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Research Article
Natural Fumigant Efficacy of Giloy Leaf Essential Oil for Control of Fungal Infections and Enhancement of Shelf Life of Pigeon Pea Seeds

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Abstract

Background and Objective: Pigeon pea [*Cajanus cajan* (L.) Millsp.] suffers great losses due to fungal infections. For protection farmers use sulphos which have negative effects. So essential oils from higher plants need to be tried for its antifungal potential and safe storage of pigeon pea having no negative effects. **Materials and Methods:** The fungal investigations on stored food seeds of Pigeon pea were done through agar plate as well as blotter paper methods. Essential oils were separately isolated from 20 plants. MIC, nature, spectrum, effect of physical factors were decided and *in vivo* efficacy of *Tinospora cordifolia* (*T. cordifolia*) leaf oil was studied for safe storage of pigeon pea. **Results:** The fungal investigations on stored food seeds of stored food seeds of Pigeon pea [*Cajanus cajan* (L.) Millsp.] showed presence of 16 fungal species. Out of these fungal species *Aspergillus flavus*, *A. niger* was found to be dominant on the basis of frequency (%). The *Tinospora* leaf oil showed absolute toxicity (100%) against frequently present *Aspergillus flavus*, *Aspergillus niger* at concentration of 400 ppm and fungicidal at 600 ppm. At 500 ppm inhibited 10 fungi and at 800 ppm concentration 15 fungi. There was no adverse effect of physical variants viz., temperature treatment up to 100°C, autoclaving at 15 lb/square inch pressure at 120°C and storage up to 120 days on potential of leaf oil. *In vivo* experiments revealed that *Tinospora* oil when applied as seed dressing agent and as a fumigant was able to preserve food seeds of pigeon pea up to 120 days having minimal changes in organoleptic behaviour during storage. **Conclusion:** It revealed that *T. cordifolia* leaf essential oil have more fungitoxic efficacy in comparison to synthetic pesticides.

Key words: Pigeon pea, *T. cordifolia* leaf oil, biodeterioration, giloy, synthetic fumigant

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Pigeon pea-[*Cajanus cajan* (L.) Millsp.] belongs to the genus-*Cajanus* (subtribe-*Cajaninae*, tribe-Phaseoleae, order-Fabales) comes in family-Fabaceae/sub-family Faboideae. Pigeon pea *(Cajanus cajan* (L.) Millsp.) is an important food legume of perennial member of the family Fabaceae[1]. This is a rich source of certain minerals along with protein and carbohydrate. The protein content comes in the range between 17.9 and 24.3 g/100 g for whole grain samples[1].

Seed mycoflora is a major factor affecting seed health. Wide ranges of fungi have been encountered with pigeon pea seed. Although, some of them have been reported to be pathogenic, many other aspect of seed pathology of pigeon pea needs to be understand. Seed mycoflora of pigeon pea as well as their culture filtrates caused considerable reduction in germination percent as compare to untreated[2].

Ghangaokar and Kshirsagar[3] identified seed borne fungi associated with different legumes seeds by using blotter paper method from selected untreated and treated seeds. The seeds have been found associated with highest number of seed borne fungi viz., *Alternaria alternata*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Rhizopus nigricans*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium solani*, *Curvularia lunata*, *Penicillium citrinum*, *Macrophomina* sp., *Monilia* sp., *Chaetomium* sp., *Rhizoctonia* sp, *Trichoderma* etc. These have potential to spoil the quality of pigeon pea during storage therefore pigeon pea seed, needs to be protected against these fungi to achieve a uniform plant stand and vigorous seedlings.

The present study therefore, aimed to investigate the seed mycoflora of pigeon pea at Gurgaon, biodeterioration due to fungi and *in vivo* preservative potential of most active oil of neem giloy (*Tinospora cordifolia* leaf essential oil) for storage of pigeon pea [*Cajanus cajan* (L.) Millsp.] seeds.

MATERIALS AND METHODS

Fungal analysis: Twenty samples of pigeon pea seeds were collected from Grocery stores of nearby Gurugaon area in year 2014-17.

Method of mycological analysis: This was done following Kumar[4] adopting agar plate and standard blotter method with slight modification.

