



E-ISSN: 2320-7078  
P-ISSN: 2349-6800  
JEZS 2017; 5(4): 996-1001  
© 2017 JEZS  
Received: 08-05-2017  
Accepted: 09-06-2017

**Arvind Malik**  
Department of Zoology,  
CCS Haryana Agricultural  
University, Hisar, India

**Rachna Gulati**  
Department of Zoology,  
CCS Haryana Agricultural  
University, Hisar, India

**Komal Duhan**  
Department of Zoology,  
CCS Haryana Agricultural  
University, Hisar, India

**Asha Poonia**  
Department of Zoology,  
Panjab University, Chandigarh,  
India

#### Correspondence

**Asha Poonia**  
Department of Zoology,  
Panjab University, Chandigarh,  
India

## Comparative efficacy of different concentrations of *Withania somnifera*, *Pongamia pinnata* and *Azadirachta indica* against *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) in wheat grains

**Arvind Malik, Rachna Gulati, Komal Duhan and Asha Poonia**

#### Abstract

In present study, bioassay of leaf powder of *Withania somnifera*, *Pongamia pinnata* and *Azadirachta indica* was done against *T. putrescentiae* in wheat grains. All the concentrations (0.5%, 0.6%, 0.7%, 1% and 2%) of all the three botanical powders were significantly better than the control (untreated wheat grains) except 0.5 percent concentration of *P. pinnata*. Among the botanicals, *W. somnifera* and *A. indica* at 0.7 percent concentration showed comparable mite population of 39.05 and 40.05 mites, respectively after 45 days as compared to 161.89 mites in untreated wheat grains. Protection against *T. putrescentiae* was 15.9 to 100, 45.7 to 100 and 33.9 to 100 percent with *A. indica* leaf powder, 2.3 to 100, 8.2 to 100 and 9.8 to 100 percent with *P. pinnata* leaf powder and 28.4 to 100, 43.7 to 100 and 22.1 to 100 percent population reduction with *W. somnifera* leaf powder after 15, 30 and 45 days post-treatment at various concentrations.

**Keywords:** *Withania somnifera*, *Pongamia pinnata*, *Azadirachta indica*, *Tyrophagus putrescentiae*, pest management

#### 1. Introduction

Wheat is one of the most produced and utilized cereal grains worldwide [1] as it comprises various valuable nutrients including starch (60-70%), proteins (10-15%), non-starch polysaccharides, minerals, vitamins and dietary fiber, which provide 60% of the calories and proteins daily [2, 3].

The complex interaction between grain including wheat, micro-environment and organisms leads to bio-deterioration of the grain. The degree of a product's infestation depends on the pests involved, the environmental conditions (e.g. temperature and moisture content), the degree of proper hygiene conditions [4] and the type of storage technology [5]. Earlier studies suggest that damage to stored grains and grain products by biotic factors may amount to 5–10 percent in the temperate zone and 20–30 percent in the tropical zone. Such damage may reach up to 40 percent, in countries where modern storage technologies have not been introduced [6]. Recently, Bashir *et al.* [7] estimated about 8 percent post-harvest losses in wheat due to storage pests.

Among storage pests, mites belonging to family Acaridae are gaining importance due to their increasing incidence and their association/ interaction with fungi and insects causing rapid qualitative and quantitative deterioration of grains [8]. The stored grain mites are major pests of wheat during its storage and accountable for the qualitative as well as quantitative losses [9]. Mites cause not only a direct weight loss in food materials, but also reduce the viability of seed stocks, as their attack is mainly confined to the embryo [10]. Among Acaridae, which includes about 400 species, *Tyrophagus putrescentiae* [11] is a ubiquitous, agriculturally and medically important mite species which is considered as severe pest of number of stored commodities all over the world especially those with high fat and protein contents [12, 13].

