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In vitro comparative efficacy of various concentrations of methanolic leaf and seed extract of *Pongamia pinnata* against *Polyphagotarsonemus latus* (Banks) in chilli

Monika Jangra, Rachna Gulati, Sonika and VK Batra

Abstract
In present investigation, the bioefficacy of methanolic leaf and seed extracts of Karanj (*Pongamia pinnata*) were tested to evaluate their toxic effects at different concentration (10.0, 7.5, 5.0, 2.5, 1.25, 0.75 and 0.37%) against mixed population of *Polyphagotarsonemus latus* on chilli during 2016 under *in vitro* conditions. *P. pinnata* responded to the mite in a concentration dependent manner i.e. lowest number of live mites and highest mortality in population was obtained with highest concentration tested (10.0%). Out of an initial number 10 mite, significantly less number of mites were recorded at 10.00 percent concentration of *P. pinnata* seed extract followed by 7.50, 5.00, 2.50 and 1.25 per cent concentration than 0.75, 0.37 percent treatment and control (CD=0.10; p=0.05). The mean number of live mites were (3.61 to 6.61 mites) out of the initial 10 mites in different treatments of *P. pinnata* leaf extract were significantly lower (CD=0.11; p=0.05) than the water alone (9.89 Mites) treatments. The number of mites recorded in treatments 2.50 and 1.25 percent concentrations were at par with each other. The methanolic seed and leaf extract of *P. pinnata* caused 67.27 and 63.89 percent mortality of *P. latus* at 10.00 percent concentration.

Keywords: *Pongamia pinnata, Polyphagotarsonemus latus, pest management*

1. Introduction

Chilli (*Capsicum annuum* L.) belongs to family Solanaceae [1]. Chillies constitute about 20 per cent of Indian spice exports in quantity and about 14 per cent in value [2]. It is grown in almost all the states throughout the country. Chilli is an important source of vitamin A, B, C, E, oleoresin, red pigment and minerals which helps in digestion [3]. Capsaicin, (8-methyl-N-vanillyl-6-enamide) an alkaloid or a lipophilic chemical; responsible for pungency that can produce a strong burning sensation in the mouth and has medicinal properties and prevents heart attack by dilating the blood vessels [4]. Although, the crop has got great export potential besides huge domestic requirement, a number of limiting factors may be attributed to low chilli productivity, of which ravages caused by insect, mite pests and occurrence of viral diseases are significant ones [5]. Chilli cultivation is attack by a multitude of pests during crop is of the most concern. The incidence of pest on chilli crop is complex with more than 293 insects and mite species [6]. Amongst these, thrips *Scirtothrips dorsalis* Hood, green peach aphid *Myzys persicae* Sulzer, *Aphis gossypi* Glover, whiteflies *Bemisia tabaci*, yellow/broad mite *Polyphagotarsonemus latus* (Banks) and fruit borer *Helicoverpa armigera* Hubner are the most vital production constraints [7].

Among these pests, the yellow mite, *Polyphagotarsonemus latus* (Banks) is a serious one causing severe damage to reproductive buds resulting in substantial yield loss. The nymphs and adults actively feed on the tender leaves causing elongation of petiole of older leaves, downward curling of leaves in an inverted boat shaped manner which results in narrowing of leaves and reduced size [8]. To control this pest, the farmers used chemical insecticides that lead to many undesirable problems like pest resurgence, environmental pollution, adverse effects on non-target organisms etc. The presence of pesticide residues seriously affected the export of chilli. Hence it is imperative to produce pesticide free chilli by adopting eco-friendly management practices for the management of mite pest. The plant products may also serve as a protective function against insect pests of stored products and field vegetable crops. Plant-derived extracts, powders [9] and essential oils may be options for mite control [10].

Plant-derived alkalis, alcohols, aldehydes, terpenoids and some mono-terpenoids show fumigant properties [11]. *Withania somnifera* and *Pongamia pinnata* are the other botanicals which showed acaricidal activity against phytophagous mite, *Tetranychus Urticae* [12]. Keeping these views in mind, the present study was made to study the effect of methanolic extracts of leaves and seeds of karanj (*Pongamia pinnata*) for management of this pest of chilli under field condition.

