Alkaloid extract of *Prosopis juliflora* (Sw.) DC. on sorghum seed mould

M. P. Raghavendra[1, 2]* S. Satish[1, 3] and K. A. Raveesha[1]

ABSTRACT

Pooled Alkaloid Extract (PAE) isolated from fractionation of methanol extract of leaves of *Prosopis juliflora* (Sw.) DC. was tested *in vivo* against sorghum seed biodeterioration during storage for six months period. The percent incidence of seed borne fungal pathogens, seed germination and seedling vigor, total water soluble protein, carbohydrate, lipid and dry matter content of the treated seeds were recorded at the interval of one month through six months period. The treatment revealed that the PAE treatment of seeds significantly reduced percent incidence of moulds and mould induced biodeterioration up to 180 days storage along with significant increase in seed germination and seedling vigor up to 90 days. Carbohydrate, protein, lipid and dry matter losses were also not observed in the treated seeds while significant loss of all the parameters was observed in untreated control seeds. The result of the present study is highly encouraging in developing herbal remedy for seed borne fungal diseases and biodeterioration of grains during storage.

Key words: Antifungal activity, alkaloid extract, *Prosopis juliflora*.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) (Moench) is a vital life-sustaining crop in many parts of the world ranking fifth after wheat (*Triticum* spp.), rice (*Oryza sativa* L.), maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.). Total world production of sorghum in the year 2002 was estimated at about 54 million tonnes (FAO, 2004) and total annual production of about 70 million metric tons of grains from 50 million hectares of land (National Academic Science, 1996). Sorghum is an important staple food crop in Africa, South Asia and Central America. It is grown in USA, Australia, and other developed countries for animal feed and it is also a principle source of energy, protein, vitamins and minerals to the poorest people of semi-arid tropics. However, the serious problem with sorghum is that it is highly susceptible to many fungi associated with grain mould disease such as species of *Alternaria*, *Curvularia*, *Fusarium*, *Drechslera* and *Phoma* (Masum *et al*., 2009). These fungi are significant destroyers of foodstuffs during storage, rendering them unfit for human consumption by retarding their nutritive value and sometimes by production of mycotoxins. Dubey *et al*. (2008) is of the opinion that priority should be given to post harvest studies, particularly in humid tropical climates, where at least half of the food supply may be lost between harvest and consumption.

Even though many chemical pesticides are available to manage post harvest loss, their residual effect after the consumption of pesticides treated seeds is of concern. In view of this plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999; El-Moughy *et al*., 2004; Ahmed *et al*., 2009). Extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails. Considering these higher plants were routinely screened in our laboratory to identify the plant with antifungal activity, during preliminary screening *Prosopis juliflora* (Sw.) DC. recorded highly significant activity, as this plant is known to contain pool of alkaloids with various biological activity, pooled alkaloid extract was selected in the present study to demonstrate its efficacy against seed borne fungal pathogens of sorghum.

MATERIALS AND METHODS

Collection and extraction of alkaloids

Healthy disease free, mature leaves of *Prosopis juliflora* were collected from Mysore, Mysore district, Karnataka (India) was used for the preparation of extract. A voucher specimen of the plant has been deposited in the herbarium of Department of Studies in Botany, University of Mysore, Mysore. The extract containing alkaloids (AE) from *Prosopis juliflora* leaves was obtained by acid/basic extraction method as described by Harborne (1998). In

brief, the air dried plant material was extracted in Soxhlet extractor with methanol (100 mL/25g) for 48 h. The methanolic extract was concentrated using rotary flash evaporator. Evaporated methanol extract was acidified with dilute HCl and extracted with ether to remove the resins, fats, oils and colouring matters. The combined aqueous-acid solution was neutralized with ammonia until it reached pH 11 and was extracted with chloroform. Aqueous ammonical solution was discarded. The resulting solution, an extract containing alkaloids, was washed with water, evaporated to dryness and confirmed for their positivity using the Dragendorff's reagent identification test (Wagner *et al.*, 1983). Chloroform solution of alkaloid was subjected to antifungal activity assay and determination of MIC.

Antifungal activity

Sorghum seed samples of cv CSH-5 which recorded high degree of mycoflora with diverse species was selected for seed treatment studies. Control and treated seed samples were subjected to Standard Blotter Method (Anonemous, 1996). Twenty-five seeds per plate were plated on three layer moistened blotter discs in petriplates. These plates were incubated at 22±2 ºC under alternating cycle of 12/ 12 h. of near ultraviolet (NUV) light and darkness for seven days. On the seventh day of incubation samples were screened for seed mycoflora with the help of stereo binocular microscope and also with the help of a compound microscope. Associated fungi were identified based on growth characteristics, colony and spore morphological characters using standard manuals (Booth, 1971 and 1977; Richardson, 1990).

One hundred seeds from each treatment (Control and PAE treated) were subjected to germination and seedling vigour test by rolled paper towel method (Anonemous, 1996). Four replicates were maintained for each treatment. Control and treated seeds were placed on the three layers of moist blotter sheets and rolled. These rolls were placed in trays containing sterile water at the bottom and covered by moist polyethylene covers and incubated for 8 days at 22±2 ºC. Root and shoot lengths of the seedlings from each treatment were recorded. Vigour Index (VI) was calculated as proposed by Abdul-Baki and Anderson (1973).

