Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
e-ISSN: 2395-3454

Vol. 16 | No. 2 | pp. 523-527 | 2017

Original Research Paper | Open Access

Modification of *Pongamia pinnata* (Linn.) Seed Chemicals and Their Fungicidal Activities

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 14-04-2016
Accepted: 24-05-2016

Key Words:
Pongamia pinnata
Seed chemicals
Saponified products
Fungicidal activity

ABSTRACT

Environmental concerns associated with the use of synthetic chemicals/petrochemical based pesticides have emphasized the importance of developing eco-friendly products with lower mammalian toxicity and a lower persistence in the environment. Seed chemicals of *Pongamia pinnata* were isolated and modified into saponified and amide products. Products derived from *P. pinnata* protein were completely soluble in water and their aqueous solutions are clear at room temperature, while saponified products showed turbidity at higher concentrations. The surface tension and viscosity of saponified products were found to be changed with dilutions, 0.03-0.05 N/m, 0.45-1.35 mpa.s at 0.1 to 5% concentrations, respectively, while surface tension and viscosity of diethanolamide product ranged from 0.036 to 0.020 N/m and 0.023 mpa.s to 1.02 mpa.s at 0.1 to 10% concentration, respectively. The properties of both products showed better surface tension lowering ability and wetting power in comparison to water. Significant (P=0.01) fungal growth inhibition was recorded at higher dilution against the tested fungus, *Flavodon flavus* and *Penicillium chrysogenum*. The diethanolamide product showed complete fungal growth inhibition at 5.0% dilution even after 7 days against *F. flavus* and *P. chrysogenum*. The present study concludes that surfactant formulation of non edible oil seeds contains fungicidal activities. The different dilutions exhibited pronounced toxicity against the target fungal species.

INTRODUCTION

Oils and fats, particularly plant based, are an important raw material for the chemical industries worldwide and can be used as pesticide due to the presence of lipid and lipid associates. They can be converted into potential pesticidal chemicals by some chemical modification utilizing the reaction sites (unsaturation, hydroxyl group) on fatty chain of the intact triglycerides or by addition of amine to protein that lead to the formation of substituted amide at the site of cleavage and free amino group.

There is a diversity of tree borne oil seeds, abundantly available in the forest and at present, hardly 7-10% of the total potential is being tapped. However, they are the potential source of non-edible oils but still underutilized due to the presence of biologically active metabolites as lipid associates in seeds.

As the demand of natural products is increasing day by day due to the increasing environmental problems, it is necessary to explore the possibilities of utilization of non-edible oils as potential pesticide or as adjuvant in pesticidal formulations against different pests viz., insects, fungi, nematodes and weeds.

Pongamia pinnata (Linn.) Pierre is a moderate sized, evergreen tree, belonging to the family Fabaceae. It is an extremely adaptable tree, hardy, resists drought, highly tolerant to salinity and a fast growing species, found in almost all parts of India. Large-scale plantation of this species has been undertaken for afforestation in dry wastelands.

The flowering season varies from April to September depending on the climatic conditions. Green pods mature after 10 to 11 weeks acquiring a tan colour. The harvesting period of pods is thus spread throughout the year, after maturity pods fall on the ground facilitated by strong breeze. Large-scale collection, however, is done by removing the bunch of pods manually. The ripe pods are flat and elliptic about 5-7 cm long enclosing one or two kidney shaped brownish red kernels and 1.5-2.5 cm wide, broad, pointed at both ends. The seeds are elliptical, reniform, reddish-brown 2 to 3 cm long (Allen & Allen 1981).

Its seed contains 25-50% oil, used for soap manufacturing and after sulphonating and sulphation used in the leather tanning industry. Root, bark, leaves, flower and seeds of this plant have medicinal properties and traditionally used as a medicinal plant. Presently, it is gaining importance due to the use of its oil for biodiesel preparation. The oil also has been known for its medicinal value for curing skin diseases such as leucoderma, psoriasis, scabies, herpes and other cutaneous diseases. It is internally administered in dyspepsia and sluggish liver (Arote & Yeole 2010).