Standard blotter method: This technique was used to detect the presence of fungi on or in the pigeon pea seeds after incubation which is useful in the sense that fast growing fungi are better detected than the slow growing ones. The 9.0 cm diameter size petri plates were taken in which three good quality blotter papers of the same diameter were kept. They were then moistened with sterilized distilled water. In each petri plate, 5 seeds were placed on the moistened blotters in such a manner that one at the center and 4 formed the outer circle. For isolation of internal fungi present on seed they were sterilized with 1.0% NaOCl for 35 sec and immediately washed twice with double distilled water. For each sample, a total of 200 seeds were studied. The petri plates were kept at 25±2°C for a period up to 7 days in alternating cycles of 12 h light and 12 h darkness. Firstly seeds of the outer ring were examined first and then the seed in the centre. The occurrence of fungal species was expressed in percent frequency as per formula.

The percent frequency was calculated by using following equation:

$$\text{Frequency (\%)} = \frac{\text{No. of plates in which individual fungal species occurred}}{\text{Total No. of plates studied}} \times 100$$

Agar plate method: About 15 mL potato dextrose agar medium (having composition potato (peeled and sliced) 200 g, dextrose 20 g, agar 20 g, distilled water 1000 mL) was poured in each sterilized petri plate. To prevent bacterial contamination, 15 mg of streptomycin sulphate was added in the medium at the time of pouring. Seeds of each sample were surface sterilized with 1.0% NaOCl for 35 sec and immediately washed twice with double distilled water thoroughly to remove NaOCl solution that adhered if any. Seeds were placed on the previously poured medium in petri plate in such a way that 4 in the outer circle and 1 at the centre. For each sample, a total of 200 seeds were taken. They were incubated similarly like blotter method and percent frequency calculated.

Fungal identifications were confirmed following Ellis[5,6], Gillman[7], Raper and Thom[8], Raper and Fennell[9] along with available literature.

Deteriorative efficacy of storage fungi on pigeon pea seeds: The deterioration caused by dominant fungal species viz., *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *A. terreus* with respect to weight loss, seed germination and nutritional composition was evaluated. For this purpose freshly harvested

sterilized pigeon pea seeds were taken in presterilized polyethylene bags (300 g seeds/bag) and inoculated by two disc (5 mm diam) of different fungal species separately. The inoculated pigeon pea seed samples were stored for 20 days under laboratory conditions at room temperature. Experiments were revised and contained 5 replicates.

Germination percentage: Five replicates of 20 seeds each were kept between moistened blotter papers and incubated at room temperature of 28±2°C. The pigeon pea seeds with 0.5 cm radicle and plumule length were considered as germinated[10].

Estimation of carbohydrate: Anthrone method of Thimmaiah[11] was followed for Carbohydrate estimation. The anthrone reaction is the basis of rapid and highly suitable method for the determination of hexoses, aldopentose and hexuronic acids either free or present in polysaccharides, carbohydrates are dehydrated by concentration H_2SO_4 to form furfural. Furfural reacts with anthrone (10-Keto-9, 10-dihydro anthracene) to form a blue-green coloured complex, which is measured calorimetrically at 630 nm.

Crude fiber: Crude fibers of the pigeon pea seeds were determined according to the standard method association of Official Agricultural Chemists[12].

Estimation of fat: The fat content was studied following the procedure of Drochioiu[13].

Estimation of protein: Estimation of protein content was done following Lowry *et al.*[14] using bovine serum albumin as standard. The optical density of each specimen was measured at 650 nm.

Isolation of volatile constituents for their toxicity against test fungi: The essential oil from leaf of 20 plants were extracted separately using Clevenger's apparatus following Kumar[15] at 90±2°C for 6-8 h. The isolated leaf essential oil was dried over anhydrous sodium sulphate and was stored at 4°C in clean glass vials. The toxicity of leaf oils was assessed following Kumar[16]. The fungi toxicity was measured following the method of Kumar[4,17] and recorded in terms of percent inhibition of mycelial growth.