VI = (Mean shoot length + Mean root length) X % germination

Seed storage studies

Control and PAE treated seeds (500, 1000 and 1500 ppm concentration for 1h. 2h. 3h. and 4h. period) were stored separately in polyethylene bags and stored in lab conditions for six months. Samples were drawn at one month interval for six months and subjected to SBM, seed germination and seedling vigor tests. Total protein, total carbohydrates and lipid contents of seeds treated with 1500 ppm of PAE for 1h. 2h. 3h. and 4h. periods were only subjected to determination of above parameters

Evaluation of the nutritional qualities

Total carbohydrate (Dubois *et al.*, 1956), water-soluble protein (Lowry *et al.*, 1951) and lipid content (Fabbri *et al*., 1980) were estimated for seeds treated with alkaloid extract in different concentration for different storage period at one month interval and compared with the seeds which served as control. More over quantification of Dry Matter Loss (DML) was determined by hot air oven method (Reed, 1987) by comparing the dry matter loss before and after storage. All the data were subjected to statistical analysis using SPSS for windows.

RESULTS

Seed storage studies

It was observed that the percentage of seedling germination and seedling vigour of the sorghum seeds increased from 1 month to 3 months in 4h. treated seeds at 1500 ppm concentration with significant decrease in seed mycoflora. Sorghum seeds treated at various concentrations in different intervals of time also revealed the significant increase in seedling vigour and seed germination with significant decrease in seed mycoflora. However, seed treated at 1500 ppm concentration for 1, 2, 3 and 4h. period recorded highly significant decrease in seed mycoflora with significant increase in seed germination and seedling vigour up to 3 months period compared with control. After 4 months storage, seedling vigour and germination decreased but was still found to be significant compared with control even at 6 months storage. It was also observed that during storage no insect infestation was observed in all the treated seeds controlling the seed biodeterioration. No change in seed morphology was observed in seeds stored. Percent incidence of *Alternaria* sp. *Fusarium* sp. *Curvularia* sp. *Penicillium* sp. *Drechslera* sp. and *Colletotrichum* sp. were significantly reduced where as no significant decrease in *Aspergillus* species was observed in all the treatments (Tables 1 to 6).

Evaluation of the nutritional qualities

The total carbohydrate and protein content of the treated seeds did not show any change even after six months storage. However a slight decrease in carbohydrate and protein content at fourth, fifth and sixth month storage in the seeds treated for all the duration was observed. The untreated seeds showed continuous marked decrease in carbohydrate and protein content respectively up to six months storage. Whereas for lipid content, untreated seeds recorded significant loss during 90 days of storage,

Table 1. Effect of PAE (500 ppm) on seed mycoflora, seed germination and seedling vigour of sorghum seeds during different storage periods

	1 month					2 months					3 months				
	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.
Germination (%)	54.00 ±0.25	54.00 ±0.25	60.33 ±0.25	64.33 ±0.16	68.67 ±0.08	53.67 ±0.25	58.67 ±0.12	65.00 ±0.12	68.67 ±0.08	71.67 ±0.12	51.67 ±0.08	60.03 ±0.23	66.00 ±0.12	70.33 ±0.24	73.00 ±0.25
Seedling vigour	701 ±0.95	764 ±0.00	779 ±0.75	944 ±1.41	1040 ±1.18	703 ±0.57	756 ±0.94	806 ±0.82	996 ±0.95	1019 ±0.25	699 ±1.19	763 ±0.50	827 ±1.22	977 ±0.70	1016 ±0.95
Seed mycoflora (Percent incidence)															
Alternaria alternata	39 ±0.70	37 ±0.47	25 ±0.40	19 ±0.25	12 ±0.25	45 ±0.47	40 ±0.25	23 ±0.47	16 ±0.28	11 ±0.25	40 ±1.77	35 ±0.25	23 ±0.47	15 ±0.00	11 ±0.47
Fusarium sp.	40 ±0.86	35 ±0.28	30 ±0.47	28 ±0.28	22 ±0.28	48 ±0.50	35 ±0.25	33 ±0.27	30 ±0.27	28 ±0.47	54 ±1.19	30 ±0.25	28 ±0.25	28 ±0.00	25 ±0.25
Curvularia sp.	06 ±0.25	05 ±0.00	05 ±0.25	05 ±0.25	05 ±0.25	09 ±0.00	08 ±0.28	07 ±0.25	06 ±0.25	06 ±0.40	08 ±0.00	07 ±0.00	06 ±0.25	06 ±0.258	05 ±0.00
Aspergillus flavus	10 ±0.25	10 ±0.25	05 ±0.25	05 ±0.25	05 ±0.25	20 ±1.22	15 ±0.28	15 ±0.25	16 ±0.28	15 ±0.25	25 ±0.25	20 ±0.47	20 ±0.47	19 ±0.28	20 ±0.25
Penicillium sp.	04 ±0.25	04 ±0.00	03 ±0.25	01 ±0.25	00	10 ±0.47	08 ±0.25	07 ±0.25	05 ±0.00	05 ±0.28	10 ±0.25	09 ±0.25	08 ±0.00	06 ±0.25	05 ±0.00
Nigrospora sp.	06 ±0.25	04 ±0.25	02 ±0.25	01 ±0.00	01 ±0.25	06 ±0.25	04 ±0.25	00	01 ±0.00	00	10 ±0.25	09 ±0.25	07 ±0.25	05 ±0.25	00
Aspergillus niger	10 ±0.57	10 ±0.28	05 ±0.25	06 ±0.25	03 ±0.25	17 ±0.28	17 ±0.47	16 ±0.25	17 ±0.25	15 ±0.25	30 ±0.25	25 ±0.25	20 ±0.28	20 ±0.28	18 ±0.28
Drechslera sp.	07 ±0.25	04 ±0.28	02 ±0.00	02 ±0.25	03 ±0.00	08 ±0.25	05 ±0.25	04 ±4.25	01 ±0.25	01 ±0.00	07 ±0.25	06 ±0.00	04 ±0.25	02 ±0.00	01 ±0.00
Trichothecium sp.	05 ±0.75	05 ±1.45	01 ±0.25	00	00	04 ±1.41	04 ±0.25	03 ±0.75	01 ±0.25	01 ±0.50	06 ±0.94	06 ±0.25	06 ±0.00	03 ±0.25	02 ±0.25
Chaetomium sp.	03 ±0.57	02 ±0.25	02 ±0.25	00	00	04 ±0.53	03 ±0.25	03 ±0.57	02 ±0.28	00	12.5 ±1.44	00	00	00	00
Phomopsis sp.	01 ±0.57	00	00	00	00	02 ±0.70	02 ±0.25	00	00	00	01 ±0.47	00	00	00	00
Colletotrichum sp.	03 ±0.95	04 ±0.25	04 ±0.00	03 ±0.25	03 ±0.28	05 ±0.00	02 ±0.25	02 ±0.25	01 ±0.25	01 ±0.25	02 ±0.00	02 ±0.00	01 ±0.00	01 ±0.25	01 ±0.25
Cladosporium sp.	05 ±0.95	04 ±0.00	03 ±0.25	03 ±0.28	02 ±0.25	05 ±1.25	03 ±0.25	02 ±0.25	01 ±0.00	01 ±0.25	08 ±0.95	05 ±0.28	03 ±0.25	01 ±	01 ±
Rhizopus sp.	07 ±0.12	5.50 ±0.50	05 ±0.25	04 ±0.00	03	06 ±0.25	06 ±0.28	04 ±0.25	02 ±0.00	02 ±0.28	08 ±0.47	10 ±0.28	08 ±0.47	06 ±0.25	06 ±0.28
Phoma sp.	01 ±0.85	01 ±0.28	01 ±0.00	02 ±0.15	00	4.50 ±0.28	04 ±0.00	1.75 ±0.25	02 ±0.57	02 ±0.00	09 ±0.57	7.25 ±0.25	07 ±0.00	5.50 ±0.28	5.25 ±0.47