2. Materials and Methods

Under *in vitro* conditions, bio-efficacy of methanolic leaf and seed extracts were evaluated against *P. latus* to determine their acaricidal activity against *P. latus* under standardized conditions (30±1^{0}C, 80-85% RH) in the Acarology Lab, Department of Zoology, CCS HAU during, 2016. Infested leaves were plucked from the field crop and brought to the laboratory. Under stereo zoom microscope, mobile stages of mite were picked with the help of bird's feather pick and released on the separate untreated leaf. Each subset was replicated three times containing 10 mites in each replicate.

2.1 Preparation of extracts of *Pongamia pinnata*

Karanj leaves and seed from University Campus were collected. Leaves (200 g) and seed (500 g) of karanj were properly cleaned and shade dried. Methanolic extract of both seeds and leaves were prepared following the standard procedure of refluxing and distillation [13]. After getting a stock concentration of 100% further dilutions were made using distilled water to obtain different concentrations *viz.*, 10.0, 7.5, 5.0, 2.5, 1.25, 0.75 and 0.37% of methanolic extracts. Suitable control (without extract) was maintained for all the experiments as a standard check.

2.2 The efficacy of *Pongamia pinnata* extract against *P. latus*

To evaluate the efficacy of methanolic leaf and seed extract of *P. pinnata* against *P. latus*. The chilli leaf containing ten mites as described above was sprayed with *P. pinnata* leaf and seed extract @ 10.0% concentration with the help of hand automizer. The leaf was air dried and then placed on moist cotton bed of the covered petri plate. Likewise, other concentrations were sprayed separately on leaf, each containing 10 mites. Simultaneous control of alone water sprayed leaves in case of methanolic extracts was maintained. Each treatment was replicated three times.

Observation on the live mite stages was recorded after every 24 h under Stereo Zoom Binocular Microscope in each treatment. These were compared with control data. Before considering the mite stage as dead, it was probed lightly with the help of bird's feather pick to detect any movement. Observations were continued after every 24 h till the appearance of the next stage or mortality of the mite stage. At the end of experiments, percent reduction was calculated for each concentration. The percent reduction in mite count as compared to pre- treatment count was calculated with the help of following formula:

$$\text{Percent Reduction (\%)} = \frac{\text{(Pre- treatment count - Average number of live mites after treatment)}}{\text{Pre- treatment count}} \times 100$$

2.3 Statistical Analysis

For assessing the effectiveness of the treatments, mean numbers of *P. latus* were pooled and analyzed statistically. Critical difference (CD) was calculated between the treatments to see the impact of population buildup of *P. latus* on chilli by single and factorial CRD (*in vitro*) method. Data for evaluating the effect of botanicals against *P. latus* under *in vitro* conditions was subjected to two factorial CRD. Data transformation was applied wherever necessary. CD was calculated in each case and means of treatments were compared to see the significant difference between the treatments and with control at different observation periods. CD was also used to find out the most effective extract and its concentration.

3. Results

3.1 *Pongamia pinnata* leaf extract

Experiments were conducted to test the bioefficacy of methanolic leaf and seed extract of Karanj (*Pongamia pinnata*) against *P. latus* under *in vitro* condition. Bioassay results clearly revealed that both the extracts possessed acaricidal activity. In the experiment, water spray served as control. *P. pinnata* extracts exhibited toxicity to the various developmental stages of *P. latus* (Table 1-2). *P. pinnata* extracts responded to the mite in a concentration dependent manner i.e. lowest number of live mites and highest reduction in population was obtained with highest concentration tested (10.0%) followed by 7.5, 5.0, 2.5, 1.25, 0.75, 0.37 percent, and water alone treatment. The latter three treatments i.e. 0.75, 0.37 percent and water alone were statistically comparable for recording the mean number of mites after the treatment. This suggested that low doses of extract (0.75 and 0.37%) did not cause significant mortality in mites.