Chemicals are popularly used to prevent or control insect and mite infestations as they offer the simplest and most cost-effective means of dealing with stored product pests. However, insecticides have serious drawbacks such as pest resurgence and resistance, lethal effects on non-target organisms, the risk of users contamination, food residues, and environmental pollution [14].

In addition, the precautions necessary to work with traditional chemical insecticides [15], and the poor storage facilities of traditional farmers in developing countries, which are unsuitable for effective conventional chemical control [14], emphasize the necessity of new and effective methods for insect pest control of stored products.

Plant-derived extracts, powders [16, 17, 18] and essential oils may be options for mite control [19]. Plant-derived alkalis, alcohols, aldehydes, terpenoids and some monoterpenoids show fumigant properties [20]. There are several studies which showed the effectiveness of plant essential oils for control of stored products pests [19]. Among botanicals, *Allium sativum*, *Curcuma longa*, *Azadirachta indica*, *Glycyrrhiza glabra*, *Ocimum* sp. reported to have toxic and repellent effects on storage mites [8, 16, 21, 22] and insects [23]. *Withania somnifera* and *Pongamia pinnata* are the other botanicals which showed acaricidal activity against phytophagous mite, *Tetranychus urticae* [24].

Keeping in view the above facts and need for search of effective botanical control measures against the notorious stored grain pest *T. putrescentiae*, the present study investigated the impact of mite infestation levels in stored wheat grains and flour during six month storage and its management to evaluate the efficacy of some botanicals against *T. putrescentiae* in stored grains.

## 2. Material and methods

Bio-efficacy of leaf powder of *Withania somnifera*, *Pongamia pinnata* and *Azadirachta indica* was measured to determine their acaricidal activity against *T. putrescentiae* under standardized conditions (27±1°C, 80-85% RH) as per Anita *et al.* [8] in the Acarology Lab, Department of Zoology, CCSHAU from April to June, 2014, in terms of the population buildup of the *T. putrescentiae* as affected by changes in the concentration of botanical and duration of exposure in wheat. Copulatory pairs of mite were picked from the stock culture rearing dishes with the help of bird feather pick.

### 2.1 Preparation of botanical leaves powder

Ashwagandha leaves from Medicinal Plants Farm Area and karanj, neem leaves from University Campus were collected. Leaves of botanicals (100 g each) were properly cleaned, washed and shade dried. When leaves dried, they were ground in a grinder to make a fine powder. Each powder was kept in moisture free closed packing.

### 2.2 The efficacy of botanicals against *T. putrescentiae* in stored grains

To evaluate the efficacy of *W. somnifera*, *P. pinnata* and *A. indica* leaf powder against *T. putrescentiae*, wheat grains were treated with five dosages viz., 0.5, 0.6, 0.7, 1 and 2 g botanical/100g wheat grains as weight by weight basis in separate sets through uniform mixing. Each set was replicated three times. In each replicate, 100 mite pairs/ 100 g grain were released separately. For each dosage, three sets of three replicates each for 15, 30 and 45 days duration were prepared. After fifteen days, first set was removed from desiccators and live mites were counted in each replicate with the help of counting dish. Likewise, second and third set was removed and numbers of mites were counted. The effects of leaf powder treatment were evaluated after 15, 30 and 45 days of treatment. Non-infested grains were kept as control under three sets of 15, 30 and 45 days duration. Mites which did not show any movement after probing with bird feather pick were considered as dead. At the end of experiments, percent protection (Gulati, 2007a; b) was calculated for each concentration and storage duration of both the extracts, in order to get an idea of the effectiveness of the test extract against *T. putrescentiae* with the help of following formula [8]:

$$\text{Protection (\%)} = \frac{\text{Number of mites recovered in UG} - \text{Number of mites recovered in TG}}{\text{Number of mites recovered in UG}} \times 100$$

Where, UG = Untreated grain; TG = Treated grain

### 2.3 Statistical Analysis

Critical Difference (CD) was calculated between the treatments to see the impact of population buildup of *T. putrescentiae* on quantitative and qualitative composition of wheat by single and factorial CRD method. Data for evaluating the effect of botanicals against *T. putrescentiae* under *in vitro* conditions was subjected to two factorial CRD. CD was calculated in each case and means of treatments (concentrations) 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 botanical and its concentration.