Results of four trials of 100 seed each ± Standard Error.

Table 2. Effect of PAE (500 ppm) on seed mycoflora, seed germination and seedling vigour of sorghum seeds during different storage periods

	4 months					5 months					6 months				
	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.
Germination (%)	51.67 ±0.53	58.00 ±0.50	65.00 ±0.25	67.67 ±0.15	67.80 ±0.11	48.33 ±0.72	59.00 ±0.31	59.33 ±0.37	62.33 ±0.16	60.33 ±0.21	43.33 ±0.5	54.33 ±0.81	57.00 ±0.22	61.33 ±0.30	64.67 ±0.11
Seedling vigour	680 ±2.39	733 ±1.75	806 ±1.00	858 ±0.57	910 ±0.86	660 ±4.33	815 ±1.25	825 ±3.14	818 ±0.57	840 ±1.25	669 ±4.58	701 ±5.25	722 ±2.59	740 ±2.25	750 ±0.50
Seed mycoflora (Percent incidence)															
Alternaria alternata	25 ±1.25	20 ±0.70	18 ±0.25	15 ±0.28	10 ±0.28	20 ±1.10	15 ±0.25	13 ±0.47	13 ±0.28	10 ±0.57	20 ±0.86	10 ±0.57	10 ±0.47	10 ±0.47	08 ±0.25
Fusarium sp.	50 ±2.92	35 ±0.70	33 ±0.95	30 ±0.57	28 ±0.25	40 ±1.65	33 ±1.18	30 ±0.50	25 ±0.25	24 ±0.25	40 ±1.22	33 ±0.25	30 ±0.50	26 ±0.47	25 ±0.47
Curvularia sp.	06 ±0.47	05 ±0.00	05 ±0.25	04 ±0.28	03 ±0.00	06 ±0.57	05 ±0.28	05 ±0.25	04 ±0.25	03 ±0.28	05 ±0.50	04 ±0.00	03 ±0.28	03 ±0.28	02 ±0.25
Aspergillus flavus	30 ±1.25	25 ±1.36	20 ±0.50	21 ±0.75	20 ±0.50	29 ±1.35	25 ±0.40	22 ±0.50	20 ±0.47	20 ±0.47	30 ±1.03	25 ±0.75	25 ±0.75	21 ±0.40	20 ±0.25
Penicillium sp.	10 ±0.94	08 ±0.28	07 ±0.50	06 ±0.25	04 ±0.25	08 ±0.47	07 ±0.25	04 ±0.28	04 ±0.50	03 ±0.25	09 ±0.70	07 ±0.50	04 ±0.25	04 ±0.25	03 ±0.25
Nigrospora sp.	06 ±0.95	06 ±0.28	04 ±0.25	02 ±0.25	02 ±0.25	05 ±0.85	04 ±0.25	03 ±0.00	01 ±0.28	01 ±0.25	05 ±0.75	04 ±0.25	03 ±0.25	01 ±0.00	01 ±0.00
Aspergillus niger	25 ±1.03	20 ±0.50	15 ±0.50	15 ±0.00	15 ±0.50	23 ±1.22	20 ±0.50	19 ±0.28	19 ±0.25	20 ±0.70	20 ±0.75	19 ±0.25	19 ±0.47	20 ±0.50	21 ±0.57
Drechslera sp.	06 ±0.98	05 ±0.50	04 ±0.25	02 ±0.28	01 ±0.00	06 ±1.31	05 ±0.25	04 ±0.00	02 ±0.00	01 ±0.00	05 ±0.81	04 ±0.47	03 ±0.28	02 ±0.25	01 ±0.00
Trichothecium sp.	05 ±0.28	05 ±0.25	04 ±0.28	01 ±0.00	01 ±0.28	04 ±0.95	04 ±0.28	03 ±0.28	02 ±0.28	01 ±0.00	05 ±0.25	04 ±0.00	04 ±0.25	02 ±0.00	02 ±0.25
Chaetomium sp.	04 ±0.48	02 ±0.25	02 ±0.25	02 ±0.25	02 ±0.25	03 ±0.47	03 ±0.25	02 ±0.00	02 ±0.00	02 ±0.00	01 ±0.25	01 ±0.25	00	00	00
Phomopsis sp.	03 ±0.50	02 ±0.25	01 ±0.00	00	00	02 ±0.00	01 ±0.25	00	00	00	01 ±0.00	01 ±0.00	01 ±0.00	01 ±0.00	00
Collectotrichum sp.	03 ±0.00	03 ±0.00	02 ±0.25	01 ±0.00	01 ±0.00	03 ±0.00	03 ±0.00	02 ±0.00	02 ±0.25	01 ±0.00	02 ±0.28	01 ±0.25	01 ±0.00	00	00
Cladosporium sp.	05±1.32	04±0.25	02±0.25	01±0.00	01±0.00	03±0.25	02±0.00	00	00	00	3.75±0.47	02±0.00	00	00	00
Rhizopus sp.	06 ±0.94	05 ±0.25	05 ±0.25	05 ±0.25	04 ±0.25	08 ±0.50	06 ±0.00	05 ±0.25	05 ±0.25	04 ±0.25	06 ±0.25	05 ±0.28	05 ±0.28	04 ±0.00	04 ±0.00
Phoma sp.	4.50 ±0.50	3.50 ±0.28	3.50 ±0.28	3.25 ±0.25	3.25 ±0.25	04 ±0.40	03 ±0.00	02 ±0.00	02 ±0.28	1.50 ±0.28	3.75 ±0.25	2.75 ±0.25	02 ±0.00	02 ±0.00	01 ±0.00

