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POST-HARVEST FUNGAL AND INSECT DETERIORATION OF PIGEON PEA SEEDS AND THEIR MANAGEMENT BY PLANT VOLATILES

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Thirty five places of fifteen districts of Eastern Uttar Pradesh were surveyed for collection of stored samples of pigeon pea seeds. In all 20 fungal species of eight genera and two insects (*Callosobruchus chinensis* and *C. maculatus*) from pigeon pea seeds were isolated and identified. Maximum number of fungal species was recorded from Ballia district while minimum from Kushinagar. *Aspergillus flavus, A. niger, A. ochraceus* and *A. terreus* were dominant and showed higher per cent occurrence. The grains deterioration due to insects was higher in Gorakhpur and Maharajganj districts. Insects caused heavier deterioration than fungi. The essential oils extracted from 25 aromatic plants were screened for their antifungal and insect repellent activities against dominant fungi and both insects respectively at 500 ppm concentration. The oils of *Citrus aurantifolia*, *Mentha arvensis* and *Ocimum sanctum* exhibited absolute toxicity against test fungi while oils of *Mentha arvensis*, *Ocimum sanctum* and *Vitex negundo* showed greater repellency against test insects. These potent oils had promising fungistatic/fungicidal and insecticidal properties. *Mentha* and *Ocimum* oils were more potent than *Citrus* and *Vitex* oils. Thus, these oils may be used for the management of pigeon pea deterioration during storage after successful *in vivo* trials.

Key words: Antifungal, Essential oils, Insect repellent, Pigeon pea seeds.

Pigeon pea (*Cajanus cajan* L.) is a legume of semiarid tropics, cultivated more than 25 tropical and sub-tropical countries of the world. During storage seed borne fungi and insects cause considerable loss in terms of quality and quantity of pigeon pea seeds (Arya and Mathew 1991, Dasbak *et al*. 2009, Aboua *et al.* 2010). The larvae of bruchids feed on the pulse seed contents reducing their degree of usefulness and making them unfit either for planting or for human consumption (Ali *et al*. 2004). The use of synthetic fumigants as pesticide has greatly contributed to the management of such losses but their indiscriminate application has posed a substantial risk to health, environment and non target organisms (Owens 1986, Omura *et al.* 1995). Recent reports on fruitfulness of botanicals have attracted the attention of researchers to search an alternative for their utilization as biopesticides. Such alternative (essential oils/ extracts) would be biodegradable, renewable in nature and safe to mankind (Tripathi and Kumar 2007, Tripathi *et al.* 2009).

North-Eastern Uttar Pradesh is ideal for pigeon pea cultivation. But due to high humidity and warm storage condition fungi and insects are responsible for biodeterioration of stored pigeon pea seeds. Present investigation was conducted to ascertain the fungal and bruchid infestation of freshly harvested pigeon pea seeds during storage. Further the efficacy of some essential oils against dominant fungi and both insects was also evaluated for the protection of pigeon pea seeds during storage.

MATERIALS AND METHODS

During 2007-2008 (September-November), places of different districts viz., Azamgarh, Ballia, Basti, Chandauli, Deoria, Faizabad, Ghazipur, Gonda, Gorakhpur, Jaunpur, Kushinagar, Maharajganj, Mau, Siddharthnagar and Varanasi of Eastern Uttar Pradesh were surveyed for collection of stored seeds of *C. cajan*. The samples were taken in

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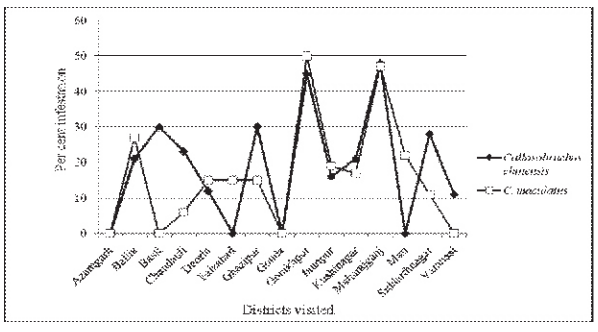
Table 1: Per cent occurrence of seed mycoflora on *Cajanus cajan* by agar plate (A) and standard blotter paper (B) methods in different districts of Eastern Uttar Pradesh

