J. Phytol. Res. 11 (1): 45-49, 1998

EFFECT OF HOMOEOPATHIC DRUGS ON THE PRODUCTION OF AFLATOXIN B_1 BY *ASPERGILLUS FLAVUS*

J. SHRIVASTAVA and D.C. ATRI
Plant Pathology Lab., Department of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar (M.P.) 470 003, India.

Antifungal and antiaflatoxic properties of twenty homoeodrugs were tested against Aspergillus flavus strain II, the contaminant of linseed grains under *in vitro* and *in vivo* conditions. Belladona 1000 and sulphur 30 appeared most promising preventive treatments, as oilseeds treated with them produced no aflatoxin B_1. Post-inoculation treatments were comparatively less effective. Nevertheless, Belladona 30, Bryonia 200, Carbo vegetabilis, Graphites 30, 1000, Mercurius solubilis 6, Phosphorus 30, Thuja 30 and 1000 curtailed toxin production up to 70%. Their unique properties are discussed in light of homoeopathic principles.

Keywords: *Aspergillus flavus*; Control; Homoeopathic drugs; Linseed; Prevention.

Introduction

Linseed, the plant material of the present investigation, is a crop of immense industrial and medicinal value and is also used as cattle feed. It is often attacked by toxigenic molds during storage. Though certain conventional chemicals such as captan, thiram, ammonia, formaldehyde, acetic acid and propionic acid have been suggested as treatment for the menace[1],no method is available which could securely control aflatoxion production. In fact, majority of conventional substances used in modern plant protection are obsessed with some or the other kind of adverse effects on health and ecosystem[2-5]. Hence, the demand to ban their use and search for non-toxic, ecofriendly alternatives[2,6-8]. In view of a few workers homoeopathic drugs could fulfil the promise as they have been shown to possess antifungal properties. Besides, there is at least one report indicating their anti-aflatoxic properties[9]. Conceding the facts, an attempt has been made in the present communication to control aflatoxin production in linseed through homoeopathic drugs.

Materials and Methods

Aspergillus flavus strain II was isolated from linseed grains and maintained on malt agar. For experimental purposes, twenty homoeopathic drugs belonging to centesimal potencies 6, 30, 200 and 1000 were used (as is customary, suffix 'c' standing for centesimal potency has been ommitted). They belonged to Sharda Boiron Lab. Ltd., Ghaziabad. In homoeopathy, concentration of drugs is inversely proportional to their potency. Hence, drug concentrations in 6, 30, 200 and 1000 potencies were of the order of 10^{-12}, 10^{-60}, 10^{-400}, 10^{-2000} respectively. From any standard these are ultramicrodilutious.

In vitro Studies : Fungitoxicity of the drugs was determined in terms of their inhibitory effects on mycelial growth as well as aflatoxin production. For this purpose, 250 ml flasks were dispensed with 50 ml sterilized yeast extract sucrose (YES) broth containing 20 g yeast extract, 200 g sucrose and 100 ml distilled water and were provided with 0.2 ml each of 6, 30, 200, 1000 drug potencies. In control, 0.2 ml 90% ethyl alcohol (drug medium) replaced the drug. Flasks were inoculated with the test fungus and incubated at 28°C for a period of 10 days. Thereafter, mycelial mats were removed and % inhibition of the mycelial growth over control was calculated.

Effects of homoeodrugs on aflatoxin production were determined by estimating the quantity of aflatoxin B_1 per gram dry mycelial weight in different culture filtrates following the standard methods[10-12] of toxin extraction, chromatographic separation and quantitative estimation.

In vivo Studies : For pre-inoculation treatment, 1.0 g oilseeds, after surface sterilization in 0.1% mercuric chloride and distilled water wash were soaked in different drug solutions for a period of 24 hours. Thereafter they were inoculated with 1.0 ml of acqueous spore suspension of the test pathogen and incubated at 28°C for 10 days. In post-inoculation treatment, seeds received homoeodrug treatment after inoculation with the test pathogen, rest of the procedure remaining the same. Seed lots soaked in ethylated water served as controls. All treatments were triplicated. Subsequently, 10 g seed samples from treated and control sets were processed for the quantitative estimation of aflatoxin B_1 as per the methods mentioned above.