## 3. Results

### 3.1 *Withania somnifera* leaf powder

Mites responded to *W. somnifera* leaf powder in a concentration dependent manner. Out of an initial number of 100 mite pairs, significantly less number of mites (nil) were recorded at 2 and 1 percent concentration than other treatments and control (CD=0.42; p=0.05).

**Table 1:** Efficacy of *Withania somnifera* leaf powder against *Tyrophagus putrescentiae* in wheat grains

Concentrations (%)	Number of mites after*			
	15 days	30 days	45 days	Mean
2.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00) <sup>a</sup>
1.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00) <sup>a</sup>
0.70	39.00 (6.26)	59.00 (7.72)	136.30 (11.68)	78.10 (8.56)
0.60	49.30 (7.07)	73.30 (8.10)	164.00 (12.83)	95.53 (9.50)
0.50	61.60 (7.90)	94.00 (9.74)	205.60 (14.37)	120.40 (10.67)
Control (untreated wheat grains)	102.33 (10.13)	135.33 (11.66)	248.00 (15.67)	161.89 (12.49)
Mean	42.04 (5.56)	60.27 (6.81)	125.65 (9.43)	

Figures in parentheses are  $\sqrt{n+1}$  transformation

CD (p=0.05) for Concentration = (0.42); SE(m) = (0.14)

CD (p=0.05) for Observation Period = (0.54); SE(m) = (0.18)

CD (p=0.05) for Concentration × Observation Period = (0.95); SE(m) = (0.32)

Values with the same superscript do not differ significantly

Post treatment results revealed significantly less number of mites from 0 to 120.4 mites at 2 to 0.5 percent concentrations. All the concentrations were significantly different with each other in terms of number of *T. putrescentiae* at the end of experimentation except first two concentrations. Duration of the treatment showed significant changes in the mite population. On first sampling day (15 days), *T. putrescentiae* counts remained significantly low (42.04 mites) (Table 1). Thereafter, the numbers had increased significantly (CD=0.54; p=0.05) at each sampling period showing 60.27 and 125.65 mites after 30 and 45 days post-treatment. Statistically significant interaction between the concentrations and observation periods (CD = 0.95; p = 0.05) (Table 1) was

observed which indicated that all the treatments were significantly better than control treatment.

### 3.2 *Pongamia pinnata* leaf powder

The data in Table 2 revealed that a significant lower number of live mites occurred in *P. pinnata* treated wheat grains as compared to control treatment (untreated grains). Treatment wise, higher concentrations (2 and 1%) were significantly (CD = 0.36; p= 0.05) more effective as these showed no *T. putrescentiae* counts as compared to initial pretreatment count (100 mite pairs) and other treatments including control (161.80 mites).

**Table 2:** Efficacy of *Pongamia pinnata* leaf powder against *Tyrophagus putrescentiae* in wheat grains

Concentrations (%)	Number of mites after*			
	15 days	30 days	45 days	Mean
2.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00) <sup>a</sup>
1.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00) <sup>a</sup>
0.70	52.00 (7.25)	112.60 (10.63)	165.30 (12.88)	109.97 (10.25)
0.60	67.00 (8.23)	126.00 (11.25)	204.30 (14.32)	132.43 (11.27)
0.50	84.00 (9.21)	153.30 (12.41)	238.00 (15.45)	158.43 (12.36) <sup>b</sup>
Control (untreated wheat grains)	102.33 (10.13)	135.33 (11.66)	248.00 (15.67)	161.89 (12.49) <sup>b</sup>
Mean	50.89 (6.14)	87.87 (7.99)	142.60 (10.06)	

Figures in parentheses are  $\sqrt{n+1}$  transformation

CD (p=0.05) for Concentration = (0.36); SE(m) = (0.12)