Fungal species	Technique	Azamgarh	Ballia	Basti	Chandauli	Deoria	Faizabad	Ghazipur	Gonda	Gorakhpur	Jaunpur	Kushinagar	Maharajganj	Mau	Siddarth-nagar	Varanasi
Alternaria alternata (Fr.)Keissler	A	1.15	1.37	-	-	0.51	-	-	-	-	1.31	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus flavipes Thom & Charch	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	9.28	-	-	-	-	-	-
A. flavus Link	A	32.55	21.33	14.68	20.31	22.45	20.57	24.43	19.18	18.27	19.41	23.98	18.82	18.30	34.40	19.53
	B	27.16	27.50	27.43	18.93	19.68	22.33	36.0	21.0	21.43	16.13	-	29.63	30.06	40.44	28.11
A. fumigatus Fresenius	A	-	-	-	-	-	-	-	-	1.01	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. nidulans Wint	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	4.23	-	-	-	-	-	-	-
A. niger van Tieghem	A	34.10	21.23	13.98	22.24	17.86	22.86	31.29	31.23	16.75	24.31	23.98	20	40.14	38.93	24.21
	B	37.03	26.82	22.51	30.61	33.85	33.0	42.61	28.11	18.57	22.58	23.17	23.41	37.85	39.18	16.64
A. ochraceus Wilhelm	A	5.42	6.12	12.58	13.39	3.06	11.43	9.16	9.16	11.67	-	-	15.53	10.56	5.34	3.01
	B	-	13.72	12.91	-	14.18	4.81	17.34	-	18.57	-	-	13.51	-	-	9.31
A. parasiticus Speare	A	0.77	-	0.75	-	-	-	-	-	-	0.51	-	-	-	-	-
	B	-	-	1.61	-	-	-	-	-	-	0.81	-	-	0.71	1.11	-
A. restrictus G.Smith	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	33.47	-	-	-	-
A. terreus Thom	A	3.87	4.79	7.69	7.61	8.16	6.86	4.53	4.51	6.59	10.21	9.35	9.41	-	6.87	8.23
	B	8.64	8.71	1.33	9.6	1.52	-	5.31	13.52	22.27	13.21	-	9.31	-	-	-
Cladosporium sp.	A	-	-	-	3.11	-	-	-	-	-	5.43	1.22	-	-	-	-
	B	-	-	-	-	-	3.81	-	-	-	-	-	-	-	-	-
Curvularia lunata (Wakker) Boedijin	A	-	2.05	1.52	-	1.53	2.86	2.29	2.21	-	-	-	-	0.70	-	-
	B	2.46	10.45	-	11.78	3.12	-	-	-	0.71	10.48	-	1.21	-	4.49	3.13
Fusarium acuminatum Ellis & Everhart	A	-	-	-	-	-	-	-	-	-	3.21	-	-	-	-	5.18
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. nivale (Fries)Cesati	A	5.42	16.44	14.22	-	14.28	2.86	12.98	12.98	11.67	-	13.0	8.23	-	4.58	5.61
	B	9.87	-	-	12.6	11.81	5.84	-	10.93	6.42	-	-	14.81	7.14	-	-
F. oxysporum Von Schlechtendal	A	-	6.85	9.21	11.43	13.77	11.43	4.56	4.51	-	6.44	4.0	7.05	-	3.21	-
	B	2.46	7.54	-	-	2.32	11.61	-	-	5.0	9.61	-	3.78	7.85	5.61	9.31
Mucor sp.	A	6.20	-	-	1.92	-	-	-	-	-	9.79	14.22	-	4.92	2.29	-
	B	-	0.61	-	4.51	-	-	-	13.21	-	6.43	19.74	-	7.14	-	9.31
Penicillium sp.	A	-	7.53	-	7.61	-	6.86	-	-	9.14	8.11	4.47	-	4.94	4.58	7.78
	B	3.70	-	-	12.63	-	11.53	-	4.61	-	10.48	5.15	-	5.0	2.24	-
P. chrysogenum Thom	A	4.65	-	4.57	-	8.67	-	9.91	9.91	11.16	4.81	-	5.29	-	2.29	8.23
	B	-	4.33	6.43	-	6.21	-	-	-	-	-	-	-	-	-	-
P. italicum Wehmer	A	5.42	11.64	5.23	-	9.18	-	-	-	11.67	-	6.0	10.58	-	-	-
	B	-	9.31	9.61	9.61	6.21	2.91	5.31	-	-	-	-	6.11	4.28	-	-
Rhizopus arrhizus Fischer	A	-	0.68	11.18	12.12	0.51	14.28	0.76	0.69	2.03	7.01	-	7.06	3.52	-	13.43
	B	7.40	-	14.51	-	0.71	-	0.11	-	0.71	3.71	18.02	3.72	-	5.61	10.44

