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SEED BORNE MYCOFLORA OF CASTOR BEAN (*RICINUS COMMUNIS* L.) FROM PAKISTAN

SHAHNAZ DAWAR[1*], SUMAIRA KHALID AND MARIUM TARIQ[2]

[1]*Department of Botany, University of Karachi, Karachi-75270, Pakistan*
[2]*M.A.H. Qadri Biological Research Centre, University of Karachi, Karachi-75270, Pakistan*
[*]*Corresponding author e-mail: shahnaz_dawar@yahoo.com*

Abstract

Castor bean seeds were analyzed by using ISTA (International seed testing association) for the detection of seed borne mycoflora. Thirty one fungal species belonging to 15 genera were isolated from 12 samples of castor bean seeds collected from different areas of Pakistan. *Fusarium solani, Alternaria alternata*, *Cephaliophora tropica* were most predominant fungal species isolated while the saprophytic fungi like *A. niger*, *A. flavus* were common in all samples of castor bean seed tested. Blotter method was considered to be better technique which gave maximum number of fungi followed by agar plate and deep freezing methods.

Introduction

Castor bean (*Ricinius communis* L.) is an industrial oil seed crop of the world. In Pakistan, Castor bean is cultivated in Punjab, Sindh and Baluchistan provinces where it is cultivated in arid and semi arid regions with about 300-350 mm rainfall suitable for its good growth. In Sindh it is planted over 2143 hectares annually with production of about 1546 tonnes and an average yield of 721 kgs/hectare (Anon., 2008). Castor bean contain 50-55% non ediable oil and 26-30% protein due to nature of chemical composition, its oil is used in more than 300 compounds (Mirza, 2009). In food industries castor oil is used as flavoring, candy, Chocolate is also use in food stuff industries (Wilson *et al.,* 1998; Busso & Castro-Prado, 2004). Its shell is used in organic termite control in soil while seed cake used as manure (Maiti *et al.,* 1988; Moshkin, 1986). Ricinoleic acid is the main component of castor oil and it considered to show anti-inflammatory effects (Vieira *et al*., 2000). Cheema *et al*., (2013) reported that castor bean is considered as moisture sensitive crop and its cultivation gave much better output when planted at rain-fed Pothwar region of Pakistan which provides much profit to the farmers of this region.

A survey of literature showed that many fungal species have been reported from castor bean which includes *Alternaria* sp., *Aspergillus* sp., *Curvularia* sp., *Fusarium* sp., *Helminthosporium* sp., *Mucor* sp., *Nigrospora* sp., *Penicillium* sp., *Sclerotium* sp., *Thielavia* sp., (Hafiz, 1986; Jamal & Ghaffar, 1974). Nagaraja *et al*., (2009) isolated forty seven fungal species belonging to 7 genera from 185 samples of castor bean seeds of which *F. oxysporum, Alternaria ricini, A. alternata, Culvularia lunata, Macrophomina phaseolina, Sclerotinia sclerotium, Cladosporium herbarum, Chaetomium globosum, Botryodiplodia acerina, Stachybotrys chartarum, Aspergillus ochraceus, A. niger, A. flavus, A. versicolor* and *Rhizopus stolonifer* were predominant on castor bean seeds. Saprophytic fungi particularly *A. niger, A. flavus, A. parasiticus* associated with seeds was observed to have showed development of mycotoxins. Aflatoxins producing fungi isolated from wheat, oil seeds, nut products, cereal grains (Yu *et al.,* 2004). Aflatoxins producing fungi are distributed and can grow over a wide range of environmental conditions and these aflatoxins caused various diseases of carcinogenic, hepatotoxic and teratogenicto in animals (Yu *et al*., 2002; Holmquist *et al*., 1983). The present study was carried out to explore seed borne mycoflora associated with castor bean using ISTA technique.

Materials and Methods

Collection of castor bean seeds: Twelve samples of castor bean seeds were collected from different parts of Pakistan viz., Sindh (4), Punjab (4), NWFP (2) and Baluchistan (2).