Table 1: *In vitro* bioassay (direct spray) of *Pongamia pinnata* leaf extracts against *Polyphagotarsonemus latus*

ConCen Traction (%)	Pre-treatment count	Number of live mites/leaf disc									Mean
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	(T)
10.00	10	8.50 (3.08)	7.00 (2.82)	6.00 (2.64)	5.00 (2.44)	4.00 (2.23)	1.50 (1.57)	0.50 (1.21)	0.00 (1.00)	0.00 (1.00)	3.61 (2.00)
7.50	10	9.00 (3.16)	8.50 (3.08)	7.00 (2.82)	6.50 (2.74)	5.00 (2.45)	3.00 (1.98)	1.50 (1.57)	0.50 (1.21)	0.00 (1.00)	4.56 (2.22)^a
5.00	10	9.50 (3.24)	8.50 (3.08)	7.00 (2.83)	6.50 (2.74)	6.00 (2.65)	4.00 (2.23)	2.50 (1.87)	0.50 (1.21)	0.00 (1.00)	4.94 (2.31)^a
2.50	10	10.00 (3.32)	9.50 (3.24)	8.00 (3.00)	8.00 (3.00)	7.00 (2.83)	6.00 (2.64)	4.50 (2.34)	2.00 (1.71)	0.50 (1.21)	6.17 (2.59)^b
1.25	10	10.00	10.00	9.00	8.50	7.50	6.50	4.00	2.50	1.50	6.61

		(3.32)	(3.32)	(3.16)	(3.08)	(2.91)	(2.73)	(2.23)	(1.87)	(1.57)	(2.69)[b]
0.75	10	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	9.50 (3.24)	9.00 (3.16)	8.50 (3.08)	8.50 (3.08)	9.50 (3.24)[c]
0.37	10	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	9.00 (3.32)	9.00 (3.16)	9.78 (3.28)[c]
0.00 (water alone)	10	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.16)	9.00 (3.16)	9.89 (3.30)[c]
Mean (OP)		9.63 (3.26)[b]	9.19 (3.19)[b]	8.38 (3.05)	8.06 (2.99)[a]	7.44 (2.88)[a]	6.31 (2.63)	5.25 (2.38)	4.13 (2.07)	3.56 (1.90)	

Figures in parentheses are √n +1 transformation
C.D. for Treatment (T) =(0.11), SE(m) =(0.04); C.D. for Observation Period (OP) =(0.11), SE(m) =(0.04)
C.D. for Interaction OP × T=(0.32), SE(m) =(0.11); Values with the same superscript do not differ significantly

All the other treatments were significantly better than these three treatments. Although the number of mites recorded (9.50, 9.78 and 9.89 mites) in 0.75, 0.37 percent and control treatments, respectively, was less than the initial number (Table 1). In direct spray bioassay, number of live mites was statistically comparable at 10.0 and 7.5 percent *P. pinnata* leaf extract concentration. The lowest two concentrations i.e. 0.37 and 0.75 percent also did not show any significant difference between them in all the developmental stages of *P. latus*. The mean number of live mites were 3.61 to 6.61 mites out of the initial 10 mites in different treatments which were significantly lower (CD=0.11; p=0.05) than the water alone (9.89 Mites) treatments. Mean number of mites recorded after treatments in 2.50 and 1.25 percent concentration are at par with each other (Table 1).

3.2 *Pongamia pinnata* seed extract

The data presented in Table 2 pertained to mixed populations of *P. latus* on *P. pinnata* seed extract treated chilli leaves with different investigating times. Mites responded to *P. pinnata* methanolic seed extract in a concentration dependent manner i.e. lowest number of live mites (3.27 Mites) was obtained with highest concentration of extract tested (10.00%) and highest number (9.18 Mites) with lowest concentration (0.37%) (Table 2). Out of an initial number 10 mite, significantly less number of mites were recorded at 10.00 percent concentration followed by 7.50, 5.00, 2.50 and 1.25 per cent concentration than 0.75, 0.37 percent treatment and control (CD=0.10; p=0.05). The lowest two concentrations i.e. 0.37 and 0.75 percent did not show any significant difference between them.