pre-sterilized polyethylene bags, brought to the laboratory and kept separately after labeling the name of places along with districts for analysis of their associated mycoflora and insects.

The mycoflora of pigeon pea seeds were isolated by agar plate method of Muskett (1948) and standard blotter paper method of Tempe (1953) using Czepeck's Dox Agar medium. In both the techniques seed samples (7 seeds in each plate) were incubated at 28±2ºC for 7 days. Each experiment was

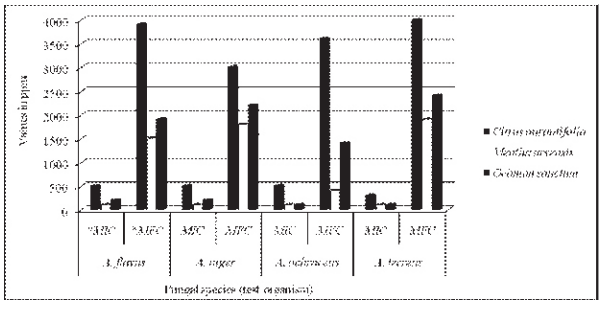
Figure 1: Per cent infestation of pigeon pea seeds due to pulse bruchids



carried out in triplicates. The fungi appearing on the seeds were isolated, purified and identified with the help of literature (Raper and Thom, 1949; Raper and Fennell, 1965; Booth, 1971 and Ellis, 1976). The culture of each purified fungal species was maintained on agar slant (CDA) at 4±1ºC for further investigation. The per cent occurrence of fungal species was calculated following the formula of Tripathi and Kumar (2007).

The samples were observed separately

Figure 2: Minimum inhibitory and minimum fungicidal concentration (*MIC/*MFC value in ppm) of potent essential oils against *Aspergillus* spp.



with hand lens for insects infestation and identified with the help of literature (Drees and

Table 2: Screening of essential oils of aromatic plants for their toxicity against fungi and insects