Performance of ISTA technique: Detection of seed borne mycoflora was carried out by using ISTA techniques (International Seed Testing Association). Methods which follows ISTA technique includes standard blotter method, agar plate method and deep freezing method. 400 seeds of each sample of castor bean seed were tested (Anon., 1993).

a. Standard blotter method: In this method, untreated and seed after treatment for 5 minutes with 1 % sodium hypochlorite and then placed on three layers of moistened blotter paper @ 20 seeds per Petri dish. These dishes were incubated for 7 days at 24±1°C under 12 hour alternating cycle of artificial day light (ADL) and darkness.

b. Deep freezing method: Seeds after treatment for 5 minutes with 1% sodium hypochlorite and untreated seeds were placed on three layers of moistened blotter paper and plates was incubated for 24 hours each at 20°C and -20°C which was then followed by 5 days of incubation at 24±1°C under 12 hour alternating cycle of artificial day light (ADL) and darkness.

c. Agar plate method: In this method treated and untreated seeds were placed on Potato Dextrose Agar (PDA) of pH 5.5 containing Benzyl Penicillin Potassium Salt (0.1 g^{-1}) and Streptomycin Sulphate (0.2 g^{-1}) at the rate of 20 seeds per plate. The dishes were incubated for 7 days at 24±1°C under 12 hour alternating cycle of artificial day light (ADL) and darkness.

Identification of mycoflora: Fungal species were identified to the generic or species level according to Ellis (1971), Barnett (1960), Booth (1971), Domsch *et al*., (1980), Nelson *et al*., (1983), Raper & Fennell (1965), Thom & Raper (1945).

Analysis of data: Data were subjected to analysis of variance (ANOVA) or factorial analysis of variance (FANOVA) following the procedure as given by Sokal & Rohlf (1995).

Results

Fungi on seeds of castor bean were detected by ISTA technique. Result showed total number of 15 genera and 31 species of fungi of which **Absidia corymbifera* (Cohn) Sacc & Trotter., **A .cylindrospora* Hagem, **A. glauca* Hagem, **Alternaria alternata* (Fr.) Keissler.,**A. longissima*, **Aspergillus candidus* Link ex link., **A .clavatus* Desm.,**A. flavus* Link ex Gray., **A. fumigatus* Fres.,**A. japonicus* Saito., **A. niger* Van Tieghem., **A. oryzae* (Ahlburg) Cohn., **A. sclerotiorum* Huber., **A. sulphureus,* **A. terreus* Thom., * *A .versicolor* (Vuill) Tiraboschi., **A. wentii* Wehmer., *Botrytis cinerea* Pers. ex Nocca & Balb., **Cephaliophora tropica* Thaxt, **Chaetomium globosum* Kunze ex Steud., **C. indicum* Corda., **Curvularia lunata* (Wakker) Boedijn., **Dreschlera dematioidea* (Bubak & Worblewski)., **Fusarium oxysporum* Schlecht. Emend. Sny. & Hans **F. solani* (Mart.) Appel & Wollenw., **Monilia* sp., **Mucor* sp. Mich. Ex St., **Myrothecium roridum* Tode ex Steudel., **Penicillium* sp. Link ex Fr., **Rhizopus oryzae* Went & Prinsen Geerligs., **R. stolonifer* (Ehrenb. ex Link) Lind. Fungal species marked with asterisk are new report (Hafiz, 1986; Jamal & Ghaffar, 1974; Ahmad *et al*., 1993; Nagaraja *et al*., 2009). Six seeds samples out of 12 were found to be infected by *C. tropica* while all samples of castorbean found to be contaminated with *A. flavus* and *A. niger* (Table 1). Followed to *A. niger* and *A. flavus,* other species of *Aspergillus* also found in highest percentage namely *A. fumigatus, A. terreus, A. versicolor* and *A. wentii.* Apart from saprophytic fungi, some pathogenic fungi were also obtained in greater amount which includes *C. tropica, C. globossum, M. roridum* from agar plate and blotter methods. Surface sterilized seeds with 1% sodium hypochlorite significantly reduced ($p<0.01$) the incidence of *A. flavus*, *A.niger, F. solani* and *M. roridum.* No significant difference was found in the incidence of *F. oxysporum, C. tropica* before and after surface sterilization on caster bean seeds. Seed sample collected from Karachi showed the highest incidence of fungi viz., *A. glauca, A. alternata, A. longissima, C. tropica, C. lunata, D. demotiodea, C. globosum, A. flavus* and *A. niger* on castorbean. Of the three different techniques used in ISTA, blotter method was observed to be best for isolation of mycoflora (Table 1).