Table 2: *In vitro* bioassay (direct spray) of *Pongamia pinnata* seed extracts against *Polyphagotarsonemus latus*

Concentration (%)	Pre-treatment count	Number of live mites/leaf disc											Mean (T)
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	
10.00	10	9.00 (3.16)	8.50 (3.08)	7.00 (2.82)	5.00 (2.41)	3.50 (2.09)	2.00 (1.71)	1.00 (1.37)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	3.27 (1.88)
7.50	10	9.50 (3.24)	9.00 (3.16)	8.00 (3.00)	7.00 (2.82)	5.50 (2.53)	3.50 (2.09)	1.50 (1.57)	1.00 (1.41)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	4.09 (2.08)
5.00	10	10.00 (3.32)	9.50 (3.24)	8.50 (3.08)	8.00 (3.00)	7.00 (2.82)	6.00 (2.64)	5.50 (2.55)	2.50 (1.87)	1.50 (1.57)	0.50 (1.21)	0.00 (1.00)	5.36 (2.39)
2.50	10	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	8.50 (3.08)	8.00 (3.00)	7.00 (2.83)	7.00 (2.83)	5.50 (2.55)	3.50 (2.12)	2.00 (1.73)	0.00 (1.00)	6.50 (2.64)
1.25	10	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	9.50 (3.24)	9.00 (3.16)	8.50 (3.08)	8.00 (3.00)	7.50 (2.91)	7.00 (2.83)	6.00 (2.65)	3.50 (2.12)	8.09 (2.99)
0.75	10	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	9.50 (3.24)	9.00 (3.16)	9.00 (3.16)	9.00 (3.16)	8.50 (3.08)	7.50 (2.91)	7.50 (2.91)	7.50 (2.91)	8.86 (3.14)[a]
0.37	10	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	9.50 (3.24)	9.50 (3.24)	9.00 (3.16)	9.00 (3.16)	8.50 (3.08)	8.00 (3.00)	7.50 (2.91)	9.18 (3.19)[a,b]
0.00 (water alone)	10	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	9.50 (3.24)	9.50 (3.24)	9.00 (3.16)	9.00 (3.16)	9.00 (3.16)	9.64 (3.26)[b]
Mean (OP)		9.81 (3.29)[b]	9.63 (3.26)[b]	9.19 (3.19)[b]	8.44 (3.05)	7.69 (2.92)	6.94 (2.76)[a]	6.31 (2.61)[a]	5.44 (2.40)	4.63 (2.21)	4.13 (2.08)	3.44 (1.89)	

Figures in parentheses are √n +1 transformation
C.D. for Treatment (T) =(0.10), SE(m) =(0.03); C.D. for Observation Period (OP) =(0.11), SE(m) =(0.04)
C.D. for Interaction OP × T=(0.32), SE(m) =(0.11); Values with the same superscript do not differ significantly

3.3 Comparative evaluation of *Pongamia pinnata* seed and leaf extract against *P. latus*

The data pertaining to three factorial experiment (treatments × concentration × observation period) is presented in Table 3. Statistical analysis depicted a significant effect of treatments on population buildup of *P. latus* on chilli (CD= 0.30; p=0.05). Results revealed that maximum number of live mites counted in control (9.64 mites), followed by 5.72, 4.78 and 3.81 mites on chilli leaves after spray with 5.0, 7.5 and 10.0 percent concentration of *P. pinnata* extract with 10 mite as the initial inoculum. These differed significantly with each other. When the results on population build up of *P. latus* over observations were compared, a significant effect of observation period was recorded (CD= 0.64; p=0.05) (Table 3). Irrespective of the treatment or concentration, the mite number was found to significantly decrease with each observation period.

Table 3: Comparative evaluation of *Pongamia pinnata* seed and leaf extract against *Polyphagotarsonemus latus* in chilli