Plant species (essential oils)*	Part used	Family	Per cent mycelial inhibition of test organisms(PMI)#				Per cent repellency#	
			Aspergillus flavus	*A. niger*	*A. ochraceus*	*A. terreus*	*Callosobruchus chinensis*	*C. maculatus*
Acorus calamus Linn.	Rhizome	Araceae	30.56±2.10	32.24±1.23	17.34±1.12	45.0±3.0	57.0±1.27	10.7±1.0
Ageratum conyzoides Linn.	Whole part	Asteraceae	53.3±3.90	75.6±0.76	74.11±0.76	65.28±3.5	65.38±1.0	66.67±1.52
A. houstonianum Mill.	,,	,,	61.6±2.45	72.6±1.10	4.66±2.08	37.20±1.80	50.00±1.52	24.33±3.05
Artemisia nilagirica Pamp	Shoot	,,	67.28±3.75	58.51±3.18	77.92±2.29	71.37±5.53	43.48±1.53	64.00±1.0
Blumea eriantha DC.	Whole part	,,	13.21±1.52	18.57±2.36	57.17±4.93	33.94±4.44	57.14±1.0	36.36±1.52
Blumea lacera DC.	,,	,,	17.23±1.0	61.21±2.0	30.56±2.10	13.19±1.10	26.08±2.08	26.00±1.0
B. laciniata DC.	,,	,,	17.43±0.68	22.04±2.10	32.45±5.04	23.21±0.92	26.66±1.53	45.0±1.52
Caesulia axillaris Roxb.	,,	,,	66.1±1.21	50±3.39	58.82±2.21	49.32±1.60	50.00±1.0	54.00±1.05
Citrus aurantifolia Swingle	Leaf	Rutaceae	100±0.00	100±0.00	100±0.00	100±0.00	61.90±1.52	64.74±1.73
Clerodendrum inermae (L.)Gaertn.	,,	Verbenaceae	23.21±0.15	34.0±1.94	39.04±1.15	10.56±1.93	4.0±0.20	20.0±2.0
Cosmos sulphureus Cav.	,,	Asteraceae	20.78±2.47	53.3±3.90	32.21±0.65	0.00±0.09	52.17±0.58	35.32±0.5
Curcuma zedoaria Rosc.	Rhizome	Zingiberaceae	15.55±1.75	14.32±0.7	69.29±3.61	59.63±3.27	57.89±1.15	57.82±1.0
Cyperus rotundus Benth.	Whole part	Cyperaceae	34.0±0.03	64.76±3.38	9.57±0.32	24.32±2.0	30.04±2.02	32.9±2.32
Eugenia heyneana (L.)Wall.	Leaf	Myrtaceae	31.84±4.27	30.01±3.4	58.22±0.76	40.38±2.02	56.25±1.52	63.64±0.58
Hyptis suaveolens Poit.	,,	Lamiaceae	48.5±3.23	38.4±5	24±1.04	44.34±0.58	60.87±1.0	42.10±1.52
Lantana camara Linn.	,,	Verbenaceae	42.8±1.21	64.1±0.5	53.96±5.22	51.79±7.09	56.52±1.15	60.00±1.52
L. indica Linn.	,,	,,	58.4±2.30	57.1±0.5	61.21±0.22	8.34±2.0	63.63±1.52	36.36±0.58
Leucas aspera Spreng	Whole part	Lamiaceae	38.89±1.0	32.14±0.5	55.45±6.36	64.40±1.44	50.00±1.52	57.14±2.0
Mentha arvensis Linn.	Leaf	,,	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
Ochna multiflora DC.	,,	Ochnaceae	45.30±1.83	9.54±2.0	34.21±0.34	23.87±2.05	36.04±1.0	30.58±2.52
Ocimum basilicum Linn.	Twig	Lamiaceae	90±2.0	93.13±1.2	98.13±2.30	91.32±1.23	83.33±0.57	80.95±0.58
O. sanctum Linn.	,,	,,	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
Polyalthia longifolia Sonner	Leaf	Annonaceae	79.86±3.25	54.38±2.30	76.86±2.0	46.54±3.05	75.00±1.0	29.16±2.88
Vitex negundo Linn.	Twig	Verbenaceae	8.54±1.32	11.23±1.26	11.18±2.29	13.22±0.29	100±0.00	100±0.00
Zingiber officinale Rosc.	Rhizome	Zingiberaceae	45.43±2.36	59.61±2.18	79.76±2.64	63.92±4.04	70.83±1.52	52.38±0.58

* Oil concentration 500 ppm

Values given are mean of three replicates ± Standard Deviation

Jackman 1999, Beck and Blumer 2007) and authenticated at Entomology Lab, Department of Zoology, DDU Gorakhpur University Gorakhpur. The cultures of insects were reared on insecticide free fresh pigeon pea seeds at 28±2ºC for further investigation. The per cent infestation of samples by insects was calculated.

