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In vitro effect of botanical extracts and fungicides against *Bipolaris sorokiniana,* causal agent of leaf blotch of barley

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Abstract: An experiment was conducted in the Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, Khulna to evaluate the effect of five botanical extracts namely garlic, onion, ginger, neem and black cumin at different concentrations (5%, 10% and 15%) and five fungicides namely Hexaconazole, Carbendazim, Mancozeb, Difenoconazole + Propiconazole and Propiconazole at different concentrations (100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm) on the mycelial growth, fresh and dry weight and colony characters of *Bipolaris sorokiniana* causal agent of leaf blight or blotch of barley. Inhibition of mycelial growth of *B. sorokiniana* was tested using botanical extracts and fungicides. In all cases, the mycelial growth inhibition was found to be increased with the increase of concentration. Hundred percent of mycelial growth was inhibited with the application of Propiconazole, Hexaconazole and Difenoconazole + Propiconazole at all concentration. In case of botanical extracts the highest percent inhibition of mycelial growth was observed by the application of garlic extracts (67.50%) at 15% concentration where the lowest was noted with the application of different botanical extracts at lower concentration. The highest fresh weight was found in case of fungicides in Mancozeb (1.86 g) at 400 ppm concentration where lowest was found in Propiconazole, Hexaconazole and Difenoconazole + Propiconazole (0.00 g) at all concentrations. The highest fresh weight was found in case of botanical extracts in ginger (1.98 g) at 10% concentration where lowest was noted in neem (1.60 g) at 5% concentration. In case of fungicides the highest dry weight was found in Carbendazim (0.064 g) at 100 ppm concentration where lowest was found in Propiconazole, Hexaconazole and Difenoconazole + Propiconazole (0.00 g) at all concentrations. The highest dry weight was found in black cumin (0.078 g) at 5% concentration where lowest was noted in onion (0.053 g) at 15% concentration in case of botanical extracts. Very small variation was observed in colony color, margin, texture and hyphal thickness in Mancozeb and Carbendazim but no mycelia was found in Propiconazole, Hexaconazole and Difenoconazole + Propiconazole. So no colony characters were observed. Botanical extracts causes variation in colony characters with the increase of concentration.
Key words: Botanical extracts, Fungicides, *Bipolaris sorokiniana*, Leaf blotch, Barley.

Introduction

Barley is considered as one of the most important cereal crops in the world. It may be an important cereal crop for food security in Bangladesh. Its covers almost 0.99 thousand hectares of land (BBS, 2007) with a total production of 536 metric tons in Bangladesh where the world barley production is 157,644,721 metric tons (FAOSTAT, 2008). There are several constraints to the world barley production. Among them leaf blotch or spot blotch caused by *Bipolaris sorokiniana* is the major and devastating disease of barley in Bangladesh (Hossain and Azad, 1992). *Bipolaris sorokiniana* (Sacc.) (teleomorph *Cochliobolus sativus*) is the causal agent of common root rot, leaf spot disease, seedling blight, head blight, and black point of wheat and barley (Sivanesan, 1990). The fungus is one of the most serious foliar disease constraints for both crops in warmer growing areas and causes significant yield losses. High temperature and high relative humidity favour the outbreak of the disease, in particular in South Asia's intensive 'irrigated wheat–rice' production systems (Kumar *et al.* 2002). It is most important in warm climates but is also present and occasionally prevalent in temperate climates. Yield losses caused by the pathogen have been estimated in average 10% in barley and 5% in spring wheat on a long-term, region-wide basis, with losses in individual fields in some years above 30% (Stack, 1991). Botanical extracts are biodegradable (Devlin and Zettel, 1999) and their use in crop protection is a practical sustainable alternative. It reduces environmental contamination and health hazards (Grange and Ahmed, 1988). Botanical fungicides are unique because they can be produced easily by the farmers and small industries (Roy *et al*., 2005). Different plant extracts also used separately or in combination to control some other fungi by the farmers. In the last decades, a number of systemic fungicides with different modes of action and targets have been developed to reduce the losses caused by the diseases (Pasquer *et al*., 2005). Very few works have done using plant extracts and fungicides in the country to control *B. sorokiniana* causing leaf blotch of barley. It is thus dire to work extensively to examine the effect of different concentration of neem, garlic and other indigenous plant extract and natural biocides like ginger, black cumin etc and different fungicides like Hexaconazole, Carbendazim, Mancozeb, Difenoconazole + Propiconazole and Propiconazole in controlling disease which are easily available. The present study was undertaken to investigate the effectiveness of selective botanical extracts and fungicides against *B. sorokiniana*, the causal agent of leaf blotch of barley.