CD (p=0.05) for Observation Period = (0.47); SE(m) = (0.16)

CD (p=0.05) for Concentration × Observation Period = (0.82); SE(m) = (0.28)

Values with the same superscript do not differ significantly

These two concentrations were statistically comparable. Among other treatments, 0.7 (10.25 mites) and 0.6 (11.27 mites) percent concentrations were significantly better than untreated grains (Table 2). However, the lowest concentration (0.5%) was at par with the control treatment in terms of *T. putrescentiae* counts (158.43 mites). These studies also revealed the concentration dependent action of *P. pinnata* as per the earlier trend in *W. somnifera* powder. Duration of the treatment also significantly affected the efficacy of the treatment. Effect of *P. pinnata* treatments on *T. putrescentiae* was more potent at 15 days post-treatment (50.89 mites) as compared to other observation periods (Table 2). It showed significant increase in *T. putrescentiae* counts at 30 (87.87 mites) and 45 days post-treatment (142.60 mites) (CD= 0.47; p=0.05).

### 3.3 *Azadirachta indica* leaf powder

Five concentrations of *A. indica* leaf powder at 2, 1, 0.7, 0.6 and 0.5 percent were also tested against *T. putrescentiae* in wheat grains. The results clearly indicated that the treatments remained effective up to 45 days (Table 3). Mites responded to the treatments in a concentration dependent manner i.e. lowest number of live mites and highest reduction in

population was obtained with highest concentration of powder tested (2%) followed by 1, 0.7, 0.6, 0.5 and grains treatment (Table 3). Irrespective of duration, all the treatments significantly controlled the mite population as is evident from the mean of treatments (0.0, 0.0, 80.1, 95.8, 112.5 mites at 2, 1, 0.7, 0.6, 0.5% concentration) as compared to control (161.89 mites) (CD= 0.33; p=0.05).

Duration of the treatment also showed significant effect in the mite population. On first sampling day (15 days), *T. putrescentiae* counts remained significantly low (47.04 mites) (Table 3). Thereafter, the numbers had increased significantly (CD=0.43; p=0.05) at each sampling period showing 61.04 and 117.08 mites after 30 and 45 days post-treatment. The interaction was statistically significant between treatments and observation periods (CD = 0.74; p = 0.05) (Table 3) which indicated that all the treatments were significantly better than control treatment. Significantly lower counts of 0 to 72.3, 0 to 90.6 and 174.6 mites were noticed at 2 to 0.5 percent *A. indica* powder concentrations after 15, 30 and 45 days post-treatment, respectively which were statistically better than mite population in control (102.33, 135.33 and 248 mites) under same durations.

**Table 3:** Efficacy of *Azadirachta indica* leaf powder against *Tyrophagus putrescentiae* in wheat grains

Concentrations (%)	Number of mites after*			
	15 days	30 days	45 days	Mean
2.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
1.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
0.70	48.00 (6.99)	63.00 (7.98)	129.30 (11.38)	80.10 (8.79)
0.60	59.60 (7.77)	77.30 (8.84)	150.60 (12.30)	95.83 (9.64)
0.50	72.30 (8.55)	90.60 (9.56)	174.60 (13.25)	112.50 (10.46)
Control (untreated wheat grains)	102.33 (10.13)	135.33 (11.66)	248.00 (15.67)	161.89 (12.49)
Mean	47.04 (5.91)	61.04 (6.68)	117.08 (9.10)	

Figures in parentheses are  $\sqrt{n+1}$  transformation

CD (p=0.05) for Concentration = (0.33); SE(m) = (0.11)

CD (p=0.05) for Observation Period = (0.43); SE(m) = (0.14)

CD (p=0.05) for Concentration × Observation Period = (0.74); SE(m) = (0.25)

Values with the same superscript do not differ significantly

Amongst these botanical powders, *Azadirachta indica* was more effective in reducing the mite population at all the concentrations tested. However, both *W. somnifera* and *P. pinnata* powders were also effective in lowering down the *T. putrescentiae* population as compared to untreated grains which acted as control.