Results and Discussion

In vitro effects : Effects of homoeodrugs revealed in terms of responses towards mycelial growth and aflatoxin production in *in vitro* experiments could be slotted into certain specific categories (Table 1). A few cases were recorded where drugs could restrict both fungal growth and aflatoxin production of a remarkable extent. For example, Carbo vegetabilis 6, Bryonia 6 and Sulphur, all potencies. Next there were several cases where drugs were recorded as poor fungitoxicants with respect to mold growth, though they inhibited aflatoxin B_1 to a significant extent. These were, Drosera, all potensies, Dulcamara, all potencies, Graphites, all potencies. Mercurius solubilis, all potencies, Lachesis 6 and Merc. corrosivus 30. Drosera 200 was an extreme example which stimulated mycelial growth (7.94%) but suppressed a good deal of toxin synthesis (81.16%). Further, there were cases where drugs were found to stimulate aflatoxin B_1 production, despite a great deal of mold reduction, e.g., Arsenicum album, all potencies. Iodium 6, 200, 1000, Plumbum 6. Special mention must be made of Arsenicum album potencies, particularly Arsenicum album 6 which was responsible for more than 15-fold rise in aflatoxin production. This dramatic shoot up could be understood in terms of polyunsaturated fatty acids especially linoleic acid which are abundant in linseed grains. Lipoperoxidation activated by Arsenicum album might be considered as playing a crucial role in inducing aflatoxin production tremendously. Such a dramatic boost in aflatoxin production was also observed in *A. parasiticus* and *A. flavus* cultures amended with synthetic lipoperoxides[13].

The lack of correlation in mold growth and aflatoxin production in *A. falvus* strain II as mentioned, has also been recorded earlier[9,14,15].

In vivo effects : It is obvious from the data (Table 2) that detoxification responses differed with respect to mode of treatment of the drugs. Some drug potencies performed better as preventives. These were : Belladona 6, 200, 1000; Bryonia 6, Sulphur 30, 200 and Thuja occidentalis 6. These curtailed aflatoxin production by more than 80%. But most successful among these were Belladona 1000 and Sulphur 30 as the oilseeds pretreated with them were completely rendered free of any aflatoxin B_1. It was interesting to note that preventive performances of all these drug potencies were on par with their *in vitro* antiaflatoxic efficacies. However, these drugs did not perform so well as curatives. In fact, homoeodrugs were not as effective in the form

Table 1. Effect of homoeodrugs on mycelial growth and aflatoxin B_1 production ability of *A. flavus* Strain II.

Drugs	POTENCY							
	6		30		200		1000	
	Per Cent Inhibition or Stimulation (-)							
	MG	AP	MG	AP	MG	AP	MG	AP
1. Aconitum napellus	19.23	18.16	31.36	13.58	5.78	26.24	35.24	3.16
2. Arnica montana	15.89	19.39	6.92	36.27	13.83	20.22	15.57	15.68
3. Arsenicum ablum	65.21	-1502.92	61.57	-508.57	56.55	-254.94	50.55	-739.67
4. Belladona	33.84	92.31	38.04	97.07	28.04	95.25	37.42	97.29
5. Bryonia	49.63	100.00	36.22	94.68	29.61	97.59	43.74	90.96
6. Carbo vegetabilis	63.02	86.25	30.39	75.65	36.37	84.02	30.48	78.13
7. Drosera	12.60	84.48	12.25	82.62	7.94	81.16	7.11	85.40
8. Dulcamara	27.08	72.11	19.28	72.70	11.25	82.81	16.57	69.53
9. Graphites	23.66	66.20	27.61	60.20	11.47	61.71	8.42	66.69
10. Hepar Sulphur	13.43	-1.81	20.61	-3.92	7.33	10.38	19.54	-1.11
11. Iodium	30.57	-149.01	38.02	01.54	30.31	-128.62	41.33	-73.35
12. Ipecacuanha	20.41	14.82	12.69	30.11	9.11	04.89	16.25	02.85
13. Lachesis	7.06	79.94	33.69	66.77	24.29	66.42	32.14	75.02
14. Mercurius corrosious	10.42	14.85	7.33	56.11	11.91	0.7.65	16.57	14.67
15. Mercurius solubilis	16.25	87.86	22.37	80.35	17.09	91.82	19.32	76.89
16. Phosphorus	6.06	54.89	9.16	66.41	14.96	86.05	13.63	58.79
17. Plumbum	36.24	-120.30	32.44	02.15	18.36	33.57	36.63	59.88
18. Rhus toxicodendron	12.43	01.29	13.62	05.81	4.47	11.28	10.60	-0.48
19. Sulphur	58.57	50.91	55.08	88.68	50.94	72.36	54.21	85.19
20. Thuja occidentalis	28.17	83.49	38.62	43.49	38.01	91.80	9.91	0.29
Control	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00