Observation Period (C)	*P. pinnata* Leaf extract (A)					*P. pinnata* Seed extract (A)					Mean C x B				Pooled mean (C)
	Cencentration (%) (B)				Mean A×C	Cencentration (%) (B)				Mean A×C					
	Control	10.0	7.5	5.0		Control	10.0	7.5	5.0		Control	10.0	7.5	5.0	
1st day	10.0	8.50	9.00	9.50	9.25	10.0	9.00	9.50	10.0	9.63	10.00	8.75	9.25	9.75	9.44
2nd day	10.0	7.00	8.50	8.50	8.50	10.0	8.50	9.00	9.50	9.25	10.00	7.75	8.75	9.00	8.88
3rd day	10.0	6.00	7.00	7.00	7.50	10.0	7.00	8.00	8.50	8.38	10.00	6.50	7.50	7.75	7.94
4th day	10.0	5.00	6.50	6.50	7.00	10.0	5.00	7.00	8.00	7.50	10.00	5.00	6.75	7.25	7.25
5th day	10.0	4.00	5.00	6.00	6.25	9.50	3.50	5.50	7.00	6.38	9.75	3.75	5.25	6.50	6.31
6th day	10.0	1.50	3.00	4.00	4.63	9.50	2.00	3.50	6.00	5.25	9.75	1.75	3.25	5.00	4.94
7th day	10.0	0.50	1.50	2.50	3.63	9.00	1.00	1.50	5.50	4.25	9.50	0.75	1.50	4.00	3.94
8th day	9.00	0.00	0.50	0.50	2.50^a	9.00	0.00	1.00	2.50	3.13	9.00	0.00	0.75	1.50	2.81^a
9th day	9.00	0.00	0.00	0.00	2.25^a	8.50	0.00	0.00	1.50	2.50	8.75	0.00	0.00	0.75	2.38^a
Mean A x B	9.78	3.61	4.56	4.94		9.50	4.00	5.00	6.50						
Pooled Mean (A)					5.72					6.25					
Pooled Mean (B)											9.64	3.81	4.78	5.72	

CD (p=0.05) for Treatment (A) = 0.30; CD (p=0.05) for Observation Period (C) = 0.64; CD (p=0.05) for concentration (B) = 0.43; CD (p=0.05) for A×C= N/A; CD (p=0.05) for A×B= 0.60; CD (p=0.05) for B×C= 1.28; CD (p=0.05) for A×B×C= N/A; Values with the same superscript in the column wise/ row wise do not differ significantly

The mite count was 9.44, 8.88, 7.94, 7.25, 6.31, 4.94, 3.94, 2.81 and 2.38 after first to ninth days of post treatment which differed significantly with each other except last two values which are at par with each other. Three concentrations of each extract treatment were compared which showed that higher concentration (10.0%) was more potent in causing mortality than lower concentration. Observations on the population buildup of *P. latus* on chilli leaves revealed a non significant interaction between treatment and observation period. A significant interaction between the treatment and the concentration was obtained (CD= 0.60; p= 0.05) (Table 3). The interaction between concentration and observation period was also found to be significant (CD= 1.28; p= 0.05) (Table 3). A non significant interaction was observed between the treatments vs. concentration vs. observation period.

In direct spray method, among the seed and leaf extract maximum mortality was recorded (Fig. 1). Out of an initial number of 10 mites, 67.27 and 63.89 percent mortality was recorded at 10.00 percent *P. pinnata* seed and leaf extract concentration. *P. pinnata* seed extract treatment results revealed lower number of mites from 4.09 to 9.64 mites at 7.5 to 0.37 percent concentrations and caused 3.64 to 59.09 percent reduction in number. Similarly, higher range of reduction of 33.89 to 63.89 percent was noticed at 1.25 to 10 percent *P. pinnata* oil concentration which was better than control.