**3.4 Comparative evaluation of botanical powders against *T. putrescentiae***

The data pertaining to three factorial experiment (treatments × concentration × observation period) are presented in Table 4. Statistical analysis depicted a significant effect of treatments on population buildup of *T. putrescentiae* on wheat grains (CD= 0.59; p=0.05). Results revealed that maximum population developed on untreated grains (161.89 mites), followed by 55, 40.05 and 39.05 mites on grains treated with *P. pinnata*, *A. indica* and *W. somnifera* with 100 mite pairs as the initial inoculum. These differed significantly with each other except the latter two treatments which were statistically comparable with each other.

When the results on population build up of *T. putrescentiae* over fortnightly observations were compared, a significant effect of observation period was recorded (CD= 0.51; p=0.05) (Table 4). Irrespective of the treatment or concentration, the mite number was found to significantly increase with each observation period. The mite count was 42.95, 63.16 and 115.87 after 15, 30 and 45 days of post treatment which differed significantly with each other. Two concentrations of each botanical treatment were compared which showed that higher concentration (1%) was more potent in causing mortality than lower concentration (0.7%). Statistically lower

number of mites (40.47 mites) was recorded on the grains treated with 1 percent concentration of botanical powder than 0.7 percent concentration (107.52 mites) (CD= 0.42; p= 0.05) (Table 4).

Fortnightly observations on the population buildup of *T. putrescentiae* on wheat grains revealed a significant interaction between treatment and observation period (CD= 1.03; p= 0.05). At each observation period (15, 30 and 45 days), mite population was found to significantly lower in each treatment compared to untreated grain which acted as control. However mite numbers in each of the three botanical treatments were statistically at par with each other. A significant interaction between the treatment and the concentration was obtained (CD= 0.84; p= 0.05) (Table 4). Mites were significantly lower in the higher concentration (0 mite) of each botanical treatment as compared to lower concentration (0.7%) of *W. somnifera* (78.11 mites), *A. indica* (80.11 mites), *P. pinnata* (110 mites) and control (161.89 mites). The former two botanical treatments were statistically comparable.

The interaction between concentration and observation period was also found to be significant (CD= 0.73; p= 0.05) (Table 4). At each observation period, the higher concentration harboured the minimum mite population (25.58, 33.83 and 62 mites after 15, 30 and 45 days post treatment) as compared to lower concentration as can be seen by the pooled mean values (60.33, 92.5 and 169.75 mites after 15,30 and 45 days post treatment). At higher concentration, mite count after 15 and 30 days post treatment do not differed significantly with each other. A significant interaction was seen between the treatments vs. concentration vs. observation period.