Materials and Methods

An experiment was conducted in the Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, Khulna, Bangladesh during May to June, 2011. An isolate of *B. sorokiniana* was collected from the preserved isolates of Plant Protection Laboratory. The basic medium, PDA was prepared following the standard procedure (Anonymous, 1968). Five botanical extracts namely garlic, onion, ginger, neem and black cumin at different concentrations (5%, 10% and 15%) which were prepared by using standard procedure (Vijayalakshmi *et al.,* 1999) and five fungicides namely Hexaconazole, Carbendazim, Mancozeb, Difenoconazole + Propiconazole and Propiconazole at different concentrations (100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm) which were evaluated in *in vitro* condition against *B. sorokiniana* following poison food technique (Dhingra and Sinclair, 1985). The basic PDA medium was modified by using the botanical extracts and fungicides at specified

concentrations. The pH of the medium was adjusted to 6.5. The fungus was grown on plate containing 25 m1 of the medium per petridish. Four replicated petridishes were used for each treatment. The plates were inoculated with 5 mm discs from old PDA culture at one disc at centre per plate. The mycelial discs were taken from the edge of the colony. The plates were incubated at 26±2°C. The radial growths of mycelium in each plate were recorded after 14 days of incubation. The radial growth of mycelium in each plate was recorded as an average of two diameters measured at right angles to one another. The colony characters were recorded after 14 days of incubation.

Percentage inhibition of growth was calculated using the following formula (Naz *et al.*, 2006):

$$\% \text{ of inhibition} = \frac{X - Y}{X} \times 100$$

Where, X = Average growth of *B. sorokiniana* in control petridishes, Y = Average growth of *B. sorokiniana* in each fungicide/ botanical extract treated petridishes.

After 14 days of incubation, mycelia were scraped and fresh weight of mycelia growth was measured by an electric balance. Mycelia were oven dried with filter paper at 70^0C for 48 hours. Then the dry weight was measured.

Results and Discussion

Effect of fungicides on radial mycelial growth, mycelial growth inhibition, fresh weight and dry weight of *B. sorokiniana* at different concentrations

The different fungicides in different concentrations inhibited the mycelial growth, fresh weight and dry weight of *B. sorokiniana* significantly ($p<0.01$).

The highest radial mycelial growth was observed in control (30 mm) and the second highest radial mycelial growth was observed in mancozeb (14.75 mm) at 100 ppm concentration which was statistically simillar with carbendazim (13.75 mm) at 100 ppm concentration and different with all other treatment combinations. The lowest radial mycelial growth was observed in propiconazole, hexaconazole and difenoconazole + propiconazole (0.00 mm) at all concentrations which were different with all other treatment combinations (Table 1).

Table 1. Effect of different fungicides at different concentration on radial mycelia growth, mycelial growth inhibition, fresh weight and dry weight of *Bipolaris sorokiniana*