Table 1. Seed borne mycoflora of castor bean.

Fungi	Sterilized seeds						Non-sterilized seeds					
	Agar plate method		Blotter method		Deep freezing method		Agar plate method		Blotter method		Deep freezing method	
	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD	NSI	I% ± SD
**Absidia corymbifera*	-	-	2	0.16 ± 0.00	-	-	-	-	-	-	-	-
**Alternaria alternata*	-	-	1	0.20 ± 0.57	-	-	2	0.25 ± 0.00	3	0.66 ± 3.05	-	-
A. longissima	-	-	2	0.125 ± 0.0	-	-	0	-	1	0.125 ± 1.0	-	-
**Aspergillus candidus*	1	5.0 ± 3.78	2	0.54 ± 2.08	2	0.16 ± 0.00	1	3.45 ± 2.54	2	2.62 ± 0.12	2	0.083 ± 0.00
**A.clavatus*	-	-	-	-	-	-	-	-	3	0.70 ± 4.72	-	-
**A.cylindrospora*	2	3.8 ± 0.00	1	1.04 ± 0.70	1	0.08 ± 0.00	1	0.54 ± 0.00	6	0.25 ± 0.00	0	1.04 ± 0.00
A.flavus	12	38.2 ± 20.89	12	17.8 ± 10.16	10	8.08 ± 6.47	12	37.5 ± 19.52	12	22.6 ± 3.52	10	10.62 ± 9.16
**A.fumigatus*	10	18.2 ± 10.96	5	23.5 ± 10.01	4	15.62 ± 3.6	10	26.1 ± 11.82	9	6.95 ± 6.98	7	7.70 ± 6.37
**A.glauca*	2	1.31 ± 6.02	5	38.95 ± 4.65	5	2.41 ± 1.06	2	6.12 ± 3.24	6	14.95 ± 5.69	4	4.01 ± 3.60
**A.japonicus*	-	-	-	-	-	-	1	0.125 ± 0	2	0.33 ± 0.00	-	-
A.niger	12	24.5 ± 12.96	12	5.41 ± 3.70	8	4.20 ± 2.21	12	18.7 ± 11.54	12	16.75 ± 7.96	9	11.29 ± 5.04
**A.oryzae*	1	0.51 ± 0.00	-	-	-	-	1	0.04 ± 0.00	2	0.29 ± 0.707	-	-
**A.sclerotiorum*	2	4.31 ± 2.82	-	-	-	-	2	1.83 ± 0.82	2	0.225 ± 0.00	-	-
**A.sulphureus*	1	12.6 ± 6.50	2	2.43 ± 1.37	1	0.41 ± 0.00	1	0.02 ± 0.00	1	8.58 ± 2.84	1	2.70 ± 1.54
**A.terreus*	8	10.12 ± 3.59	8	1.41 ± 0.00	1	0.29 ± 0.05	8	3.12 ± 2.22	5	6.37 ± 2.86	2	0.50 ± 0.00
A.versicolor	8	2.29 ± 18.85	5	5.58 ± 14.91	5	4.58 ± 8.70	8	2.08 ± 0.71	8	4.0 ± 8.90	5	2.95 ± 6.46
**A.wentii*	5	18.29 ± 5.85	8	14.58 ± 5.95	8	8.58 ± 4.70	9	3.08 ± 2.71	5	8.04 ± 2.90	9	6.95 ± 2.46
Botrytis cinnera	-	-	-	-	-	-	-	-	2	4.45 ± 0.94	-	-
**Cephaliophora tropica*	5	25.28 ± 2.37	2	21.45 ± 8.75	5	7.66 ± 2.03	6	19.8 ± 8.49	4	33.75 ± 9.54	5	8.70 ± 3.37
Chaetomium globosum	1	0.37 ± 0.00	6	1.83 ± 5.39	-	-	1	0.45 ± 0.00	4	1.16 ± 3.55	-	-
**C. indicum*	2	2.75 ± 0.82	4	2.45 ± 5.25	-	-	1	0.45 ± 0.00	6	4.54 ± 8.849	1	0.75 ± 9.89
Curvularia lunata	1	0.21 ± 0.00	1	0.08 ± 0.00	-	-	-	-	1	0.20 ± 0.00	-	-
**Drechslera demotiode*	2	0.37 ± 2.12	-	-	1	0.08 ± 0.00	-	-	1	0.58 ± 2.82	2	0.08 ± 0.00
Fusarium oxysporum	1	2.75 ± 12.9	-	-	2	3.91 ± 5.26	1	1.21 ± 1.41	-	-	1	2.70 ± 13.79
**F.solani*	3	0.54 ± 0.00	-	-	5	1.16 ± 2.51	3	0.37 ± 0.00	-	-	3	1.16 ± 0.00
**Monilia* sp	1	0.66 ± 3.05	-	-	3	2.26 ± 1.82	2	0.125 ± 0.00	-	-	4	3.21 ± 0.86
**Mucor* sp	2	0.41 ± 0.577	1	2.58 ± 6.71	3	1.91 ± 3.96	1	1.16 ± 4.24	2	4.20 ±7.08	2	0.08 ± 0.00
**Myrothecium roridum*	6	9.75 ± 0.89	6	2.33 ± 0.00	-	-	5	0.54 ± 0.00	6	0.08 ± 0.00	-	-
**Penicillium* sp	2	4.16 ± 9.15	2	5.5 ± 11.53	4	2.29 ± 13.3	1	9.70 ± 11.28	2	3.5 ± 9.86	5	4.37 ± 35.38
R.stolonifer	-	-	4	0.20 ± 0.57	3	1.83 ± 0.00	-	-	-	-	4	2.54 ± 0.00
**Rhizopus oryzae*	7	20.4 ± 15.83	6	4.66 ± 0.69	2	7.79 ± 0.50	9	11.62 ± 9.4	6	9.04 ± 3.60	3	4.54 ± 2.79