**Table 4:** Comparative susceptibility of botanical powders against *Tyrophagus putrescentiae* in wheat grains

Treatments (T)	Number of mites								Mean (T×OP)			Pooled mean (T)
	Concentration (1.0%) (C)				Concentration (0.7%) (C)							
	Observation period (OP)			Mean (T×C)	Observation period (OP)			Mean (T×C)				
	15days	30days	45days		15days	30days	45days					
<i>Withania somnifera</i>	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00 <sup>a</sup> )	39.00 (6.26)	59.00 (7.72)	136.00 (11.68 <sup>a</sup> )	78.11 (8.55)	19.50 (3.63 <sup>a</sup> )	29.50 (4.36 <sup>a</sup> )	68.16 (6.34 <sup>a</sup> )	39.05 (4.77 <sup>a</sup> )
<i>Pongamia pinnata</i>	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00 <sup>a</sup> )	52.00 (7.25)	112.66 (10.63)	165.33 (12.88)	110.00 (10.25)	26.00 (4.12 <sup>a</sup> )	56.33 (5.81 <sup>a</sup> )	82.66 (6.94 <sup>a</sup> )	55.00 (5.62)
<i>Azadirachta indica</i>	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00 <sup>a</sup> )	48.00 (6.99)	63.00 (7.98)	129.33 (11.38 <sup>a</sup> )	80.11 (8.78)	24.00 (3.99 <sup>a</sup> )	31.50 (4.49 <sup>a</sup> )	64.66 (6.19 <sup>a</sup> )	40.05 (4.89 <sup>a</sup> )
Untreated Grains	102.33 (10.13)	135.33 (11.66)	248.00 (15.67)	161.89 (12.49)	102.33 (10.13)	135.33 (11.66)	248.00 (15.67)	161.89 (12.49)	102.33 (10.13)	135.33 (11.66)	248.00 (15.67)	161.89 (12.49)
Mean (C × OP)	25.58 (3.28 <sup>a</sup> )	33.83 (3.66 <sup>a</sup> )	62.00 (4.66)		60.33 (7.66)	92.50 (9.50)	169.75 (12.90)					
Pooled mean (C)				40.47 (3.87)				107.52 (10.02)				
Pooled mean (OP)									42.95 (5.47)	63.16 (6.58)	115.87 (8.78)	

Values in parentheses are √n+1 transformation

CD (p=0.05) for Treatments (T) = 0.59

CD (p=0.05) for Concentration (C) = 0.42

CD (p=0.05) for Observation Period (OP) = 0.51

CD (p=0.05) for T × C = 0.84

CD (p=0.05) for T × OP = 1.03

CD (p=0.05) for C × OP = 0.73

CD (p=0.05) for T×C × OP = 1.46

Values with the same superscript in the column wise/ row wise do not differ significantly

Protection of wheat grains against *Tyrophagus putrescentiae* after different botanical powder treatments were also calculated and presented in Table 5. *A. indica* leaf powder provided 15.9 to 100.00, 45.7 to 100.00 and 33.9 to 100.00 percent population reduction against *T. putrescentiae* after 15, 30 and 45 days post-treatment. Similarly, *P. pinnata* leaf powder provided 2.3 to 100.00, 8.2 to 100.00 and 9.8 to 100.00 percent population reduction against *T. putrescentiae* after 15, 30 and 45 days post-treatment. Likewise, *W.*

*somnifera* leaf powder provided 28.4 to 100.00, 43.7 to 100.00 and 22.1 to 100.00 percent population reduction against *T. putrescentiae* after 15, 30 and 45 days post-treatment. The treatments provided 100 percent protection against *T. putrescentiae* infestation up to 45 days post treatment at higher concentrations (2 and 1%). This showed that the effectiveness of these treatments with respect to *T. putrescentiae* control was comparable (Table 5).

**Table 5:** Protection of wheat grains against *Tyrophagus putrescentiae* after treatment with botanical powder

Concentration (%)	Protection against <i>Tyrophagus putrescentiae</i> (%)								
	<i>Withania somnifera</i>			<i>Pongamia pinnata</i>			<i>Azadirachta indica</i>		
	15 Days	30 days	45 Days	15 days	30 days	45 days	15 days	30 days	45 days
2.00	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1.00	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.70	54.7	64.7	48.4	39.5	32.6	37.4	44.2	62.3	51.0
0.60	42.7	56.1	37.9	22.1	24.6	22.6	30.7	53.7	43.0
0.50	28.4	43.7	22.1	2.3	8.2	9.8	15.9	45.7	33.9

#### 4. Discussion

For the control of insect pests of stored grains, some work involving the extracts/oils of certain plants, has been conducted but little information on mites is available. Therefore, the present discussion encompasses the work on both mite and insect pests of stored grains and mites inhabiting other habitats.