Fungicides	Concentration (ppm)	Radial growth (mm)	Inhibition percentage	Fresh weight (g)	Dry weight (g)
Control	0	30 a	-	1.84	0.093
Mancozeb	100	14.75 b	50.83 f	1.67 e	0.063 b
	200	12.50 c	58.33 e	1.62 g	0.058 d
	300	10.25 d	65.83 b	1.70 d	0.059 c
	400	8.75 e	70.83 c	1.86 a	0.048 f
	500	7.00 f	76.67 b	1.85 ab	0.045 h
Propiconazole	100	0.00 g	100.0 a	0.00 i	0.000 j
	200	0.00 g	100.0 a	0.00 i	0.000 j
	300	0.00 g	100.0 a	0.00 i	0.000 j
	400	0.00 g	100.0 a	0.00 i	0.000 j
	500	0.00 g	100.0 a	0.00 i	0.000 j
Carbendazim	100	13.75 b	54.17 f	1.85 ab	0.064 a
	200	11.75 c	60.83 e	1.65 ef	0.058 d
	300	10.25 d	65.83 d	1.62 g	0.051 e
	400	9.00 e	70.00 c	1.81 c	0.047 g
	500	8.25 e	72.50 c	1.61 gh	0.042 i
Hexaconazole	100	0.00 g	100.0 a	0.00 i	0.000 j
	200	0.00 g	100.0 a	0.00 i	0.000 j
	300	0.00 g	100.0 a	0.00 i	0.000 j
	400	0.00 g	100.0 a	0.00 i	0.000 j
	500	0.00 g	100.0 a	0.00 i	0.000 j
Difenoconazole + Propiconazole	100	0.00 g	100.0 a	0.00 i	0.000 j
	200	0.00 g	100.0 a	0.00 i	0.000 j
	300	0.00 g	100.0 a	0.00 i	0.000 j
	400	0.00 g	100.0 a	0.00 i	0.000 j
	500	0.00 g	100.0 a	0.00 i	0.000 j
CV (%)		7.43	2.96	1.35	3.08

Means followed by a different letters are significantly different at 1% level.

No mycelial growth of *B. sorokiniana* was found at any concentration of propiconazole, hexaconazole and difenoconazole + propiconazole i.e. percentage inhibition were 100% at all the concentrations. The lowest percent inhibition was observed in mancozeb (7.00%) at 500 ppm concentration which was statistically different with all other treatment combinations (Table 1). Regression equation (Fig. 1) between concentrations of mancozeb and mycelial growth inhibition percentage revealed that more than 99% of inhibition was obtained with increasing concentration followed by 97% of carbendazim. Complete inhibition was found in case of propiconazole, hexaconazole and difenoconazole + propiconazole.

The highest fresh weight was found in mancozeb (1.86 g) at 400 ppm concentration which was statistically simillar with carbendazim (1.85 g) at 100 ppm concentration and different with all other treatment combinations. The lowest fresh weight was found in propiconazole, hexaconazole and difenoconazole + propiconazole (0.00

g) at all concentrations which were statistically different with all other treatment combinations (Table 1).

The highest dry weight of the fungus was found in control (0.093 g) and second highest dry weight was found in carbendazim (0.064 g) at 100 ppm concentration which is statistically different with all other treatment combinations. The lowest dry weight was found in propiconazole, hexaconazole and difenoconazole + propiconazole (0.00 g) at all concentrations which were statistically different with all other treatment combinations (Table 1).

Effect of botanical extracts on radial mycelial growth, mycelial growth inhibition, fresh weight and dry weight of *B. sorokiniana* at different concentrations

The different botanical extracts in different concentrations inhibited the mycelial growth, fresh weight and dry weight of *B. sorokiniana* significantly ($p<0.01$).

The highest radial mycelial growth was observed in control (30 mm) and the second highest radial mycelial growth was observed in black cumin (25.25 mm) at 5% concentration which was statistically simillar with ginger (24.25 mm) at 5% concentration and different with all other treatment combinations. The lowest radial mycelial growth was observed in garlic (9.75 mm) at 15% concentration which was different with all other treatment combinations (Table 2).

Table 2. Effect of different botanical extracts at different concentration on radial mycelial growth, mycelial growth inhibition, fresh weight and dry weight of *Bipolaris sorokiniana*

Botanical extracts	Concentration (%)	Radial growth (mm)	Inhibition percentage	Fresh weight (g)	Dry weight (g)
Control	0	30 a	-	1.84	0.093
Garlic	5	17.75 f	40.83 f	1.82 bc	0.074 cd
	10	13.00 hi	56.66 cd	1.62 fg	0.070 efg
	15	9.75 k	67.50 a	1.90 ab	0.059 ijk
Onion	5	23.00 c	23.33 i	1.81 bcd	0.075 bc
	10	19.00 ef	36.66 fg	1.77 cde	0.072 de
	15	12.00 ij	60.00 bc	1.82 bc	0.053 j
Ginger	5	24.25 bc	19.16 ij	1.81 bcd	0.076 b
	10	20.00 de	33.33 gh	1.98 a	0.068 gh
	15	13.25 hi	55.83 cd	1.70 efg	0.065 i
Neem	5	19.75 de	34.16 gh	1.60 g	0.074 cd
	10	15.75 g	47.50 e	1.62 fg	0.070 efg
	15	11.25 j	62.49 b	1.64 fg	0.060 ij
Black cumin	5	25.25 b	15.83 j	1.71 def	0.078 a
	10	21.00 d	30.00 h	1.70 efg	0.075 bc
	15	14.00 h	53.33 d	1.68 efg	0.071 def
CV (%)		3.57	7.65	3.08	2.81

Means followed by a different letters are significantly different at 1% level.