25 aromatic plants collected from forests of Gorakhpur Division were brought to the laboratory and identified with the help of flora (Srivastava 1976). The volatile constituents in the form of essential oil from each plant were extracted separately through hydrodistillation by Clevenger's apparatus at 90±2 ^{0}C. Each essential oil was dried over anhydrous sodium sulphate and was stored at 4±1^{0}C under sterilized condition for further experimentation.

The antifungal activity of essential oil of individual plant species was evaluated separately against dominant test fungi viz., *Aspergillus flavus*, *A. niger, A. ochraceus* and *A. terreus* at 500 ppm by Inverted Petri plate technique of Bocher (1938) with slight modification (Tripathi and Kumar, 2007). Each experiment contained three replicates. The fungitoxicity was recorded in terms of per cent mycelial inhibition following Pandey *et al.* (1982).

The minimum inhibitory concentration (MIC) of each potent oils viz., *Citrus aurantifolia*, *Mentha arvensis* and *Ocimum sanctum* was determined against dominant test fungi following Tripathi and Kumar (2007). Further minimum fungicidal concentration (MFC) of potent oils was also determined following Garber and Houston (1959). Each experiment contained three replicates.

The repellent activity of essential oils against two insects viz., *Callosobruchus chinensis* and *C. maculatus* at 500 ppm was tested using Y tube Olfactometer following Tripathi and Kumar (2007). Each experiment contained three replicates. The repellent activity was recorded in terms of per cent repellency.

The insecticidal activity of potent oils (*M. arvensis, O. sanctum* and *Vitex negundo*) was evaluated against *C. chinensis* and *C. maculatus* following modified method of

Table 3: Insecticidal activity of potent essential oils against pulse bruchids

Potent essential oils*	Per cent mortality (±SD) of insects at different exposure periods			
	Callosobruchus chinensis		*C. maculatus*	
	24h	48h	24h	48h
Mentha arvensis	100±0.00[a]	100±0.00[a]	100±0.00[a]	100±0.00[a]
Ocimum sanctum	76.6±0.57[b]	100±0.00[a]	100±0.00[a]	100±0.00[a]
Vitex negundo	26.6±0.58[c]	56.6±1.52[b]	26.6±0.57[b]	43.3±1.53[b]

Each data represents the mean of three replicates. Per cent mortality (±SD) followed by same letter within a column are not significantly different at 0.05 level (DMRT).
* Concentration -5µl

Singh *et al.* (2005) at 5µl dose. The per cent mortality of insects was recorded at intervals of 24 and 48 h exposure. A control set was maintained without oil. Each experiment contained three replicates and data was statistically analyzed using standard deviation and two-ways ANOVAs.

RESULTS AND DISCUSSION

In all 20 fungal species belonging to eight genera were isolated from pigeon pea seeds by both methods. The fungal species identified as *Aspergillus flavus, A. niger, A. ochraceus, A. parasiticus, A. terreus, Cladosporium* sp., *Curvularia lunata, Fusarium nivale, F. oxysporum, Penicillium* sp., *P. chrysogenum, P. italicum, Mucor* sp. and *Rhizopus arrhizus* were observed in both the techniques. *A. flavipes*, *A. restrictus* and *A. nidulans* were isolated by blotter paper method while *Alternaria alternata*, *Aspergillus fumigatus* and *F. acuminatum* were isolated by agar plate method only. Maximum number of fungal species was recorded from Ballia district and minimum from Kushinagar by agar plate method, however, on blotter paper Deoria samples showed maximum number of fungi and minimum from Kushinagar. *Aspergillus flavipes*, *A. nidulans*, *A. restrictus* and *F. acuminatum* were exclusively reported from Gorakhpur, Gonda, Kushinagar and Varanasi districts respectively.