Results of four trials of 100 seeds each ± Standard Error.

Table 3. Effect of PAE (1000 ppm) on seed mycoflora, seed germination and seedling vigour of sorghum seeds during different storage periods

	1 month					2 months					3 months				
	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.
Germination (%)	55.00 ±0.47	57.00 ±0.25	58.67 ±0.20	68.33 ±0.90	68.33 ±0.39	56.67 ±0.31	61.33 ±0.28	64.00 ±0.28	68.33 ±0.24	69.33 ±0.18	52.69 ±0.09	63.67 ±1.25	64.67 ±0.16	68.33 ±0.15	71.11 ±0.38
Seedling vigour	701 ±0.47	913 ±0.25	966 ±0.25	961 ±0.00	971 ±0.25	703 ±0.47	870 ±0.47	899 ±8.82	971 ±0.55	1010 ±1.25	699 ±1.10	949 ±0.28	961 ±1.55	971 ±0.94	1011 ±0.56
Seed mycoflora (Percent incidence)															
Alternaria alternata	39 ±1.43	33 ±0.47	22 ±0.47	18 ±0.47	10 ±0.40	38 ±1.65	30 ±0.47	22 ±0.23	20 ±0.28	10 ±0.50	35 ±1.22	28 ±0.23	20 ±0.50	13 ±0.25	10 ±0.25
Fusarium sp.	42 ±1.22	33 ±0.47	21 ±0.29	20 ±0.47	18 ±0.50	48 ±1.03	31 ±0.40	16 ±0.23	13 ±0.23	10 ±0.28	54 ±1.50	33 ±0.47	17 ±0.12	15 ±0.37	15 ±0.12
Curvularia sp.	06 ±0.28	04 ±0.57	04 ±0.25	04 ±0.25	04 ±0.40	09 ±1.10	06 ±0.40	03 ±0.62	02 ±0.47	02 ±0.47	08 ±0.28	06 ±0.28	03 ±0.47	02 ±0.47	02 ±0.40
Aspergillus flavus	10 ±0.50	11 ±0.23	07 ±0.25	05 ±0.25	05 ±0.25	20 ±0.18	21 ±0.33	15 ±0.47	14 ±0.47	15 ±0.28	25 ±0.47	21 ±0.23	20 ±0.27	20 ±0.27	19 ±0.31
Penicillium sp.	04 ±0.28	02 ±0.28	02 ±0.25	01 ±0.25	01 ±0.25	10 ±0.47	08 ±0.28	07 ±0.25	04 ±0.25	02 ±0.25	11 ±0.43	07 ±0.43	04 ±0.25	04 ±0.25	04 ±0.25
Nigrospora sp.	06 ±0.28	03 ±0.50	01 ±0.25	00	00	06 ±0.28	02 ±0.25	02 ±0.28	00	00	08 ±0.47	05 ±0.25	03 ±0.37	01 ±0.00	00
Aspergillus niger	10 ±0.57	15 ±0.47	07 ±1.22	02 ±0.86	01 ±0.75	17 ±0.75	17 ±0.75	15 ±0.47	15 ±0.50	16 ±0.47	25 ±0.75	20 ±0.50	19 ±0.25	17 ±0.57	17 ±0.70
Drechslera sp.	07 ±0.45	05 ±0.47	03 ±0.25	02 ±0.00	02 ±0.25	08 ±0.47	03 ±0.48	02 ±0.25	01 ±0.25	01 ±0.25	07 ±0.57	03 ±0.28	02 ±0.25	01 ±0.00	01 ±0.00
Trichothecium sp.	05 ±0.50	06 ±0.28	00	00	00	04 ±0.28	04 ±0.25	01 ±0.25	00	00	06 ±0.25	04 ±0.25	02 ±0.00	00	00
Chaetomium sp.	03 ±0.57	01 ±0.25	01 ±0.25	00	00	04 ±0.57	03 ±0.25	01 ±0.25	00	00	10.50 ±0.50	00	00	00	00
Phomopsis sp.	01 ±0.50	00	00	00	00	02 ±0.25	01 ±0.25	00	00	00	01 ±0.25	00	00	00	00
Collectotrichum sp.	03 ±0.57	03 ±0.57	03 ±0.50	05 ±0.57	02 ±0.00	03 ±0.50	03 ±0.50	03 ±0.50	03 ±0.00	02 ±0.00	02 ±0.28	01 ±0.28	00	00	00
Cladosporium sp.	03 ±0.28	02 ±0.25	00	00	00	06 ±0.57	04 ±0.57	2.25 ±0.25	00	00	9.25 ±0.75	4.75 ±0.47	00	00	00
Rhizopus sp.	05 ±0.50	04 ±0.25	02 ±0.25	02 ±0.28	00	06 ±0.25	04 ±0.25	03 ±0.50	02 ±0.00	01 ±0.00	08 ±0.25	05 ±0.25	04 ±0.62	02 ±0.00	02 ±0.00
Phoma sp.	01 ±0.25	00	00	00	00	5.75 ±0.75	04 ±0.40	1.25 ±0.25	00	00	11 ±1.00	5.25 ±0.62	05 ±0.40	4.50 ±0.28	3.50 ±0.28