The cake left after oil extraction has been used as a manure or combination with nitrogenous fertilizers. As the demand of bio-products are increasing day by day due to the harmful effects of deadly poisonous synthetic chemicals, screening and exploitation of non traditional oil seeds are the need of the hour.

Therefore, the present study was initiated to explore the possibilities of utilization of non-edible oil seed chemicals of *P. pinnata* and their potential as pesticide against fungal pathogen.

MATERIALS AND METHODS

Matured pods of *Pongamia pinnata* (Karanja) were collected in the month of May from Mandla Road, Jabalpur. Seed coat was removed by hammering or sticking and kernels were separated by winnowing. The seed kernels were weighed before and after removing the seed coat. The seeds were ground and used for chemical analysis.

Extraction of oil: 50 g of the seed kernel powder was weighed accurately, thimble was prepared and placed in the Soxhlet extraction apparatus. Sample was extracted with petroleum ether (60-80°C) for 6h. Petroleum ether was evaporated with the help of vacuum evaporator under reduced pressure at 40°C .

Modification of oil: 50 mL of karanja oil was taken in conical flask and added 150 mL of 90% ethanol, then 100 mL of 30% KOH. Reaction temperature was maintained 85°C. The conical flask was covered with watch glass and ice cubes were kept above the watch glass to avoid alcohol evaporation. After the completion of reaction, the solution was tested for its solubility.

Isolation of protein from oil cake: Oil seeds of *Pongamia pinnata* was defatted by hexane extraction and oilcake (OC) was collected from soxhlet thimble. Proteins were extracted with 0.5% aqueous NaOH solution (1:20 w/v cake: alkali) at 50°C for 3 hr. Alkali extract was filtered and the residue was discarded. Filtrate was brought to isoelectric point (pH 4.5-5.2) by addition of diluted HCl. Protein thus precipitated was filtered and dried.

Modification of karanja protein by diethenolamine: Diethanolamines (60 mL) was heated to 190°C for one hour in a double necked round bottom flask. Protein was added slowly to it and reaction temperature was maintained as 180° C. After completion of protein addition, refluxing was continued till aqueous solution of the product showed no precipitate at isoelectric point of protein. The product was cooled to room temperature and stored under anhydrous condition.

Physico-chemical properties of modified products:

***Solubility*:** Solubility was assessed in different solvents i.e., ethanol, methanol, diethyl ether, acetone, petroleum ether, water, sulphuric acid, hydrochloric acid, sodium chloride, butanol, 15% sodium carbonate, and 15% sodium bicarbonate. 100 mg material was dissolved in 5 mL solvents.

***Wetting power*:** The Draves-Clarkson method as reported in Bureau of Indian Standard. 1×1" strips were cut from cotton fibre cloths. Sinking times were determined for different concentrations of the product.

***Foaming power*:** A standard cylinder method was used and the foam volume was determined for different concentrations of sample as described by Bureau of Indian Standard.

***Emulsifying power*:** 40 mL of liquid paraffin and 40 mL of aqueous face containing different concentrations of test sample in a 500 mL stoppered conical flasks were shaken vigorously ten times, then the mixture was transferred to a measuring cylinder and the time taken for 10 mL of the aqueous phase to separate was noted.

***Surface tension*:** Surface tension of modified oil's different concentrations was determined with the help of stalegmometer.