Fungitoxic properties of neem giloy-*T. cordifolia* leaf oil: For MIC of most active giloy-*T. cordifolia* leaf essential oil was determined by following Kumar[4,17] method. Different concentration of the *T. cordifolia* leaf oil ranging from 200-600 ppm were prepared by dissolving requisite amount of oil in 0.5 mL acetone and then mixing with 9.5 mL czapeks dox agar medium separately. In control sets the petri plates having acetone and medium without oil were used. From periphery of 7 days old culture of each of test fungi, fungal discs (5 mm diam) were obtained and aseptically inoculated in each of control and the treatment sets. All these sets were incubated at 28±2°C for 6 days. In treatment/control sets diameters of fungal colony were measured in mutually perpendicular directions on the 7th day and the average was used to calculate the percent inhibition of mycelia growth of test fungi separately. The leaf oil treated discs of the fungi showing complete inhibition of their mycelia growth up to 7 days were washed with sterile water and placed again on fresh solidified medium to observe the revival of mycelia growth. The fungitoxic spectrum of the neem giloy *T. cordifolia* leaf oil was studied against various fungi isolated from pigeon pea seed samples. In addition effect of temperature, autoclaving and storage on the fungi toxicity of oil was determined following Kumar[4]. The each experiment was repeated thrice and contained 5 replicates.

Comparison of neem giloy *T. cordifolia* leaf oil efficacy with synthetic fumigant: This was determined following Kumar[4] with slight modification. For fumigant potential efficacy (giloy *T. cordifolia* leaf oil determination), requisite amount of *T. cordifolia* leaf oil was soaked in cotton swab (100 mg weight) and was introduced in separate polyethylene bags (17.0 cm diameter×20.4 cm height) which had 200 g of pigeon pea seed sample to attain 1 μL mL^{-1} (v/v) concentration. Synthetic fumigants viz., aluminium phosphide (sulphos) and ethylene dibromide (EDBA ampule) were also treated similarly and introduced at 500 ppm concentration on samples of 200 g pigeon pea seeds each.

This was determined following Kumar[4,17] with slight modification. To measure its protectant power of neem giloy *T. cordifolia* leaf oil as seed dressing the stock solution of 100 μL of *T. cordifolia* leaf oil was prepared by dissolving 100 μL of oil in 1 mL of acetone. About 200 g of pigeon pea seed were filled in plastic containers, treated with 1 mL stock solution of the leaf oil. Pigeon pea seeds were dressed by continuous shaking for 5 min for proper coating. Similarly, two contact fungicides such as copper oxychloride and carbendazim (1000 mg/200 g seeds) were taken to run parallel for comparison purpose.

The pigeon pea seeds in control sets were dressed with requisite amount of acetone in place of the *T. cordifolia* leaf oil and fungicide. The polyethylene containers were sealed to make it airtight and kept at room temperature at 75±2% humidity. The fungal species were recorded for presence/absence after 15-120 days separately.

Statistical analysis: Five parallel measurements were taken and Mean±SD was calculated. The observed data on antifungal activity against storage fungi, MIC, spectrum, effect of physical factors on the giloy *T. cordifolia* leaf essential oil were obtained by taking average of 5 replicates.

RESULTS AND DISCUSSION

A total of 16 fungal species viz., *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *A. phoenicis*, *A. tamari*, *A. terreus*, *A. sydowi*, *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *P. glabrum*, *Rhizopus nigricans*, *Trichoderma viride* and *Trichothecium roseum* were found to be present on all collected 20 samples of pigeon pea. In both methods blotter and agar plate on unsterilized seeds, sterilized seeds which *A. flavus* Link showed 19.1, 11.9, 18.3, 9.1 and *A. niger* van Tieghem 17.0, 10.9, 17.1, 9.7% frequency, respectively. Table 1 records that *Aspergillus flavus*, *A. niger*, *A. ochraceus* and *A. terreus* were the dominant species on the basis of percent frequency and *Aspergillus* genera dominated on seeds of pigeon pea.