Results of four trials of 100 seed each ± Standard Error.

Table 4. Effect of PAE (1000 ppm) on seed mycoflora, seed germination and seedling vigour of sorghum seeds during different storage periods

	4 months					5 months					6 months				
	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.
Germination (%)	51.67 ±0.8	66.00 ±0.47	67.00 ±0.20	69.33 ±0.39	73.33 ±0.36	48.33 ±0.59	65.33 ±0.43	67.00 ±0.25	69.67 ±0.35	69.00 ±0.20	43.33 ±0.62	52.67 ±.031	54.30 ±0.23	55.17 ±0.16	57.67 ±0.50
Seedling vigour	680 ±2.12	860 ±2.04	797 ±1.03	962 ±2.30	1000 ±22.95	660 ±6.12	770 ±1.75	797 ±2.21	897 ±3.4	879 ±1.50	669 ±4.47	703 ±2.02	713 ±1.75	737 ±1.87	779 ±6.86
Seed mycoflora (Percent incidence)															
Alternaria alternata	25 ±0.62	15 ±0.28	09 ±0.50	05 ±0.47	04 ±0.25	20 ±0.70	15 ±0.25	12 ±0.47	10 ±0.47	09 ±0.28	20 ±0.27	15 ±0.44	14 ±0.42	10 ±0.25	09 ±0.25
Fusarium sp.	50 ±1.77	30 ±0.25	21 ±0.47	14 ±0.12	09 ±0.25	40 ±0.75	25 ±0.28	20 ±0.35	15 ±0.35	09 ±0.25	40 ±0.62	26 ±1.03	23 ±0.50	16 ±0.25	10 ±0.27
Curvularia sp.	06 ±0.35	04 ±0.21	02 ±0.00	02 ±0.28	01 ±0.28	06 ±0.00	05 ±0.20	03 ±0.20	03 ±0.28	01 ±0.57	05 ±0.47	03 ±0.28	03 ±0.28	02 ±0.28	01 ±0.00
Aspergillus flavus	30 ±0.32	25 ±0.23	20 ±0.50	17 ±0.28	20 ±0.23	29 ±1.71	25 ±0.28	25 ±0.23	24 ±0.47	24 ±0.31	30 ±0.29	28 ±0.59	30 ±0.40	28 ±0.23	25 ±0.23
Penicillium sp.	10 ±0.50	07 ±0.50	04 ±0.20	04 ±0.00	02 ±0.28	08 ±0.75	05 ±0.28	04 ±0.35	04 ±0.25	02 ±0.28	09 ±0.57	06 ±0.25	04 ±0.14	04 ±0.20	02 ±0.25
Nigrospora sp.	06 ±0.25	02 ±0.00	02 ±0.25	00	00	05 ±0.47	02 ±0.00	02 ±0.25	00	00	05 ±0.50	02 ±0.25	02 ±0.25	00	00
Aspergillus niger	25 ±0.75	20 ±0.65	20 ±0.23	17 ±0.23	16 ±0.23	23 ±0.28	20 ±0.23	20 ±0.25	18 ±0.37	16 ±0.23	20 ±0.35	20 ±0.23	18 ±03.6	16 ±0.23	15 ±0.42
Drechslera sp.	06 ±0.25	03 ±0.28	02 ±0.25	00	00	06 ±0.28	04 ±0.28	03 ±0.00	02 ±0.25	00	05 ±0.50	04 ±0.28	03 ±0.25	02 ±0.00	00
Trichothecium sp.	05±0.25	04±0.25	02±0.28	00	00	04±0.28	03±0.25	02±0.25	00	00	05±0.57	03±0.00	02±0.28	00	00
Chaetomium sp.	04 ±0.28	03 ±0.25	01 ±0.00	00	00	03 ±0.28	02 ±0.28	01 ±0.00	00	00	01 ±0.00	00	00	00	00
Phomopsis sp.	03±0.50	02±0.00	01±0.25	00	00	02±0.25	01±0.00	00	00	00	01±0.00	01±0.00	00	00	00
Collectotrichum sp.	03 ±0.50	03 ±0.00	03 ±0.00	03 ±0.25	02 ±0.25	03 ±0.50	03 ±0.28	03 ±0.28	03 ±0.25	03 ±0.25	02 ±0.25	02 ±0.25	02 ±0.25	01 ±0.00	01 ±0.00
Cladosporium sp.	5.50 ±0.28	2.25 ±0.25	00	00	00	3.50 ±0.50	01 ±0.00	00	00	00	2.75 ±0.25	01 ±0.00	00	00	00
Rhizopus sp.	06 ±0.28	04 ±0.28	03 ±0.28	02 ±0.25	01 ±0.00	08 ±0.25	06 ±0.14	04 ±0.14	02 ±0.00	02 ±0.25	06 ±0.25	04 ±0.28	02 ±0.00	02 ±0.25	02 ±0.28
Phoma sp.	5.50 ±0.28	2.50 ±0.28	1.75 ±0.25	00	00	4.75 ±0.75	2.75 ±0.25	02 ±0.25	01 ±0.00	01 ±0.00	4.50 ±0.28	2.75 ±0.25	02 ±0.25	01 ±0.00	01 ±0.00