***Viscosity*:** Viscosity of modified oil's different concentrations was determined with the help of a viscometer.

Screening for antifungal activity: The *in vitro* tests were carried out to measure the effects of the karanja seed chemicals by assessing its contact effects toward mycelial radial growth of the tested plant pathogenic fungi (*Flavodone flavus* and *Penicillium crysogenum*), by the product amended plates (Huang et al. 2005). Potato dextrose agar (PDA) medium was used in the study. Different dilutions (0.5%, 1.25%, 2.5% and 5.0%) were prepared utilizing saponified and diethanolamide products in sterile potato dextrose agar medium in Petri dishes. The solution in each Petri dish was gently swirled and allowed to solidify. The amended medium in the Petri dishes were inoculated at the centre with 5 mm inoculum-disc of each test fungus and incubated at 25°C for 7 days. Fungal growth in different treatments were recorded after 24 hr, 48 hr, 72 hr and 7 days.

The mean fungal radial growth of the pathogen was determined by measuring the diameter of the colony in two directions at right angles. For each concentration, four replicate plates were used. The mean growth values were obtained and converted into the inhibition percentage or mycelial growth in relation to the control treatment by using the formula,

$$\text{Percentage of inhibition} = (c\text{-}t) \times 100 / c$$

Where, c and t represent fungal growth diameter in control and chemical amended plates, respectively. The medium with inoculum disc, but without any chemical served as control.

Statistical analysis: Data obtained from experiments were

Table 1: Solubility of modified products of karanja seed chemicals in different solvents.

Sl. No.	Solvents	Solubility	
		Saponified product	Diethanolamide product
1	Ethanol	S	S
2	Methanol	S	S
3	Diethyl ether	MS	MS
4	Acetone	S	S
5	Pet.ether	S	S
6	Butanol	S	S
7	Water	S	S
8	H_2SO_4	S	S
9	HCl	S	S
10	$15Na_2CO_3$	MS	MS
11	$15NaHCO_3$	MS	MS

S-Soluble, MS-Mildly Soluble, NS-Not Soluble

statistically analysed by SPSS software (14.0) and accordingly CD values were determined (Chandel 1984).

RESULTS AND DISCUSSION

The seed chemicals - oil and cake protein were modified with saponification using ethanolic KOH and diethanolamide with diethanol amine at specific temperature and time conditions.

Solubility of modified products was assessed in different organic and inorganic solvents (Table 1). Product showed solubility in polar solvents and water while it was dispersible in salt solutions.

The results of different properties of modified products of karanja oil are presented in Table 2 and 3.

The surfactant properties i.e. surface tension, viscosity, foaming power, wetting power and emulsifying power of both the products were determined. The results of different properties of modified products of karanja seeds are presented in Tables 2 and 3.

The viscosity of saponified and amide product varied from 1.45 mpa.s to 7.58 mpa.s and 0.7 to 1.18 mpa.s at 0.1 to 10% concentration, respectively. Saponified product showed good emulsifying power (28.43 min) and foaming power (10.4 cm) at 10% concentration.

The surface tension of different dilutions of karanja saponified and amide products were found to be changed in different dilutions, 0.071N/m and 0.068 N/m at lowest dilution. Surface tension at higher concentration could not be measured with the help of Stalegmometer due to high turbidity. The surfactant properties of both products showed better surface tension lowering ability and wetting power in comparison to water.

Similar trend of surface tension lowering ability has been reported by Vedanayagam et al. (1984) in diethanol amides of other oils.

The antifungal activities in modified products of seed chemicals of *P. pinnata* were assessed against plant pathogenic fungi i.e. *Flavodon flavus* and *Penicillium chrysogenum* under laboratory conditions.

Significant (P=0.01) fungal growth inhibition was recorded at higher dilution against the tested fungus, *Flavodon flavus* (Tables 4 and 5). The complete fungal inhibition of *F. flavus* was recorded at 2.5% and higher concentrations while 24.37 and 52.31% inhibition at 0.5% and 1.25%, respectively after 7 days. Similarly, against *P. chrysogenum*, 35.62, 55.57, 100 and 100% fungal inhibition was recorded at 0.5,1, 2.5 and 5% dilutions, respectively.

The diethanolamide product showed complete fungal growth inhibition at 5.0% dilution after 7 days against *F. flavus* and *P. chrysogenum* (Tables 6 and 7).