Time to time various other workers reported fungal species on pigeon pea seeds viz., *Aspergillus flavus*, *A. niger*, *Penicillium* sp., *Cladosporium herbarum*, *Rhizopus stolonifer*, *Alternaria alternata*, *Macrophomina phaseolina* and *Fusarium udum*[18], *Xanthomonas campestris*, *Alternaria alternata*, *Alternaria tenuissima*, *Aspergillus flavus*, *Aspergillus niger*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Drechslera halodes*, *Fusarium moniliforme*, *F. oxysporum*, *Penicillium* sp., *Phoma betae*, *Rhizopus nigricans* and *Trichothecium roseum*[19], *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Macrophomina*, *Penicillium*, *Phoma*, *Rhizopus* and *Trichothecium*[20], *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger*, *Helminthosporium* sp., *Fusarium* sp. and *Cladosporium* sp.[21], 17 fungal species[22], *Aspergillus flavus*, *A. niger*, *A. nidulans*, *A. fumigatus*, *A. Oryzae*, *Penicillium citrinum*, *Fusarium oxysporum*, *Alternaria solani*, *A. alternata*, *Drechslera tetramera*, *Curvularia lunata*, *Rhizopus stolonifer*, *Cheatomium* sp., *Mucor* sp. and mycelia sterilia[23], *Alternaria alternata*, *Aspergillus niger*[24], *Aspergillus flavus*, *A. niger*, *Penicillium notatum* and *Cladosporium herbarum*[2], 16 fungi[25], *Alternaria alternata*, *Chaetomium* sp., *Penicillium citrinum*, *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Rhizopus nigricans*, *Fusarium oxysporum*, *F. moniliforme*, *F. solani*, *Curvularia lunata*, *Macrophomina* sp., *Monilia* sp., *Rhizoctonia* sp., *Trichoderma* sp.[3].

The variation in fungal species may be due to different climatic conditions, isolation periods and storage containers in which pigeon pea seeds were kept.

In present investigation untreated seeds were found to be associated with highest percent frequency of mycoflora. In case of untreated seeds the percent incidence of *Aspergillus flavus* was the highest followed by *A. niger*, *A. ochraceus* and *A. terreus.* Patil *et al*.[2] also found the percent incidence of *Aspergillus flavus* was the highest followed by *A. niger* using agar plate method. In a study Ghangaokar and Kshirsagar[3] reported that untreated seeds were found to be associated with highest number of seed borne fungi.

Table 1: Frequency (%) of different fungi on the stored seeds of pigeon pea

	Moist blotter method		On potato dextrose agar medium	
Fungi recorded	US	SS	US	SS
Alternaria alternata (Fr.) *Keissler*	1.0	1.1	3.1	-
Aspergillus candidus Pers ex.	1.1	-	2.2	-
Aspergillus flavus Link	19.1	11.9	18.3	9.1
Aspergillus niger van Tieghem	17.0	10.9	17.1	9.7
Aspergillus ochraceus Wilhelm	10.2	4.7	11.1	4.7
Aspergillus tamarii Kita	1.3	-	2.3	-
Aspergillus terreus Thom	11.1	1.3	9.9	6.1
Aspergillus sydowi (Bainier and Sartory) Thom and Church	2.7	1.3	5.1	2.1
Fusarium moniliforme Sheldon	2.0	1.3	3.0	-
Fusarium oxysporum von Schlechtendal	1.5	1.7	1.9	1.1
Fusarium solani (Mart.) Sacc.	3.2	2.3	1.1	0.7
Penicillium glabrum (Wehmer) Westling	3.0	-	0.5	-
Rhizopus nigricans Ehr.	1.7	-	-	-
Trichoderma viride Pers.ex.Fr.	2.3	-	0.3	-
Trichothecium roseum (Persoon) Link ex	1.7	-	0.7	-

Fungus not reported *Values are given as percent of mean of 20 samples studied. US: Unsterilized seeds, SS: Sterilized seeds

Table 2: Deteriorative efficacy of dominant fungi in pigeon pea seeds after 20 days storage

	Weight loss (g^{-1})		Germination (%)	
Fungal species	Control	Treatment	Control	Treatment
Aspergillus flavus	Nil	0.189±0.21	86.43±0.11	46.57±0.13
A. niger	-	0.177±0.12	87.43±0.11	50.30±0.12
A. ochraceus	-	0.131±0.13	85.00±0.12	78.33±0.11
A. terreus	-	0.050±0.14	90.00±0.15	79.24±0.12

Table 3: Nutritional composition of mature pigeon pea seeds treated with 4 dominant fungal species per 100 g