Results of four trials of 100 seed each ± Standard Error.

Table 5. Effect of PAE (1500 ppm) on seed mycoflora, seed germination and seedling vigour of sorghum seeds during different storage periods

	1 month					2 months					3 months				
	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.
Germination (%)	55.00 ±0.50	61.33 ±0.75	66.00 ±0.75	69.67 ±0.58	69.67 ±0.43	56.07 ±0.75	62.63 ±0.33	65.00 ±0.35	70.33 ±0.50	70.67 ±0.45	52.67 ±2.28	66.00 ±0.25	69.67 ±0.55	73.00 ±0.50	73.67 ±0.39
Seedling vigour	701 ±2.65	735 ±4.71	827 ±5.34	965 ±6.87	981 ±2.02	705 ±2.77	938 ±1.75	982 ±1.03	1005 ±24.10	1044 ±14.00	699 ±22.83	954 ±1.43	981 ±2.36	1084 ±6.60	1243 ±9.43
Seed mycoflora (Percent incidence)															
Alternaria alternata	39 ±1.03	20 ±0.47	18 ±0.23	13 ±0.51	05 ±0.25	38 ±0.23	25 ±0.23	15 ±0.23	10 ±0.75	03 ±0.28	35 ±0.23	25 ±0.31	18 ±0.37	10 ±0.51	05 ±0.25
Fusarium sp.	42 ±0.25	20 ±0.28	17 ±0.28	10 ±0.25	06 ±0.25	48 ±0.25	20 ±0.28	15 ±0.25	08 ±0.25	05 ±0.25	54 ±0.70	30 ±0.70	15 ±0.25	05 ±0.25	05 ±0.50
Curvularia sp.	06 ±0.00	04 ±0.47	02 ±0.25	02 ±0.25	02 ±0.25	09 ±0.47	05 ±0.50	03 ±0.25	00 ±0.00	00 ±0.00	08 ±0.25	06 ±0.25	03 ±0.57	00 ±0.00	00 ±0.00
Aspergillus flavus	10 ±0.25	10 ±0.40	05 ±0.50	01 ±0.00	01 ±0.00	20 ±0.47	15 ±0.28	10 ±0.25	09 ±0.00	09 ±0.25	25 ±0.25	15 ±0.40	12 ±0.47	06 ±0.25	05 ±0.25
Penicillium sp.	04 ±0.25	01 ±0.00	00	00	00	10 ±0.25	04 ±0.25	02 ±0.25	00	00	11 ±1.03	04 ±0.28	02 ±0.00	00	00
Nigrospora sp.	06 ±0.70	02 ±0.25	01 ±0.00	00	00	06 ±0.25	02 ±0.00	02 ±0.25	00	00	08 ±0.25	03 ±0.25	01 ±0.00	00	00
Aspergillus niger	10 ±0.47	08 ±0.47	03 ±0.28	02 ±0.00	01 ±0.00	17 ±0.47	15 ±0.25	10 ±0.47	10 ±0.25	09 ±0.28	25 ±0.25	18 ±0.28	13 ±0.25	10 ±0.25	08 ±0.28
Drechslera sp.	07 ±0.25	03	03	03	00	08 ±0.25	05 ±0.28	03	02	00	07 ±0.00	03 ±0.28	00	00	00
Trichothecium sp.	05 ±0.25	04 ±0.25	00	00	00	04 ±0.25	01 ±0.00	00	00	00	06 ±0.25	01 ±0.00	00	00	00
Chaetomium sp.	03 ±0.00	00	00	01 ±0.25	00	04 ±0.28	01 ±0.25	00	00	00	01 ±0.00	01 ±0.00	00	00	00
Phomopsis sp.	01 ±0.25	00	00	00	00	02 ±0.00	01 ±0.28	00	00	00	01 ±0.00	01 ±0.00	00	00	00
Collectotrichum sp.	04 ±0.57	3.75 ±0.75	2.75 ±0.25	2.50 ±0.28	1.50 ±0.28	03 ±0.28	03 ±0.25	02 ±0.28	01 ±0.00	01 ±0.00	02 ±0.28	01 ±0.00	01 ±0.00	00	00
Cladosporium sp.	2.75 ±0.25	1.75 ±0.25	00	00	00	4.75 ±0.25	2.75 ±0.25	1.75 ±0.25	00	00	8.25 ±0.25	3.75 ±0.25	00	00	00
Rhizopus sp.	05 ±0.57	04 ±0.25	02 ±0.25	01 ±0.00	00	06 ±0.25	03 ±0.25	02 ±0.28	02 ±0.28	01 ±0.00	08 ±0.40	03 ±0.25	02 ±0.70	02 ±0.00	01 ±0.00
Phoma sp.	02 ±0.40	00	00	00	00	4.75 ±0.75	2.75 ±0.25	1.75 ±0.25	00	00	8.50 ±0.28	04 ±0.00	03 ±0.00	02 ±0.00	02 ±0.28