Mg = Mycelial Growth; AP = Aflatoxin Production.

of post-inoculation treatments. The most promising ones among these, however, were: Belladona 30, Bryonia 200, Carbo vegetabilis 200, Graphites 30, 1000, Mercurius solubilis 6, Phosphorus 30 and Thuja occidentialis 30 and 1000. These curtailed aflatoxin B production up to 70%.

Moreover, *in vitro* efficacies of certain homoeodrugs were found to be more or less modified on host front. For example, antiaflatoxic potentials of carbo vegetabilis, Drosera, Dulcamara and Lachesis were rendered weaker and those of the Hepar sulphur and Iodium were made stronger, both as preventives and curatives. Some host factors of unknown nature were presumably responsible for such alterations[6,16].

Besides, a perusal of data would also reveal certain unusual and unconventional features of homoeodrug action. Among the 80 drug potencies applied, though many emerged as fungicides, yet none could inhibit mycelial growth completely. Such observations have also been recorded by earlier workers dealing in homoeopathy[6,7,16,17] The reasons are far from known. Perhaps homoedrugs do not act against the pathogens *in vitro* as effectively as they do against them *in vivo*. Unlike allopathy, homoeopathy considers host as the primary site of action

Table 2. *'In vivo'* effect of homoeopathic drugs on Aflatoxin production on linseed grains by *A. flavus* strain II.

Drugs	POTENCY							
	6		30		200		1000	
	Per Cent Inhibition or Stimulation (-)							
	MG	AP	MG	AP	MG	AP	MG	AP
1. Aconitum napellus	18.90	3.62	1.42	42.52	34.96	29.29	5.51	37.48
2. Arnica montana	65.67	16.85	35.12	2.61	41.73	54.33	8.03	18.58
3. Arsenicum album	6.77	15.28	20.00	7.09	-13.07	24.09	2.20	-14.17
4. Belladona	91.81	42.36	57.75	60.63	98.74	19.69	100.00	22.52
5. Bryonia	88.82	59.06	48.50	39.21	71.65	67.24	20.63	31.18
6. Carbo vegetabilis	39.69	10.24	21.41	32.28	51.34	72.28	44.09	-9.92
7. Drosera	3.15	31.02	8.35	52.76	49.61	01.73	37.01	11.81
8. Dulcamara	15.43	37.17	53.07	25.67	43.15	62.20	33.39	19.37
9. Graphites	55.28	51.02	7.56	62.20	31.34	-12.60	28.50	67.09
10. Hepar Sulphur	53.54	42.20	51.50	54.65	70.80	36.22	70.71	5.51
11. Iodium	15.75	11.50	41.10	28.66	31.18	3.62	9.61	48.82
12. Ipecacuanha	3.15	5.04	21.26	-22.99	-9.92	51.65	23.46	18.74
13. Lachesis	31.50	-2.83	46.77	31.02	15.12	15.75	23.94	11.02
14. Mercurius corrosious	12.60	40.94	9.92	19.21	36.22	7.24	38.43	30.71
15. Mercurius solubilis	57.64	60.00	44.41	4.25	34.02	58.58	47.24	40.31
16. Phosphorus	59.06	38.58	12.28	68.19	3.46	15.91	27.09	9.29
17. Plumbum	31.34	53.86	5.67	2.52	27.56	11.34	43.62	8.82
18. Rhus toxicodendron	3.46	17.01	7.40	20.31	-44.09	01.42	8.35	-30.87
19. Sulphur	61.26	40.47	100.0	60.79	80.00	63.62	64.25	35.59
20. Thuja occidentalis	80.47	21.10	73.54	60.31	45.67	38.11	31.97	68.82
Control	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00

PR = Preinoculation treatment
PO = Post-inoculation treatment

where fundamental contradictions of health and disease operate, wherefrom the drugs marshal their powers to fight against the pathogen, the latter being considered as playing the auxillary role in producing the disease[6,7,16,17].