The study clearly demonstrated the suppressive effect of karanja products on fungal growth of selected species, this might be due to the fatty acid with karanjin, further saponification enhance the bioactivity of fatty oil. Isman & Akhtar (2007) and Collavino (2006) have also reported that pesticidal properties of fatty acids can be maximized through saponification and esterification.

The biodegradable and antibacterial properties of diehanolamides from proteins are reported by Tamer et al. (1967) and Chakrabarty et al. (1957).

The findings are in accordance with the studies reported by Wagh et al. (2007). They evaluated antifungal and antibacterial activity of different concentration of oil obtained from *P. pinnata* against *Aspergillus niger, A. fumigatus, Staphylococcus aureus* and *Pseudomonas aeruginosa,* employing Minimum Inhibitory Concentration (MIC) determination and dry-weight method and suggested the use of fatty oil of this plant for developing plant derived antimicrobial drugs. Similarly, kananja oil showed antibacterial activity against both Gram-positive and Gram-negative organisms (Bhat et al. 1962, Patel & Trivedi 1962). However, soap prepared from the oil has been found to be devoid of any antibacterial activity. Basawa et al. (2001) reported that antimicrobial property of karanja oil is probably due to inhibition of cell membrane synthesis. Same results were obtained by Vohra Shailja (2001), who reported that the karanja extracts exhibited outstanding antifungal activity against the soil-borne phytophagous fungus *Sclerotium rolfsii* (Sacc.) and found that karanja oil with karanjin was more active than karanja oil without karanjin. Karanjin, however, exhibited moderate antifungal activity.

Table 2: Surfactant properties of *P. pinnata* oil saponified product.

Concentration %	Viscosity (mpa.s)	Surface Tension (N/m)	Emulsifying Power (min)	Wetting power (min)	Foaming Power (cm)
0.1	1.45±0.02	0.071±0.12	1.3±0.10	1.26±0.01	1.4±0.1
0.25	1.51±0.01	0.048±0.03	1.5±0.05	1.12±0.02	1.7±0.02
0.5	1.56±0.01	0.039±0.14	4.12±0.12	0.87±0.12	2.7±0.03
1	1.57±0.02	0.034±0.05	8.41±0.05	0.78±0.01	3.4±0.05
2.5	1.79±0.0	0.033±0.10	9.13±0.03	0.55±0.05	4.6±0.0
5	2.35±0.0	0.032±0.06	11.8±0.11	0.45±0.02	7.2±0.1
7.5	2.58±0.02	*	20.0±0.05	0..35±0.03	9.2±0.1
10	7.58±0	*	28.43±0.1	0.25±0.02	10.4±0.1

* Surface tension could not be measured due to high viscosity. Values are the mean of three determinations ± standard deviation

Table 3: Surfactant properties of *P. pinnata* amide product.

Concentration %	Viscosity (mpa.s)	Surface Tension (N/m)	Emulsifying Power (min)	Wetting power (min)	Foaming Power (mL)
0.1	0.70±0.01	0.068	6.15±0.15	6.10±0.04	5.1±0.05
0.25	0.72±0.01	0.066	6.45±0.15	4.3±0.05	5.3±0.0.5
0.5	0.76±0.01	0.063	6.55±0.15	3.25±0.36	5.4±0.2
1	0.78±0.02	0.062	7.3±0.05	3.2±0.05	5.9±0.2
2.5	0.86±0.01	0.056	7.5±0.05	3.0±0.05	6.0±0.05
5	1.08±0.02	0.046	8.15±0.15	1.4±0.02	6.4±0.1
7.5	1.12±0.02	*	8.35±0.05	0.5±0.05	6.8±0.1
10	1.18±0	*	8.05±0.10	0.10±0.04	7.2±0.2

* Surface tension could not be measured due to high turbidity. Values are the mean of three determinations ± standard deviation.