In present study higher numbers of fungal colonies were isolated by agar plate in comparison to blotter paper method as observed by Kumar and Tripathi (2002). Out of 20 fungal species isolated, *A. flavus*, *A. niger*, *A. ochraceus* and *A. terreus* showed higher per cent occurrence in all districts (Table 1).

The pigeon pea seed samples showed numerous coloured beetles of about 0.30 cm long with serrated antennae (*C. chinensis*) and 1-8 inch long, reddish brown slightly elongated beetles with two black spots near middle (*C. maculatus*). These insects were reported earlier by Dongre *et al*. (1993) and Silim *et al.* (1998). Both the insects exhibited higher per cent infestation in samples of Gorakhpur and Maharajganj districts (Fig. 1). The seed samples of Azamgarh, Basti, Faizabad Gonda, Mau and Varanasi districts were free from insects infestation. On account of higher occurrence of fungi viz., *A. flavus*, *A. niger*, *A. ochraceus* and *A. terreus* and insects viz., *C. chinensis* and *C. maculatus*, these were selected as test organisms.

During antifungal screening of essential oils from 25 aromatic plants at 500 ppm concentration, only essential oils of *C. aurantifolia*, *M. arvensis* and *O. sanctum* showed complete inhibition of mycelial growth of all test fungi. However, other oils at this concentration showed a considerable variation in inhibition (Table 2). In addition to this, during insect repellent testing of 25 essential oils, the oils of *M. arvensis*, *O.*

sanctum and *V. negundo* could repel all the insects of both species at 500 ppm dose showed 100% repellency while rest of the essential oils exhibited lower level of repellent activity (Table 2). The repellent plants may contain certain active volatile compounds that elicit antifeedant behavior by the visiting insects.

In present investigation MICs of *M. arvensis* and *O. sanctum* oil were lower than that of *C. aurantifolia* oil against all the test fungi. *Mentha* oil was fungistatic at its MIC of 100 ppm against all test fungi; however MIC of *Mentha* oil has been reported to be 0.10 mg mL^{-1} against *A. flavus* by earlier worker (Kumar *et al.,* 2007). *Ocimum* oil was fungistatic at its MIC of 200 ppm against *A. flavus* and *A. niger* while at 100 ppm against *A. ochraceus* and *A. terreus*. Both the oils were fungicidal in nature against all test fungi at higher concentration. It is interesting to note that MFC of *Mentha* oil against *A. ochraceus* was 400 ppm (Fig. 2).

Further during insecticidal investigation, *Mentha* and *Ocimum* oil applied at dose of 5µl caused significant mortality of both insects within 24 and 48 h exposure than *Vitex* oils (Table 3) and the order of efficacy was *Mentha>Ocimum>Vitex* against *C. chinensis* and *Mentha=Ocimum>Vitex* against *C. maculatus*. In earlier report, 10µl dose and 24h exposure of *M. arvensis* oil caused 55% mortality of *C. maculatus* (Raja *et al.*, 2001); however in present study 5µl dose of *Mentha* oil was able to cause 100% mortality of test insect. Rajapakse (2006) investigated that at 0.05 and 0.17µl concentration (24h exposure), *O. sanctum* oil exhibited 100% mortality of *C. chinensis* and *C. maculatus* respectively. On the contrary in our study 100% mortality was observed by increasing the concentration of the *Ocimum* oil. In contrast to the report of Sahaf and Moharramipour (2008) who investigated the *Vitex* oil as strong insecticidal agent against *C. maculatus*, is reported to be weak insecticidal agent in present investigation. The differences observed among the mortality of insects by essential oils are may be due to the differences in their chemical composition and place of occurrence.

Based on the observed toxicity of *Mentha* and *Ocimum* essential oils against dominant fungi and insects they may be recommended as a plant based pesticidal agents for the protection of stored bigeon seeds and other food commodities.

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