International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 08-12-2020; Accepted: 27-12-2020: Published: 07-01-2021
www.botanyjournals.com
Volume 6; Issue 1; 2021; Page No. 61-65

In vitro studies on phytotoxic effect of *Cassia tora* essential oil on germination and seedling growth of paddy and its insect repellent efficacy against *Sitophilus oyyzae*

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Abstract
The work was undertaken to investigate the Fungitoxic, Phytotoxic potential and Insect repellent activity of essential oil of *Casssia tora against* two test fungi *viz*., *Aspergillus flavus* and *Fusarium moniliforme* and test insect *Sitophilus oryzae.* The essential oil of *Cassia tora* remained fungitoxic against both the test fungi at 1.0 X 10^3 µL/L concentration. When paddy seeds were exposed to selected essential oil at the same concentration, no phytotoxicity was observed in seed germination and seedling growth under *in vitro* conditions. The selected essential oil also showed repellent activity against the test insect *Sitophilus oryzae*, which damages the stored grains and reduces the nutritional value.

Keywords: *cassia tora*, phytotoxic effect, insect repellent

Introduction

Rice is the staple food in India and is cultivated in almost all the states in our country. It contributes about 42 % of country's food grain production and about 70% of the population dependent for their livelihood (Prasad *et. al* 2012) [37]. However several types of pathogens are the causal factors for the reduction in the quality of rice. One of the major pathogens is fungi, which cause tremendous reduction in the quality of rice. These fungal pathogens produce symptoms such as seed abortion, seed rot, seed necrosis, reduction or elimination of seed germination resulting in the development of disease at later stage of plant growth (Khanzada *et al*. 2002) [42].

The most common fungal pathogens which attack rice seeds are the species of *Aspergillus* and *Fusarium.* These are of most concern as they produce mycotoxins, *i.e.* toxic secondary metabolites and occur in major food crops in the field and continue to contaminate during storage (Reedy *et al*., 2010b). Several strategies and methods including chemical, physical or biological treatments are being adapted to control the fungal attack, their growth and further mycotoxins production and food contamination; however such treatments require expensive sophisticated equipment and chemicals or reagents (Reddy *et al*., 2010a) [40]. Many Chemicals are being used for the last few decades to control the fungal growth (Daferera *et al*. 2003) [7]. However, these chemicals are not suitable as antifungal agents because of several disadvantages associated with them. Indiscriminate use of the chemicals may produce several side effects to consumers. The ill effects of these chemicals have always been overlooked and are being used without critical assessment of their carcinogenic, treatogenic and pollutive effects (Detroy *et al.* 1971) [9]. Due to this concern consumer now demands less use of chemical fungicides due to the non-biodegradability, pollutive nature and residual toxicities of chemicals (Basilico and Basilico 1999) [5]. In recent years, because of awareness of the toxicity to man and animals, the regulatory agencies have banned the use of Several such chemicals (Devi and Raha, 2013) [10]. The natural chemicals extracted from plants are now being used as an excellent alternative to synthetic chemical fungicides (Regnault-Roger & Philogene, 2008 [43]; Sithisut, Fields, & Chandrapathya, 2011) [45]; however the antimicrobial potential of these plant based natural chemicals was recognized since prehistoric times (Kishore 1983 [25]; Mohammed 1983) [32]. Plant natural chemicals have recently proven their usefulness in providing less phytotoxic, more systemic, non pollutive and easily biodegradable fungicides, in contrast to many synthetic fungicides that have several adverse effects (Hal & Harman, 1991) [14]. The alternative choice is therefore the use of plant based fungicides that are easily biodegradable and safe, with minimal environmental impact and danger to consumers (Fawecett & Spencer, 1970) [12]. Plants are the store house of biologically active compounds to combat various pathogens. Plant essential oils have been used for centuries as fumigants (Maia MF *et al.* 2011) [31]. Essential oils are volatile oil(s) that have strong aromatic components and that give distinctive odour, flavour or scent to a plant. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites (Koul *et al.* 2008) [28]. Essential oils have a long history in medical and dietary uses (Regnault *et al*. 2012) [43] and are "generally recognized as safe" (Akhtar *et al.* 2007) [2]. They may act as antifungal Fumigants (Park *et al*. 2003 [36], Rajendran *et al.* 2008) [39], contact insecticides (Zapata *et al*. 2010,) [50], antifeedants (Tripathi *et al.* 2009) [48] or repellents (Tapondjou *et al.* 2005). However, there are several reports indicating that many phytochemicals including essential oils produce phytotoxicity at high concentrations and show detrimental effects on plant growth and seed germination (Putnam and Tang, 1986) [38]. Therefore present investigation aimed to access the fungi-toxic efficacy against *Aspergillus flavus* and *Fusarium moniliforme*, phytotoxic effect on the seed germination and insect repellent efficacy of *Cassia tora* essential oil.