NSI = No. of samples infected
SD = Standard deviation
I % = Infection %
* = New reports on castor bean seeds

Discussion

Present results showed that treatment of seeds using sodium hypochlorite @ 1% successfully reduced superficial fungi on castor bean seeds. Similar results were reported by some other researchers (Limonard, 1968; Tariq *et al*., 2005; Niaz & Dawar, 2009). Blotter method was considered to be better followed by agar plate and deep freezing methods. Jovicevic (1980) also reported that filter paper method was the more practical method for routine analysis of seed health. Such similar results have been observed by Khan *et al*., (1988) on rice; Tariq *et al*., (2005) on soybean; Niaz & Dawar (2009) on maize; Rasheed *et al*., (2004) from groundnut seed. *A. flavus* and *A. niger* were predominant fungi of castor bean seed. Deep freezing method considered being better for isolation of *F. oxysporum, F. solani, Monilia* spp., and *Penicillium* spp., Rahim *et al*., (2013; 2010); Niaz & Dawar (2009) observed the similar results on lentil and maize respectively. It was reported that *Penicillium* sp., reduced viability of seed (White *et al*., 1979). However, Mathur *et al*., (1975) observed that *Fusarium* spp., was best isolated by deep freezing method.

The pathogenic fungi induce disease in plants and humans due to production of some toxic chemicals named mycotoxins. Our result showed highest percentage of *A. flavus* and *A. niger* where these were found to be an important mycotoxigenic species and associated with seed damage (Horn, 2005; El-Maraghy, 1996). Mycotoxigenic fungi grow on almost every kind of nourshing medium (Petzinger & Weidenbach, 2002). Scussel (1998) reported production of mycotoxins by three genera viz., *Aspergillus, Fusarium* and *Penicillium. Aspergillus* strains particularly *A. flavus, A. niger, A. parasiticus* were responsible for the production of aflatoxin B_1, B_2, G_1, G_2 which produce liver cancer (Purchase, 1974). Aflatoxin B_1 affects serum protein resulting in hepatotoxicity (Quezada *et al.*, 2000). Another mycotoxin, zeralenone, produced from *Fusarium* spp., causing haemorrhage and necrosis in bone marrow (Desjardins *et al.*, 2006). *Penicillium* species are reported to produce diseases in animals like mycotoxicoses in man and domestic animals (Scott *et al.,* 1972). Fifty maize samples from 59 samples were found to be contaminated with aflatoxins and 43 seed samples were contaminated with zearalenone (Niaz *et al.,* 2012).