Another feature conspicuous in majority of cases was that majority of drug responses were not proportional to the concentration (potency) of the drug. This is unlike conventional substances where drug responses are mostly dose dependent. The mode of drug preparation which involves not only dilution but also potentization, might account for this distinction[18-20]. The process of potentization presumably generates varied forms (physical states) of the drug molecules corresponding to different drug potencies, each form endowed with a distinct functional property (medicinal value) corresponding to a particular optimum, suggestive of multiple site action of homeopathic drugs[18-20]. Hence, multiple peaks of responses over a range of drug potencies. This seems consistent with observation made earlier[6,7,16]. If this be the case, then it would be exceedingly difficult for the pathogen to develop resistance against homeodrugs, by operation of alternative pathways[6]. This is not so with conventional substances which are site specific selective fungicides. Perhaps, this could also be the reason why more pathogens evolved resistance against benomyls

(site specific fungicide) than against dithiocarbamates (multiple site fungicide)[21-24].

Acknowledgement

The authors are grateful to Prof. K. M. Vyas, Head of Botany Department, Dr. H.S. Gour Vishwavidyalaya, Sagar, for providing laboratory facilities.

References

1. Dubey GL 1980, Ph. D. Thesis Dr. H.S. Gour V.V. Sagar
2. Eckert JW and Sommer NF 1976, *Ann. Rev. Phytopath.* **5** 391
3. Gullino ML and Kuijpers LAM 1994, *Ann. Rev. Phytopathol.* **32** 559
4. Hocart MJ, Lucus JA, Peberdy JA 1990, *Mycol. Res.* **94** (1) 9
5. Jalali BL and Sharma OP 1993, *Ind. J. Microbiol.* **33** (2) 83
6. Chandra S and Khanna KK 1981, *Recent Advances in the Biology of Microorganisms* Part II Eds. Bilgrami KS and KM Vyas
7. Goswami N and Das D 1980, *Hahn. Glean.* **47** 832
8. Klingauf F 1982, *Plant Res. and Development* **16** 75
9. Sinha KK and Singh PL 1983, *Indian Phytopath.* **36** (2) 356
10. Eppley RM 1968, *JAOAC* **51** (1) 74
11. Maggon KK, Vishwanathan L, Venkitasubramanian TA and Mukerji KG 1969, *J. Gen. Microbiol.* **59** 119
12. Nabney J and Nesbitt BF 1965, *Analyst* **19** 155
13. Passis, Nazzaro-Porro M, Fanelli C, Fabbri AA and Fasella P 1984, *Appl. Microbiol. Biotechnol.* **19** 186
14. Prasad RB 1983, *Proc. Symp. Mycotoxin in food and feed* Bhagalpur 207
15. Sahay M 1983, *Proc. Symp. Mycotoxin in food and feed* Bhagalpur 199
16. Khare D and Atri DC 1995, *J. Phytol. Res.* **8**(1) 49
17. Dua VK and Atri DC 1986-87, *Bull. Bot. Soc.* **33-34** 4
18. Gibson RG 1968, *Br. Hom. J.* **57**(3) 157
19. Pelican W and Unger G 1971, *Br. Hom. J.* **60** 233
20. Rawson DS 1976, *The Hahn. Glean.* **43**(12) 538
21. Dekker J 1976, *Ann. Rev. Phytopathol.* **14** 405
22. Dimond AE, Horsfall JG, Heuberger JW and Stoddard EM 1941, *Connecticut Agri, Exp. Sta. Bull.* **451** 635
23. Georgopulos SG 1977, *Antifungal Compounds*, Eds. Sesler HS and Siegel SR Vol.2, NY
24. Owens RG 1969, *Fungicides an Advanced Treatise* Ed. Torgeson, Academic Press Vol. 2 NY