Table 4: Antifungal activities of *P. pinnata* saponified seed product against *Flavodon flavus.*

Dilution	% Fungal inhibition over control			
	24 hr	48hr	72hr	7th day
0.5%	100 (90.04)	25.32 (30.12)	19.12 (25.71)	24.37(31.29)
1.25%	100 (90.04)	69.60 (56.52)	71.88 (58.01)	52.21 (48.65)
2.5%	100 (90.04)	100 (90.04)	100 (90.04)	100 (90.04)
5.0%	100 (90.04)	100 (90.04)	100 (90.04)	100 (90.04)
C.D. (P = 0.01)	N.S.	13.35	3.5	4.77

Values are the mean of 4 replications. Figures in parentheses are arcsine transformed values.
N.S. = Non significant

Table 5: Antifungal activities of *P. pinnata* saponified seed product against *Penicillium chrysogenum.*

Dilution	% Fungal inhibition over control			
	24 hr	48hr	72hr	7th day
0.5%	100 (90.04)	53.29 (50.36)	20.09 (26.15)	35.62(36.41)
1.25%	100 (90.04)	43.38 (39.36)	29.19 (32.26)	55.57 (48.41)
2.5%	100 (90.04)	100 (90.04)	100 (90.04)	100 (90.04)
5.0%	100 (90.04)	100 (90.04)	100 (90.04)	100 (90.04)
C.D. (p= 0.01)	N.S.	20.40	9.054	7.936

Values are the mean of 4 replication. Figure in parentheses are arcsine transformed values.
N.S. = Non significant

Table 6: Antifungal activities of *P. pinnata* diethanolamide product against *Flavodon flavus.*

Dilution	% Fungal inhibition over control			
	24 hr	48hr	72hr	7th day
0.5%	7.14 (11.10)	20.49 (26.88)	9.96 (18.59)	19.87 (24.45)
1.25%	22.62 (28.41)	31.48 (34.11)	36.61 (37.19)	16.94 (24.08)
2.5%	100 (90.04)	100 (90.04)	100 (90.04)	70.22 (62.05)
5.0%	100 (90.04)	100 (90.04)	100 (90.04)	100 (90.04)
C.D. (p = 0.01)	N.S.	3.48	8.51	2.12

Values are the mean of 4 replication. Figures in parentheses are arcsine transformed values.
N.S. = Non significant

Table 7: Antifungal activities of *P. pinnata* diethanolamide product against *Penicilium cryspgenum.*

Dilution	% Fungal inhibition over control			
	24 hr	48hr	72hr	7th day
0.5%	11.80 (17.39)	28.92 (31.63)	52.81 (46.64)	59.41(50.62)
1.25%	24.53 (29.48)	34.11 (35.37)	60.76 (55.27)	38.84 (38.56)
2.5%	100 (90.04)	100 (90.04)	100 (90.04)	100 (90.04)
5.0%	100 (90.04)	100 (90.04)	100 (90.04)	100 (90.04)
C.D. (p = 0.01)	10.44	2.32	4.32	12.74

Values are the mean of 4 replication. Figures in parentheses are arcsine transformed values.

CONCLUSION

Day by day conventionally applied synthetic chemicals become ineffective due to the generation of gradual resistance in pests and mammalian toxicity. Thus, this study suggests that the karanja (*Pongamia pinnata*) seeds can be used as a potential source of oil and protein which can be used for the development of bioactive chemicals by some chemical modifications. The study clearly indicates that the *P. pinnata* seeds chemical products possess potential antifungal activities under laboratory conditions. However, further field studies are needed. These chemicals are biodegradable, environment friendly, and available in sufficient quantities as cheap raw material and can be effectively used for the development of eco-friendly lead molecules in pesticidal formulations for the management of fungal pathogens of agriculture as well as forests.

ACKNOWLEDGEMENT

Authors are thankful to the Director for providing necessary facilities to conduct the study.

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