Material and Methods

Collection of plant material

Twelve plant species belonging to different families of angiosperms, collected from different parts of Gorakhpur and Maharajganj districts, were identified with the help of identification keys of floras (Hooker 1872–1897; Srivastava 1976) [16]. The identity of each collected plant was confirmed against their authentic herbarium specimens lodged in the herbarium of the Department of Botany, St. Andrew's College, Gorakhpur. The healthy, fresh plant parts free from any visible contamination were used for the extraction purpose.

Oil Extraction

The steam distillation was done using a Clevenger apparatus containing 5 L round bottom glass flask were added 500 g of fresh parts (leaves) and 2.5 L of distilled water (vegetal material/ extraction solvent rate = 1/5 (m/v). The mixture was left under reflux for 3 hours at 50°C. The volatile oil was collected and stored at 4° C for further analysis.

Test Fungi

The essential oil extracted from the plant materials was assayed for fungitoxic activity against the fungal strains *Aspergillus flavus* and *Fusarium moniliforme.* These fungi were grown on PDA plates at 27^0C ± 2°C and maintained with periodic sub – culturing at 4^0C. Both of these fungi are pathogens of paddy plants.

Screening of the essential oil against the test fungi

The fungitoxic efficacy of essential oil extracted from different parts of plant was determined against the test fungi by 'Poisoned Food Technique' (Arora and Dwivedi, 1979). 10 mg of streptomycin was added thoroughly to prevent any bacterial contamination. For treatment sets, 1 mL of the essential oil was mixed with 9 mL of molten PDA medium in a presterilized Petri plate and the contents were agitated in a circular mode in order to mix the essential oil homogeneously.In control sets, 1 mL of sterilized double distilled water was added in place of the essential oil. A fungal disc (5 mm in diameter) cut from the periphery of 7 days old culture of different test fungi with the help of flame sterilized cork borer, served as inoculums. The plates were incubated for 7 days at 27 ± 2°C. Colony diameters in mutual. Perpendicular directions were measured on the seventh day in assay plates. Fungitoxicity was recorded in terms of the percent inhibition of mycelial growth and calculated using the following formula (Vincent 1947) [49]:

$$\text{Percent Inhibition} = \frac{dc - dt}{dc} \text{ X } 100$$

Where,

Dc = average diameter of fungal colony in control sets

Dt = average diameter of fungal colony in treatment sets

The experiments were repeated twice and each set contained four replications.

Determination of Phytotoxicity

The phytotoxicity of selected essential oil of *Cassia tora* was studied with respect to seed germination and seedling growth of paddy.

Effect on Seed Germination

For treatment sets, surface sterilized seed of paddy were soaked in 1.0 X 10^3 µL/L of essential oil for 5 hours. Same amount of seeds were soaked in sterilized water for the same period for control sets. Treated as well as control seed were separately placed on three layers of moist filter paper for 6 days in petriplates, which were incubated at room temperature.

Observations were recorded in terms of the percent seed germination (Table 2). Each set contained three replicates.

Effect on Seedling Growth (shoot length and root length)

The germinated seeds of previous sets were allowed to grow upto eight days and the length of plumule of each germinated seed in control and treatment sets were recorded (Table 3 & 4).

Test Insect

Sitophilus oryzae adults free of exposure to insecticides and pathogens were reared and cultured in uncooked rice. The plastic containers used for rearing of *Sitophilus oryzae* were covered with muslin cloth and the culture was maintained at 27 ±2°C, 65-70% R.H. and light: dark cycle of 12 hours. For the bioassays, the F1 generation of the adults from the culture was used.