Nutritional composition	Control	*Aspergillus flavus*	*A. niger*	*A. ochraceus*	*A. terreus*
Carbohydrates	62.78 g±0.10	32.17 g±0.11	31.11 g±0.12	52.17 g±0.10	53.13 g±0.13
Dietary fiber	15.00 g±0.12	7.30 g±0.10	7.10 g±0.12	12.30 g±0.13	12.20 g±0.15
Fat	1.49 g±0.05	0.49 g±0.03	0.47 g±0.02	1.10 g±0.13	1.12 g±0.12
Protein	21.70 g±0.10	11.70 g±0.11	10.70 g±0.10	17.70 g±0.11	17.90 g±0.10

Table 4: Minimum inhibitory concentration of *T. cordifolia* leaf oil against test fungi

Dose of oil (ppm)	*Aspergillus flavus*	*A. niger*
200	45±0.11	45±0.12
300	75±0.23	85±0.27
400	100±0.20	100±0.23
500	100±0.12	100±0.13
600	100*±0.31	100*±0.21

*Fungicidal, Data are mean of five replicates, ±Standard error

As evident from Table 2, *Aspergillus flavus*, *A. niger* played important role in seed weight loss and seed germination. The *Aspergillus flavus* inoculated seeds showed 32.17 g±0.11 *A. niger* 31.11 g±0.12/100 g while *A. ochraceus* inoculated showed 52.17 g±0.10/100 g carbohydrate content, respectively. The *Aspergillus flavus* inoculated seeds showed 11.7 g±0.11/100 g, *A. niger* 10.7 g±0.10/100 g while *A. ochraceus* inoculated showed 17.7 g±0.11/100 g protein content, respectively (Table 3). On account of wide occurrence and their pathogenicity *Aspergillus flavus*, *A. niger* were selected as test organisms. Similarly Arya *et al*.[26] reported that *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium udum* and *Rhizopus nigricans*, *Xanthomonas campestris*, strongly inhibited the seed germination. Another study revealed that *Alternaria alternata*, *Alternaria tenuissima*, *Aspergillus flavus*, *Aspergillus niger*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Drechslera halodes*, *Fusarium moniliforme*, *F. oxysporum*, *Penicillium* sp., *Phoma betae*, *Rhizopus nigricans* and *Trichothecium roseum* were found dominant and adversely affected the seed germination and seedling vigour and caused seedling diseases[12].

Out of 20 leaf essential oils tested giloy-*T. cordifolia* leaf essential oil showed highest toxicity against all test fungi isolated from pigeon pea seeds and showed complete inhibition (100%) of the mycelial growth at 500 ppm.

The MIC of *T. cordifolia* leaf essential oil against dominant fungi *A. flavus* and *A. niger* was found to be 400 ppm (Table 4). It was found fungicidal at 600 ppm. It showed MIC against 10 fungi at 500 ppm while at 800 ppm it controlled 15 fungi (Table 5). There was no ill effect of temperature treatment up to 100°C for duration 60 min, autoclaving at 15 lb/square inch pressure at 120°C and storage up to 120 days (Table 6).

Tinospora cordifolia oil was more effective than commercial pesticides (copper oxychloride and carbendazim) during *in vivo* experiments both as seed dressing and fumigation studies (aluminium phosphide and ethylene dibromide). The seed fumigation method was more effective than seed dressing method, protected seeds of pigeon pea up to 120 days from fungal infestation thereby increasing its shelf life. The seeds of control sets showed proliferation of several fungal species after 15 days of storage. Seeds stored with *T. cordifolia* leaf essential oil as preservative showed better smell and taste in comparison to ones stored with pesticides. This study revealed that *T. cordifolia* leaf essential oil was more fungi toxicants than tested fungicides, thereby indicating the possibility of its exploitation as an ideal antifungal agent for protection of pigeon pea seeds during storage. Since the plant, *T. cordifolia* grow luxuriantly, its essential oil is an easily available, indigenous and renewable source of fungi toxicant with no known mammalian toxicity.