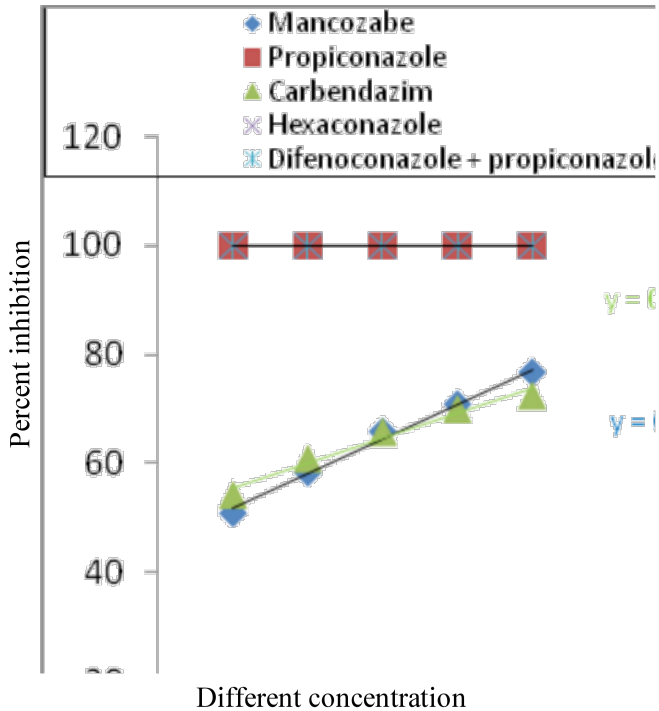

Fig. 1. Functional relationship between concentration and mycelial growth inhibition percentage of fungicides

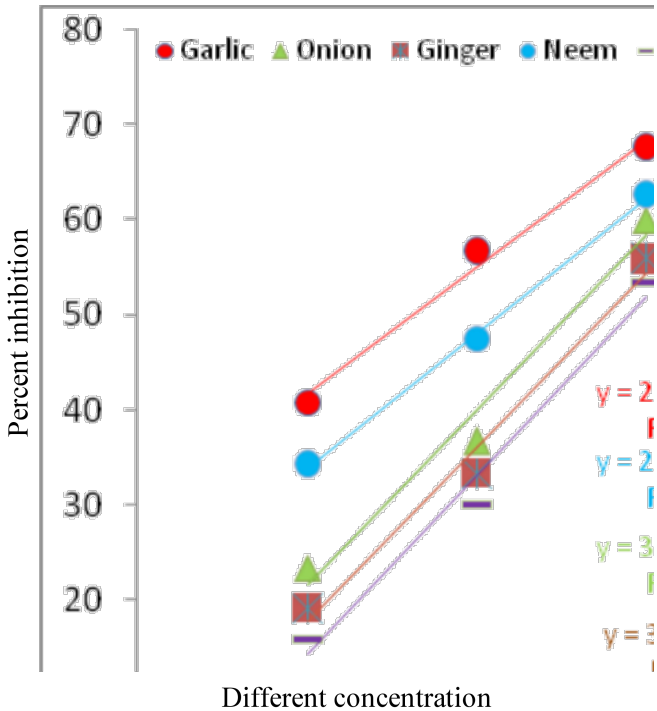

Fig. 2. Functional relationship between concentration and mycelial growth inhibition percentage of botanical extracts

The highest percent inhibition was found in garlic (67.5%) at 15% concentration which is statistically different with all other treatment combinations. The lowest percent inhibition was observed in black cumin (15.83%) at 5% concentration which was statistically different with all other treatment combinations (Table 2). Regression equation (Fig. 2) between concentrations of garlic extract and mycelial growth inhibition percentage revealed that more than 98% of inhibition was obtained with increasing concentration followed by 99% of onion, 97% of ginger, 98% of neem and 98% black cumin respectively.