Many fungi on castor bean seeds indicates that these fungi produce disease on plants and also produce mycotoxin. Suitable management practices should be taken by improving the storage condition for the reduction of these fungi and obtaining high yield of crop.

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References

Ahmad, I., S. Iftikhar and A.R. Bhutta. 1993. *Seed-bone microorganism in Pakistan:* Checklist 1991. PARC., Islamabad, pp. 32.

Anonymous. 1993. International rules for seeds testing. S*eed Science and Technol*., 21: 1-288.

Anonymous. 2008. *Agriculture Statistics of Pakistan.* Ministry of Food, Agriculture & Livestock. Economic Wing, Govt. of Pakistan, Islamabad. pp. 274.

Barnett, H.L. 1960. *lllustared genera of Imperfect Fungi (second edition)*.Burgess Pub. Co., pp. 225.

Booth, C. 1971. *The genus Fasarium.* CMI, Kew, Surrey, England. pp. 237.

Busso, C. and M.A. Castro-Prado. 2004. "Cremophor EL stimulates mitotic recombination in uvsH//uvsH diploid strain of *Aspergillus nidulans*". *An. Acad. Bras. Cienc*., 76(1): 49-55. PMID 15048194.

Cheema, N.M., U. Farooq, G. Shabbir, M.K.N. Shah and M. Musa. 2013. Prospects of castor bean cultivation in rainfed tract of Pakistan. *Pak. J. Bot.,* 45(1): 219-224.

Desjardins, A.E., M. Busman, R. Proctor and R.J. Stessman. 2006. *Wheat kernel black point and fumonisin contamination by Fusarium proliferatum (abstract).* National *Fusarium* Head Blight Forum Proceedings. pp. 115.

Domsch, K.H., W. Gams and T.H. Anderson. 1980. *Compendium of soil fungi*. Vol. 1. Academic Press, London. pp. 859.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes* . CMI, Kew, Surrey, England. pp. 608.

El-Maraghy, S.S.M. 1996. Fungal Flora and Aflatoxin contamination of feedstuff samples in Beida Governorate, Libya. *Folia Microbiologica,* 41(1): 53-60.

Hafiz, A. 1986. *Plant Diseases* Publ. by Pak. Agric. Res. Coun., Islamabad, p. 4-5.

Holmquist, G.U., H.W. Walker and H.M. Stahr. 1983. Influence of temperature, pH, water activity and antifungal agents on growth of *A. flavus* and *A. Parasiticus*. *J. Food Science.,* 48: 778-782.

Horn, B.W. 2005. Colonization of wounded peanut seeds by soil fungi: selectivity for species from *Aspergillus* section Flavi. *Mycologia,* 97: 202-217.

Jamal, A. and A. Ghaffar. 1974. Mycoflora of poultry feeds. *Pak. J. Bot.,* 6(6): 165.

Jovicevic, B. 1980. Contribution to the knowledge of harmfull mycoflora on seeds and seedling of wheat, maize and sunflower. *Zastita Bilja*, 31: 101-119.

Khan, S.A.J., A.K. Khanzada, N. Sultan and M. Aslam. 1988. Evaluation of seed health testing techniques for the assessment of seed borne mycoflora of rice. *Pak. J. Agric. Res*., 9: 502-505.

Limonard, T. 1968. *Ecological aspects of seed health testing.* International seed testing Association, Wagoningen , Netherland. pp. 167.

Maiti, S., M.R. Hegde and S.B. Chattopadhyay. 1988. *Hand Book of Oilseed Crops*. Oxford & IBH Publishing Co. (Pvt). Ltd. New Delhi.

Mathur, S.K., S.B. Mathur and P. Neergaard. 1975. Detection of seed borne fingi in sorghum and allocation of *Fusarium moniliforme. Seed. Sci. & Technol.,* 683-690.

Mirza, M.Y. 2009. Oil seed Program. NARC, Islamabad. http://www.parc.gov.pk/1SubDivision/NARCCSI/oil.html.