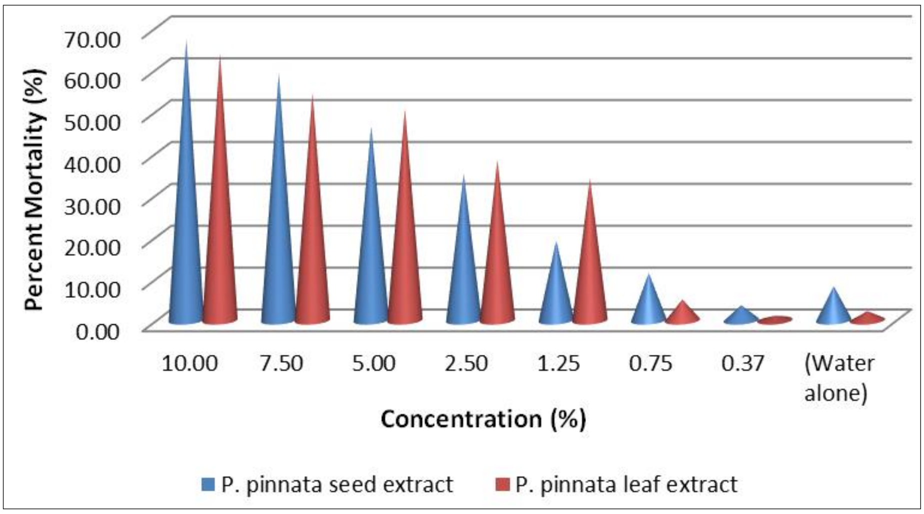

Fig 1: Percent mortality of *Polyphagotarsonemus latus* after spray of various concentration of seed and leaf extract *Pongamia pinnata*

4. Discussion

Pongamia pinnata is regarded as medicinally plant as well as earth-friendly herbal pesticide. In present investigation, the bioefficacy of methanolic leaf and seed extracts of *P. pinnata* responded to the *P. latus* in a concentration dependent manner i.e. lowest number of live mites and highest mortality in population was obtained with highest concentration (10.0%). Kumar *et al*. [14] reported that methanolic extract of neem and karanj at 1% concentration was effective in control of *Tetranychus* sp. upto 78.6 and 71.9% respectively. During the present study, 10.00 percent concentration of *P. pinnata* seed and leaf extract caused 67.27 and 63.89 percent mortality, respectively. Methonalic leaf and seed extract of *P. pinnata* showed the maximum feeding deterrent activity, reduction in growth regulation and egg hatchability against *P. latus* due to the synergistic action of Karanj flavonoids. It contains two major flavonoids, *i.e*., karanjin and pongapin [15], that possess pesticidal properties [16, 17]. A number of studies have demonstrated that pongam contains pesticidal properties against pests such as pulse beetle *Callosobruchus chinensis*

[18], mosquitos [19] and the termite *Odototermes obesus* [16]. Thus, it is revealed that pongam also contains chemicals that should be useful for pest management.

Leaf powder of *Withania somnifera, Pongamia pinnata* and *Azadirachta indica* showed concentration dependent activity i.e. higher concentration (2 and 1%) showed significantly no population as compared to lower concentrations (0.7, 0.6 and 0.5%) after 45 days post treatment [20]. In many cases, farmers are misguided by spraying synthetic insecticides to combat this pest which are harmful to our health and environment. Thus, pongam extracts are a promising tool to combat insect pests. Pongam leaf extract can be recommended as an organic-based pesticide to manage the turnip aphid, *L. pseudobrassicae* [21]. The various parts of *P. pinnata* tree have been also used as insecticidal, nematicidal, antifungal, antibacterial and antiviral activities of *P. pinnata* [22]. The oil of *Pongamia* repelled brown plant hopper (*Nilaparvata lugens* Stal.) and significantly reduced its ingestion and assimilation of food and both brown plant hopper and white back plant hopper (*Sogatella furcifera* Horv.) suffered heavy mortality [23]. Schoonhoven [24] also attributed the mode of action of oils towards interference in normal respiration, resulting in suffocation. Kumar and Singh [25] also reported that *P. pinnata* was effective against insect pests among stored grains, field and plantation crops as oviposition deterrents. Thus natural extracts from plants or botanical extracts have been noticed for their safety to the environment and human health as well as their effective function to kill pest insects**.** Further, the appearance of resistance strains of the pests appears to be minimal due to different mode of action when compared with synthetic pesticides. Moreover, *P. pinnata* leaves extracts may attract natural enemies [26] and hence increase parasitism levels under field conditions. Botanical insecticides based on pongam leaves are thus usable concurrently with biological control agents [27]. At last, this newly developed phytopesticide is found to be good and can be recommended for protection of chilli crops against the yellow mite, *P. latus.*

5. Conclusion
The present study focused upon management practices against yellow mite in chilli. 10.0% concentration of both extract was most potent against *P. latus.* More studies are required in this area and *P. pinnata* leaf and seed extract can be used for protection of chilli crops against *P. latus.*

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