The highest fresh weight was found in ginger (1.90 g) at 10% concentration which was statistically simillar with garlic (1.90 g) at 15% concentration and different with all other treatment combinations. The lowest fresh weight was found in neem (1.60 g) at 5% concentration which was statistically different with all other treatment combinations (Table 2).

The highest dry weight of the fungus was found in control (0.093 g) and second highest dry weight was found in black cumin at 5% concentration which is statistically different with all other treatment combinations. The lowest dry weight was found in onion (0.053 g) at 15% concentration which was statistically different with all other treatment combinations (Table 2).

Table 3. Effect of different fungicides and botanical extracts on colony characters of *Bipolaris sorokiniana*

Fungicides and Botanical Extracts	Colony characters				
	Surface	Color	Margin	Texture	Hyphal Thickness
Control	Upper	White	Regular	Compact velvety	Thick
	Lower	White	Regular		
Mancozeb 100 ppm	Upper	Off white	Regular	velvety	Moderately thick
	Lower	Light brown	Regular		
Mancozeb 200 ppm	Upper	Off white	Regular	velvety	Moderately thick
	Lower	Light brown	Regular		
Mancozeb 300 ppm	Upper	Off white	Regular	velvety	Thin
	Lower	Light brown	Regular		
Mancozeb 400 ppm	Upper	Off white	Regular	velvety	Thin
	Lower	Whitish brown	Regular		
Mancozeb 500 ppm	Upper	Off white	Regular	velvety	Thin
	Lower	Whitish brown	Regular		
Carbendazim 100 ppm	Upper	Off white	Regular	velvety	Moderately thick
	Lower	Light brown	Regular		
Carbendazim 200 ppm	Upper	Off white	Regular	velvety	Moderately thick
	Lower	Light brown	Regular		
Carbendazim 300 ppm	Upper	Off white	Regular	velvety	Thin
	Lower	Light brown	Regular		
Carbendazim 400 ppm	Upper	Off white	Regular	velvety	Thin
	Lower	Whitish brown	Regular		
Carbendazim 500 ppm	Upper	Off white	Regular	velvety	Thin
	Lower	Whitish brown	Regular		
Garlic 5%	Upper	White	Regular	Velvety	Thick
	Lower	Off white	Regular		
Garlic 10%	Upper	White	Regular	Velvety	Thick
	Lower	Whitish brown	Regular		
Garlic 15%	Upper	White	Regular	Compact velvety	Thin
	Lower	Grey	Regular		
Onion 5%	Upper	White	Regular	velvety	Thick
	Lower	Off white	Regular		
Onion 10%	Upper	White	Regular	velvety	Thick
	Lower	Whitish brown	Regular		
Onion 15%	Upper	White	Wavy	Compact velvety	Thin
	Lower	Grey	Regular		
Ginger 5%	Upper	White	Wavy	velvety	Thick
	Lower	Off white	Regular		
Ginger 10%	Upper	White	Wavy	Compact velvety	Moderately Thick
	Lower	Grey	Regular		
Ginger 15%	Upper	White	Wavy	Compact velvety	Thin
	Lower	Grey	Regular		
Neem 5%	Upper	White	Irregular	Compact velvety	Thick
	Lower	Light brown	Regular		
Neem 10%	Upper	White	Irregular	Compact velvety	Thick
	Lower	Light brown	Regular		
Neem 15%	Upper	White	Irregular	Compact velvety	Thin
	Lower	Light brown	Regular		
Black cumin 5%	Upper	Off white	Regular	Compact velvety	Thick
	Lower	Blackish	Regular		
Black cumin 10%	Upper	Off white	Regular	Compact velvety	Thick
	Lower	Blackish	Regular		
Black cumin 15%	Upper	Off white	Regular	Compact velvety	Thin
	Lower	Blackish	Regular		

Effect of different fungicides and botanical extracts on colony characters of *Bipolaris sorokiniana*

Effect of different fungicides and botanical extracts on the colony characters of the *B. sorokiniana* was studied. Colony characters of both surfaces were recorder in table 3. Recorded colony characters revealed that upper surface color on mancozeb and carbendazim treated media were white to off white and the lower surface color on the mancozeb and carbendazim treated media were whitish brown to light brown. Colony margins and texture of upper and lower surface of mancozeb and carbendazim were regular and velvety respectively. Hyphal thickness of mancozeb and carbendazim were moderately thick to thin. From our visual observation no mycelia was found in propiconazole, hexaconazole and difenoconazole + propiconazole. So no colony characters were observed.