Moshkin, V.A. 1986. *Castor*. Amerind Publishing Co. (Pvt). Ltd. New Dehli. pp. 1-315.

Nagaraja, O., M. Krishnappa and A.M. Sathisha. 2009. Seed mycoflora associated with castor, *Ricinus communis* L., and their effect on germination. *Journal of Oilseed Research*, 26(2): 177-180.

Nelson, P.E., T.A. Toussoun and W.F.O.Marasas. 1983. *Fusarium* species .*An illustrated manual of identification* .The State Univ. Press, University Park, Pennsylvania. pp. 203.

Niaz, I. and S. Dawar. 2009. Detection of seed borne mycoflora in Maize (*Zea mays* L.). *Pak. J. Bot.,* 41(1): 443-451.

Niaz, I., S. Dawar and N.J. Sahar. 2012. Detection of mycotoxins in maize seed samples. *Pak. J. Bot.,* 44(3): 1075-1078.

Petzinger, E. and A. Weidenbach. 2002. Mycotoxins in the food chain: The role of ochratoxins. *Livest. Prod. Sci*., 76: 245-250.

Purchase, I.R.H. 1974. *Mycotoxin.* Elsevier Scientific Publ. Com. Amsterdam. pp. 443.

Quezada, T., H. Cuellar, F. Jaramillo-Juarez, A.G. Valdivia and J.L. Reyes. 2000. Effects of aflatoxin B1 on the liver and kidney of broiler chickens during development. *Pharm. Toxicol. Endocrinol.,* 125: 265-272.

Rahim, S., S. Dawar and M. Tariq. 2010. Mycoflora associated with Lentil (*Lens culinaris* L.) seeds of Pakistan. *Pak. J. Bot.,* 42(6): 4345-4352.

Raper, K.B. and D.I. Fennell. 1965. The *Genus Aspergillus* .The Williams & Wilkins Company, Baltimore. pp. 686.

Rasheed, S., S. Dawar, A. Ghaffar and S.S. Shaukat. 2004. Detection of seed borne mycoflora of groundnut. *Pak. J. Bot.,* 36(1): 199-202.

Scott, P.M., W.V. Walbeek, B. Kennedy and D. Anyeti. 1972. Mycotoxins (Ochratoxin A, Citrinin and Sterigmatocystin) and toxigenic fungi in grains and other agricultural products. *J. Agricult. Food. Chem.,* 20: 1103-1109.

Scussel, V.M. 1998. Mycotoxinas iem alimentos. Florianopolis: Insular. pp. 144.

Sokal, R. and F.J. Rohlf. 1995. Biometry *The principles and practices of statistical in Biological Research Freeman*, Newyork. pp. 887.

Tariq, M., S. Dawar, M. Abid and S.S. Shaukat. 2005. Seed-borne mycoflora of soybean. *Int. J. Biol. Biotech.,* 2: 711-713.

Thom, C. and K.B. Raper. 1945. *A manual of the Aspergilli.* The Williams and Wilkins Company, Baltimore, Md. pp. 373.

Vieira, C., S. Evangelista, R. Cirillo, A. Lippi, C. A. Maggi and S. Manzini. 2000. Effect of ricinoleic acid in acute and subchronic experimental models of inflammation". *Mediators Inflamm.,* 9(5): 223-8.

White, N.D.G., L.P. Henderson and R.N. Sinha. 1979. Effect of infestations by three stored-product mites on fat acidity, seed germination and mycoflora of stored wheat. *J. Eco. Ent.,* 72: 763-766.

Wilson, R., B.J. Van Schie and D. Howes. 1998. Overview of the preparation, use and biological studies on polyglycerol polyricinoleate (PGPR). *Food Chem. Toxicol.,* 36: 9-10.

Yu, J., B. Deepak and K.C. Ehrlich. 2002. Aflatoxin biosynthesis. *Rev. Iber Microbiology*, 19: 191-200.

Yu, J., P.K. Chang, K.C. Ehrlich, J.W. Cary, D. Bhatnagar, T.E. Cleveland, G.A. Payne, J.E. Linz, C.P. Woloshunk and W. Bennett. 2004. Clustered pathway genes in aflatoxin biosynthesis. *App. Environ. Microbiolology*, 70: 1253-1262.

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