Results of four trials of 100 seed each ± Standard Error.

Table 6. Effect of PAE (1500 ppm) on seed mycoflora, seed germination and seedling vigour of sorghum seeds during different storage period

	4 months					5 months					6 months				
	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.
Germination (%)	51.67 ±0.83	69.33 ±0.42	69.33 ±0.44	70.00 ±0.28	71.67 ±0.28	48.33 ±0.65	62.33 ±1.65	62.67 ±0.16	64.33 ±0.39	67.00 ±0.47	44.33 ±0.45	52.67 ±0.64	55.57 ±0.88	57.67 ±0.13	59.33 ±0.51
Seedling vigour	680 ±3.53	981 ±3.67	1018 ±4.15	1034 ±5.25	1108 ±22.41	660 ±11.25	933 ±11.33	993 ±0.75	996 ±3.94	957 ±18.69	669 ±4.19	737 ±12.34	790 ±2.65	810 ±13.42	835 ±7.84
Seed mycoflora (Percent incidence)															
Alternaria alternata	23 ±0.75	13 ±0.25	08 ±0.25	04 ±0.25	04 ±0.25	21 ±1.18	13 ±0.50	10 ±0.25	08 ±0.25	06 ±0.28	22 ±0.75	15 ±0.70	13 ±0.28	13 ±0.28	10 ±0.25
Fusarium sp.	50 ±1.35	30 ±0.50	19 ±0.25	19 ±0.25	05 ±0.25	40 ±0.94	20 ±0.57	18 ±0.23	17 ±0.47	10 ±0.50	40 ±0.28	26 ±0.28	20 ±0.23	13 ±0.25	12 ±0.47
Curvularia sp.	06 ±0.38	04 ±0.38	02 ±0.25	02 ±0.25	02 ±0.25	06 ±0.27	04 ±0.28	020.00	02 ±0.25	01 ±0.00	05 ±0.47	03 ±0.25	02 ±0.25	01 ±0.00	00 ±0.00
Aspergillus flavus	30 ±1.25	23 ±0.50	17 ±0.40	15 ±0.75	13 ±0.25	29 ±0.62	20 ±2.91	18 ±0.28	17 ±0.25	17 ±0.25	30 ±0.40	28 ±0.25	25 ±0.25	20 ±0.25	19 ±0.25
Penicillium sp.	10 ±1.22	06 ±0.47	03 ±0.25	03 ±0.25	01 ±0.25	11 ±0.47	05 ±0.25	04 ±0.25	03 ±0.25	01 ±0.00	09 ±0.25	06 ±0.25	03 ±0.25	02 ±0.25	02 ±0.28
Nigrospora sp.	06 ±0.25	02 ±0.00	02 ±0.28	01 ±0.00	00	05 ±0.28	02 ±0.25	00	00	00	05 ±0.28	02 ±0.25	01 ±0.00	01 ±0.00	01 ±0.00
Aspergillus niger	25 ±0.75	18 ±0.25	17 ±0.50	15 ±0.53	13 ±0.47	26 ±0.50	18 ±0.25	17 ±0.25	17 ±0.25	17 ±0.47	20 ±0.81	20 ±0.50	16 ±0.25	14 ±0.25	13 ±0.25
Drechslera sp.	06 ±0.25	02 ±0.00	00	00	00	06 ±0.25	03 ±0.25	02 ±0.25	00	00	05 ±0.25	04 ±0.25	02 ±0.25	00	00
Trichothecium sp.	05 ±0.25	03 ±0.25	02 ±0.25	00	00	04 ±0.25	02 ±0.00	02 ±0.00	02 ±0.00	02 ±0.25	05 ±0.25	03 ±0.25	00	00	00
Chaetomium sp.	05 ±0.57	03 ±0.00	01 ±0.00	00	00	03 ±0.28	02 ±0.00	01 ±0.00	00	00	01 ±0.00	00	00	00	00
Phomopsis sp.	03 ±0.28	02 ±0.28	01 ±0.00	00	00	02 ±0.28	01 ±0.00	00	00	00	01 ±0.00	01 ±0.00	00	00	00
Collectotrichum sp.	03 ±0.26	03 ±0.00	03 ±0.25	02 ±0.25	01 ±0.00	03 ±0.25	03 ±0.00	03 ±0.00	03 ±0.25	02 ±0.47	02 ±0.00	02 ±0.25	01 ±0.00	01 ±0.00	00
Cladosporium sp.	5.25 ±0.25	1.75 ±0.25	00	00	00	2.75 ±0.25	01 ±0.00	00	00	00	2.75 ±0.25	01 ±0.00	00	00	00
Rhizopus sp.	06 ±0.25	04 ±0.25	03 ±0.25	02 ±0.25	01 ±0.00	08 ±0.28	05 ±0.28	04 ±0.25	02 ±0.00	02 ±0.25	06 ±0.25	04 ±0.28	02 ±0.25	02 ±0.25	01 ±0.00
Phoma sp.	06 ±0.57	2.50 ±0.28	1.50 ±0.28	00	00	4.25 ±0.25	2.75 ±0.25	1.25 ±0.25	01 ±0.00	01 ±0.00	4.75 ±0.75	2.50 ±0.28	1.50 ±0.28	01 ±0.00	01 ±0.00