Table 1: Screening of essential oils against test fungi

				Aspergillus flavus			*Fusarium moniliforme*		
S.N.	**Essential oil tested**	**Parts used**	**Yield**	**1.0 X 10^3 µL/L**	**2.0 X 10^3 µL/L**	**3.0 X 10^3 µL/L**	**1.0 X 10^3 µL/L**	**2.0 X 10^3 µL/L**	**3.0 X 10^3 µL/L**
1	*Aegle marmelos*	L	0.2	18.5	35.7	75.2	21.6	49.3	70.2
2	*Ageratum conyzoides*	L	0.02	25.2	40.3	80.7	23.8	38.5	68.9
3	*Callistemon lanceolatus*	L	0.8	21.7	37.6	57.2	18.5	32.8	53.6
4	*Carum carvi*	L	0.6	15.9	32.9	45	22	35	56.2
5	*Cassia tora*	L	0.7	100	100	100	100	100	100
6	*Cyperus rotundus*	Rh	0.4	26.6	36.3	58.2	23.5	42.4	67.6
7	*Eucalyptus citriodora*	L	0.6	19.5	37.6	53.9	38.4	72.8	94.6
8	*Lantana indica*	L	0.5	38.9	64.4	93	21.6	37.2	57.2
9	*Murraya koenigii*	L	0.5	24.5	38.5	55.4	22.6	40.5	64.9
10	*Parthenium hysterophorus*	L	0.6	18.7	35.3	75.2	38.9	54.8	83.2
11	*Polyalthia longifolia*	L	0.08	26.2	40.7	61.8	18.6	32.5	53.3
12	*Xanthium strumarium*	L	0.09	41.8	70.2	100	18.5	32.2	53.8
L = Leaf Rh = Rhizome									

Table 2: Phytotoxic effect of *Cassia tora* essential oil on paddy seeds germination

Germination period (days)	Percent seed germination		
	C	T - 1	T - 2
2	65	52	50
4	83	78	75
6	96	94	90
8	100	100	96
Value of t		0.43*	0.73*

C= Control set T - 1 = 1.0 X 10^3 µL/L concentration T – 2 = 5.0 X 10^3 µL/L concentration * = Insignificant at 5% level

Table 3: Effect of *Cassia tora* essential oil on plumule length of paddy

Germination period (days)	Average length (mm)		
	C	T - 1	T - 2
4	28	27	26
5	36	35	32
6	43	42	40
7	50	50	48
Value of t		0.12*	0.47*

C= Control set, T - 1 = 1.0 X 10^3 µL/L concentration, T - 2 = 5.0 X 10^3 µL/L concentration * = Insignificant at 5% level

Table 4: Effect of *Cassia tora* essential oil on Radicle length of paddy

Germination period (days)	Average length (mm)		
	C	T - 1	T - 2
4	40	41	40
5	48	45	43
6	55	52	53
7	60	58	59
Value of t		0.35*	0.37*

C= Control set, T - 1 = 1.0 X 10^3 µL/L concentration, T - 2 = 5.0 X 10^3 µL/L concentration * = Insignificant at 5% level

Table 5: Response of test insect (*Sitophilus oryzae*) to *Cassia tora* essential oil at 1.0 X 10^3 µL/L

Number of insects in base arm (non-reactive)	Number of insects in base arm (repelled)	Number of insects in base arm (attracted)
3	17	5
3	21	1
3	22	0
5	17	3
4	18	3
2	18	5
4	21	0
6	19	0
3	22	0
5	19	1
Total = 38	194	18
Mean = 3.8	19.4	1.8

Value of Chi – square = 1.25* * = Insignificant at 5% level

Results

The essential oils isolated from different parts of 12 species of angiospermic plants screened against *Aspergillus fiavus* & *Fusarium moniliforme* at 1.0 X 10^3 µL/L, 2.0 X 10^3 µL/L, & 3.0 X 10^3 µL/L concentrations using poisoned food technique, only the essential oil of *Cassia tora* possessed absolute toxicity against *Aspergillus fiavus* & *Fusarium moniliforme*. The antifungal activity of essential oils is depicted in Table 1. The percent inhibitions exhibited by the different plant species varied considerably, ranging from 15.9 (*Carum carvi*) to 100% (*Cassia tora*).

The phytotoxicity of oil was measured in terms of percent seed germination and the seedling growth in terms of plumule & radical length of paddy. The result recorded in Table -2 showed that the germination of seeds of rice remained unaffected when seeds were soaked in the oil of *Cassia tora*. The seeds of paddy after germination were allowed to grow for eight days. The observations were recorded in Table-3 and Fig. - 4 revealed that in the treatment sets, there was no toxic effect on the growth of plumule & radical. During growth the plumule & radical length remained more or less same with the control sets. It is, therefore, apparent from above observation that the essential oil of *Cassia tora* did not have any phytotoxicity on paddy seeds.