Botanical extracts causes variation in colony characters. Here exists no difference in color of upper surface except off white but difference in various colors at the lower surface. Lower surface color on the neem treated media was brown to light brown, off white to grey for onion, ginger and garlic, off white to blackish in regard of black cumin. Differences in color lower surface in different treatment might be due to differences in pigmentation both amounts and kinds. There were a few variations in colony margins of upper and lower surface of extracts treated media. The margin of upper and lower surfaces was regular except as wavy and irregular in the upper surface. There was no difference in the surface colony margin of garlic, onion, black cumin. The wavy margin was found in ginger treated media. Irregular margin was found in neem treated media. There was no variation in colony texture except velvety for onion 5%, onion 10%, garlic 5%, garlic 10%, black cumin 5%, black cumin 10%. Variation was also found in the hyphal thickness. Very thick mycelia were found in case of control condition. Thick mycelia are found in case of black cumin, onion and ginger. Thin mycelia were found in case of neem, garlic. In most cases, hyphal thickness was reduced with increase in concentration.

No mycelia were occurred in propiconazole, hexaconazole and difenoconazole + propiconazole. So no colony characters were observed.

References

Anonymous. 1968. Plant Pathologist's Pocket Book. Commonwealth Mycological Institute, 394-395.

BBS. 2007. Statistical Year Book. Bangladesh Bureau of Statistics. Ministry of Planning, Government of the Peoples Republic of Bangladesh.

Delvin, J.F. and T. Zettel. 1999. Eco-agriculture: Initiatives in Eastern and Southern Africa. J. of Plant Mol. Biol., 6(2): 150-152.

Dhingra, O.D. and J.B. Sinclair. 1985. Basic Plant Pathology Methods. Department of Plant Pathology. University of Illionis, USA. Plant Dis., 132-163.

FAOSTAT, 2008. www.http// FAOSTAT.org/default/barly.html.

Grange, N. and S. Ahmed. 1988. Handbook of Plants with Pest Control Properties. 7(5): 75-78.

Hossain, I. and A.K. Azad, 1992. Reaction of wheat to *Helminthosporium sativum* in Bangladesh. Hereditas, 116: 203-205.

Kumar, J., P. Schafer, R. Hückelhoven, G. Langen, H. Baltruschat, E. Stein, S. Nagarajan and K. Kogel. 2002. *Bipolaris sorokiniana*, a cereal pathogen of global concern: cytological and molecular approaches towards better control. Mol. Plant Pathol., 3(4): 185–195.

Naz, F., C.A. Rauf, I.U. Haque and I. Ahmad. 2006. Management of *Fusarium oxysporum* with plant diffustates and chemicals. Pakistan J. Phytopathol., 18(1): 36-43.

Pasquer, F., E. Isdore, J. Zarn and B. Keller. 2005. Specific patterns of changes in wheat gene expression after treatment with three antifungal compounds. Plant Mol. Biol., 57: 693-707.

Roy, B., R. Amin, M.N. Uddin, A.T. Islam, M.J. Islam and B.C. Halder. 2005. Leaf extracts of Shiyalmutra (*Blumea lacera*) as botanical pesticides against lesser grain borer and rice weevile. Journal of Biological Science, 5(2): 201-204.

Sivanesan, A. 1990. *Mycosphaerella graminicola*. CMI descriptions of pathogenic fungi and bacteria No. 986. Mycopathologia, 109:51-53.

Stack, R.W. 1991. Yield losses in barley due to common root rot in North Dakota. *In*: Proceedings, International Conference on Common Root Rot. Eds. R.D. Tinline *et al*. Agriculture, Saskatoon, Sask, Canada: 1–5.

Vijayalakshmi, K., B. Subhashini and S. Koul. 1999. Plants in pest control, pongam, tulasi and aloe. Centre for Indian Knowledge Systems, Chennai, India.