Results of four trials of 100 seed each ± Standard Error.

with total loss after 180 days of storage. In case of seeds treated with PAE the lipid content was 17.6 mg/g at 90 days of storage and gradually decreased from 90-180 days of storage. In case of seeds treated with PAE, even at 90 days of storage there is no total loss of lipid. The dry matter loss was significantly low in treated seeds compared to untreated seeds even after 180 days of storage, percent dry matter loss did not exceed 1.2%.

Seedling growth

It was observed that the seedling growth was more in treated seeds compared with control seeds. Among the different concentrations tested, maximum growth promotion of the seedling was observed in seeds treated with 1500 ppm concentration for 4h. period compared with control seedlings. The seedlings were found healthy compared to control seedlings. Reduction in the percentage incidence of *Fusarium* sp., *Alternaria* sp., *Curvularia* sp., *Drechslera* sp., and other fungi was observed in all the treatments with PAE over the control. Maximum reduction was observed in 4 h. treatment at 1500 ppm concentration.

DISCUSSION

Generally, tropical conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest, and flash floods lead to fungal proliferation and mycotoxins contamination. Poor harvesting practices, improper storage, and sub optimal conditions during transport and marketing can also contribute to fungal growth and proliferation of mycotoxins (Dubey *et al*., 2008). Many reports are available in India and abroad on the contamination of grains in general and sorghum and maize in particular by species of *Fusarium, Aspergillus, Drechslera, Alternaria* and other species of fungi (Shashidhar *et al*., 1992; Shabbir and Rajasab, 2005; Islam *et al*., 2009). Many species of *Fusarium* are also known to produce toxins during storage of grains. Fumonisins produced by species of *F. moniliforme*, *F. proliferatum* are important. The results of the present investigation reveal that species of *Fusarium* are effectively controlled by PAE suggesting that the alkaloid extract has the potency to prevent Fusarial toxin production and contamination of grains during storage.

Prosopis juliflora (Sw.) DC. is known to possess several important biological activities (Raghavendra *et al*. 2009). Many alkaloids such as juliflorine, julifloricine and julifloridine (Ahmad *et al.,* 1978), juliprosine (Daetwyler *et al*., 1981), juliprosinine and juliflorinine (Ahmad *et al*., 1989) are found to be responsible for the biological activity. Recently, julifloravizole a novel alkaloid with broad spectrum antifungal activity against species of *Fusarium*, *Drechslera* and *Alternaria* was reported from the leaves of *P. juliflora* (Raghavendra, 2007). In view of these, in the present study pooled alkaloid extract was extracted from the plant to prove synergistic effect of all the alkaloids against seed borne phytopathogenic fungi of sorghum.

In vivo antifungal activity studies of PAE suggests significant inhibitory activity against the important grain mould fungi of sorghum. It is also evident from the present investigation that the seed quality parameters such as seed germination and seedling vigour are not affected by PAE even at 1500 ppm concentration treatment for four hour duration. It is evident from the present investigation that the nutritional qualities (protein, carbohydrate, lipids and dry matter weight) of the seeds treated with pooled alkaloid extract are not lost even during storage for six months. The results also suggest that insect infestation during storage is completely controlled, thus indicating the useful effects of pooled alkaloid extract in preventing fungal biodeterioration of grains during storage and also maintaining the seed quality. The alkaloid extract is an important component which could be exploited for seed treatment based on further toxicological studies.

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M. P. Raghavendra[1, 2]* S. Satish[1, 3] and K. A. Raveesha[1]

[1]Herbal Drug Technology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, 570 006, Karnataka, India.

[2]Department of Post Graduate Studies in Microbiology, Maharani's Science College for Women, J. L. B. Road, Mysore-570 005, Karnataka, India.

[3]Microbiology Laboratory, Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore-570 006, Karnataka, India.

*Department of Post Graduate Studies in Microbiology, Maharani's Science College for Women, J. L. B. Road, Mysore- 570 005, Karnataka, India, Phone: 09844037008, E.mail: mpraghavendra @gmail.com