The insect *Sitophilus oryzae* cause damage to stored grains of paddy and degrade the quality of grains by reducing their dry weight and nutritional value. The repellent activity of selected essential oil was tested against *Sitophilus oryzae*. Data depicted clearly showed that the essential oil of *Cassia tora* had the repelling activity for *Sitophilus oryzae* at concentration 1.0 X 10^3 µL/L. This repellency increases with the increase in concentration of the essential oil (Table – 5).

Discussion

Several angiospermic plant species defend themselves from pathogenic microorganisms by producing essential oils (Liu and Chu 2002) [30]. These oils are volatile compounds produced from various parts of the plant body. As natural substances, they represent a potential source of new antifungal agents. During the last few decades, extensive work has been carried out to prove the ability of essential oils as antifungal agents. Essential oils extracted from aromatic plants and spices are known to inhibit the mycelial growth of fungi. Several essential oils have been reported to have cidal effect on fungal pathogens which cause different postharvest diseases and animal and human disorders (Banihashemi and Abivardi 2011) [4]. Various essential oils could be useful alternative substances replacing synthetic chemical fungicides in the plant disease management (Gwinn *et al.* 2010 [13]; Nguefack *et al.* 2013) [35]. Aromatic plants such as members of the Asteraceae, Lamiaceae, Rutaceae, and Verbenaceae contain essential oils which have bioactivity against several fungi (Lahlou 2004 [39]; Edris 2007) [11]. The antifungal activity of essential oils against pathogenic fungi can be attributed to morphological changes in the cell wall and interference in enzymatic reactions of wall synthesis, which affect fungal growth and morphogenesis (Sharma and Tripathi 2008) [44]. Data of the present investigation clearly indicate that the essential oil of *Cassia tora* is found most effective against the mycelial growth of *Aspergillus flavus* & *Fusarium moniliforme* (Table – 1).

Many workers reported the potent inhibitory effect of essential oils on seed germination and seedling growth and causing morphological and physiological changes in the plant seedlings (Kordali *et al*., 2015 [27]; Bali *et al*., 2016) [3]. Experiments were conducted to study the effect of the selected essential oil on seed germination and seedling growth. The results shown in Table - 2, 3 & 4 revealed that exposed paddy seeds with the selected essential oil for different time periods had no inhibitory effect on seed germination and seedling growth, thus proving the non-phytotoxicity of the selected oil.

Essential oils were also reported to have repellent effect against insects. *Adhatoda vasica* (Acanthaceae) essential oil exhibited repellent activity against *Sitophilus oryzae* and *Brachyponera chinensis* (Kokate *et al*., 1985) [26]; oil extracted from *Ocimum suave* (Lamiaceae) and *Lippia* (Verbenaceae) repelled *Sitophilus zeamas* (Hassalani and Lwande, 1989 [15]; Mwangi *et al*., 1992) [33]. *Acorus calamus* (Araceae) essential oil showed repellent efficacy against *Tribolium castaneum* (Jilani *et al*., 1988) [21]. Essential oil extracted from the berries of *Jupinerus communis* (Cupressaceae) is a very good mosquito repellent (Kalemba *et al*., 1990) [22]. Some essential oils and their components exhibited both a repellent and a larvicidal action; volatile oils of *Ocimum* including camphor, cineole, methyl eugenol, limonene, myrcene and thymol had strong repelling activity against mosquitoes (Chokechaijaroenporn *et al*., 1994) [6]. In the present investigation, the selected oil was also tested for insect repellent efficacy. The data of table – 5 reveals that the essential oil of *Cassia tora* has repellent activity against *Sitophilus oryzae* at concentration 1.0 X 10^3 µL/L.

Conclusion

From the present study, it could be concluded that the essential oil extracted from *Cassia tora* exhibits fungitoxic efficacy against *Aspergillus fiavus and Fusarium moniliforme* and non-phytotoxic towards the test plant and does not suppress the germination and early seedling growth of the test plant. The essential oil extracted from *Cassia tora* has repellent activity against the test insect. Since essential oils contain natural ingredients hence are easily biodegradable, non pollutive, less phytotoxic and safe for human health and the environment. The findings of present investigation suggest that the essential oil of *Cassia tora* has potential to be developed into natural fungicide & repellent to control insects in stored products.

However, before making any suggestion regarding the replacement of synthetic chemical pesticide with *Cassia tora* oil on a commercial basis, continued research particularly on the mechanisms involved under *in vivo* conditions are required.

Acknowledgement

Authors are grateful to Rev. Prof. J. K. Lal, The Principal, St. Andrew's College, Gorakhpur (U.P) for providing infrastructure and all facilities.

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