The leaf oil of *T. cordifolia* showed minimum inhibitory concentration-400 ppm against both *Aspergillus niger* and *A. flavus*. The previous literature revealed that there is a marked variation in the MIC of different plant oils against *Aspergillus niger* and *A. flavus* thus-*Ocimum adscendens* willd 200 ppm[27]. *Syzygium aromaticum* (L.) Merrill and Perry 200 ppm[28], *Cedrus deodara* (Roxb.ex Lambert) G.Don 1000 ppm and *Trachyspermum ammi* (L.) Sprague 500 ppm[29] *Adhatoda vasica* 500 ppm[30]. *Cuminum cyminum* 400 ppm[16]. The variation in the MIC of different plant oils may be due to the presence of different chemical constituents.

Table 5: Spectrum of leaf oil of *Tinospora* at different doses

Fungal species	Mycelial growth inhibition of fungi (%)			
	300 ppm	400 ppm	500 ppm	800 ppm
Alternaria alternata	43.0±0.10	82.1±0.21	85.0±0.20	100.0±0.21
Aspergillus candidus	58.7±0.12	87.1±0.21	83.0±0.12	100.0±0.10
A. flavus	53.3±0.13	100.0±0.12	100.0±0.13	100.0±0.10
A. niger	44.7±0.23	100.0±0.11	100.0±0.10	100.0±0.21
A. ochraceus	47.2±0.11	100.0±0.15	100.0±0.13	100.0±0.11
A. phoenicis	49.9±0.12	100.0±0.13	100.0±0.14	100.0±0.21
A. tamari	48.3±0.11	100.0±0.12	83.0±0.15	100.0±0.11
A. terreus	45.0±0.23	100.0±0.13	100.0±0.13	100.0±0.21
A. sydowi	47.7±0.12	100.0±0.13	100.0±0.12	100.0±0.11
Fusarium moniliforme	44.3±0.23	78.4±0.12	100.0±0.23	100.0±0.21
F. oxysporum	41.5±0.27	100.0±0.21	100.0±0.17	95.0±0.11
F. solani	58.9±0.32	100.0±0.12	100.0±0.31	100.0±0.11
P. glabrum	53.4±0.22	100.0±0.22	90.0±0.01	100.0±0.21
Rhizopus nigricans	54.3±0.12	83.2±0.12	94.4±0.01	100.0±0.11
Trichoderma viride	64.5±0.13	95.1±0.20	100.0±0.11	100.0±0.01
Trichothecium roseum	63.0±0.12	95.1±0.12	93.0±0.01	100.0±0.01

Data are mean of five replicates, ±Standard error

Table 6: Physical factors vis-a-vis fungi toxicity of *Tinospora* leaf oil

Physical factors	Inhibition of mycelia growth (%) at its MIC
Temperature (°C) (time of treatment-60 min)	
40	100±0.23
60	100±0.11
80	100±0.21
100	100±0.34
Autoclaving (15 lbs/sq inch pressure at 120°C)	
For 15 min	100±0.23
Storage in days	
15	100±0.11
30	100±0.13
45	100±0.23
60	100±0.13
75	100±0.14
90	100±0.11
105	100±0.13
120	100±0.17

Data are mean of five replicates, ±Standard error

Wellman[31] reported that a fungicide should be able to retain its activity during long period of its storage. The fungitoxic factor in the oil of *Adenocalyma allicea* was lost within 21 days of storage[32] while persisted for long period in the oil of *Ageratum conyzoides*[33], *Coriandrum sativum*[16], *Cuminum cyminum*[17]. The fungal toxicity was not ill affected by storage up to 120 days during present investigation. So this show that the *T. cordifolia* leaf essential oil can be safely stored at any ambient temperature for long periods without loss in toxicity.

CONCLUSION

The study revealed that *T. cordifolia* leaf essential oil was more fungi toxicants than tested synthetic pesticides, thereby indicating the possibility of its exploitation as an agent for protection of seed of pigeon pea during storage.

SIGNIFICANCE STATEMENTS

The present study discovers storage fungi of pigeon pea and the pathogenic nature of dominant fungal species. For the control of these fungi essential oils isolated from 20 plants were tested against dominant fungi. The effect of physical parameters were tested on its efficacy for most effective *Tinospora* oil. Storage efficacy were observed. In which *Tinospora* oil showed absolute toxicity against dominant fungi at 400 ppm. This plant is available everywhere so the farmer can use it for safe storage of pigeon pea seeds and can enhance shelf life. Thus, a new theory of Botanical control from this oil may be arrived for safe storage of pigeon pea seeds.

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