 45	16. 21
Coffea cruda	97. 29	81. 08	59. 45	67. 56	59. 45	59. 45	81. 08	75. 67	21. 62	97. 29	35. 13	89. 18	72. 97	94. 59
Opium	21. 62	89. 18	78. 37	70. 27	59. 45	8. 10	67. 56	67. 56	59. 45	-18. 91	18. 91	75. 67	56. 73	59. 45
Spongia	97. 29	89. 18	32. 29	94. 59	27. 02	81. 08	8. 10	94. 59	51. 35	64. 87	72. 97	67. 56	56. 75	36. 48
Thuja occidentalis	72. 97	54. 05	94. 59	-351. 35	56. 75	-169. 05	67. 56	-224. 32	86. 48	-2. 70	24. 32	45. 96	59. 45	10. 81
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

Another characteristic striking in majority of cases was that several drug responses were not proportional to the concentration of the drug. This is unlike conventional substances where drug responses are usually concentration dependent. The mode of drug preparation which uniquely involves potentization might account for this feature [16,17,18]. The process of potentization presumably produces different physical forms of the drug molecules, each form endowed with a distinct medicinal property, suggestive of multiple site action of homoeopathic drugs[16,17,18]; hence sinusoidal responses over a range of drug potencies. It appears as if each drug potency acts as a separate drug. Such observations have already been made[13,14]. 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 such as benomyls[19,20].

CONCLUSION

As pre inoculation treatments of homoeopathic drugs such as Coffea cruda 3, Spongia 3, Thuja occidentalis 6, 200 and Bryonia 3 have curtailed *in vivo* aflatoxin B_1 production significantly. A range of homoeopathic drugs potencies such as Carbo vegetabilis 3, 6, Coffea cruda 200, 1M, 10M, Spongia 3, 6, 30, Opium 3 used as post inoculation treatments brought about a remarkable reduction in aflatoxin B_1 synthesis. Hence these can be employed as curatives.

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