Leaf powder of *Withania somnifera*, *Pongamia pinnata* and *Azadirachta indica* were bioassayed against *T. putrescentiae* in wheat grains during present study 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. *T. putrescentiae* have the potential to complete development from egg to adult within 15 days test period at 27°C and 80-85% [12]. Concentration dependent activity against *T. putrescentiae* was earlier recorded in *Curcuma* treatments (oil, powder, oleoresin) [17, 21], garlic products [22], *Ocimum* and liquorice extracts [8], Bt toxins (Bt1 and Bt2) [25]. These were effective in bringing down the expected emergence due to olfactory and contact inhibition. Schoonhoven [26] also attributed the mode of action of oils towards interference in normal respiration, resulting in suffocation. Potts and Roderiguez (1978) [27] reported that mint oil at 2 percent concentration significantly reduced the adult progeny of *T. putrescentiae*. *Detia* diatomaceous earth (DE) did not eliminate mites completely, but negative growth rate was found against *T. putrescentiae* [28].

Reports are available on efficacy of other plant extracts against *T. putrescentiae*. Eucalyptus, mint, turmeric and garlic products had pronounced effects on immature stages (egg and larvae) although nymph and adults of *T. putrescentiae* and *S. nesbitti* were also affected [16, 18]. These results are in conformity with studies on stored insect pests [28]. Lee *et al.* [19] studied the acaricidal activity (direct contact application) of 12 fennel seed oil extracts against *T. putrescentiae* and found naphthalene (4.28 µg/cm<sup>2</sup>) and Carvone (4.62 µg/cm<sup>2</sup>) to be the most toxic. In another study, the vapours of seven out of 13 natural monoterpenes tested possessed acaricidal activity against mobile stages of *T. putrescentiae* [30]. Of these, pulegone, menthone, linalool, and fenchone had the highest vapour toxicity with a LC<sub>50</sub> ≈ 14 µl/l. These compounds are similar to those reported active against *T. longior* adults treated by contact and inhalation [31], though they required three times the dose of menthone, linalool and fenchone to obtain LC<sub>50</sub> values in the range of those obtained in this work, and no activity was reported for pulegone. This large difference in susceptibility might be related to the smaller size of *T. putrescentiae* in relation to *T. longior* and/or to the specificity of the compound, as in the case of pulegone. The lack of ovicidal activity of the natural monoterpenes tested seems in accord with the view that the exochorion of eggs of the genus *Tyrophagus* functions as a barrier against desiccation, as suggested by Witalinski [32], or against uptake of the vapour. Thus, the higher acaricidal activity recorded on immature and adult stages of *T. putrescentiae* might be again

related to desiccation. However, action by interference with respiratory processes cannot be discarded, since the metabolic rate and oxygen consumption is less in eggs than in adults, which could reduce the rate of mortality of eggs, as reported in *Acarus siro* [33]. This was a first step in unravelling the complex mechanisms of action of these natural monoterpenes on the mould mite. Of the natural monoterpenes tested, pulegone, menthone, linalool and fenchone are the most promising for possible use against *T. putrescentiae* due to the low doses required to produce a high mortality in the immature and adult stages. Additionally, no residues dangerous for neither human health nor modifications of the colour, flavour, odour, and texture of stored food treated with such products have been reported [31]. However, aqueous extract of *Azadirachta indica* were found to decrease germination, root length, shoot length and biomass production in various crops including wheat [34].

During present study, protection against *T. putrescentiae* with *A. indica* leaf powder was 15.9 to 100, 45.7 to 100 and 33.9 to 100 percent, 2.3 to 100, 8.2 to 100 and 9.8 to 100 percent with *P. pinnata* leaf powder and 28.4 to 100, 43.7 to 100 and 22.1 to 100 percent population reduction with *W. somnifera* leaf powder after 15, 30 and 45 days post-treatment. Earlier, *G. glabra* and *O. sanctum* extract showed pronounced effects on the population of the mite. It provided 71.5 to 94.7, and 66 to 92 percent relative protection against *T. putrescentiae* at different durations [8]. The present results will enable the scientists to adopt appropriate control measures leading protection of wheat seeds as well as to ensure food security by minimizing the storage losses.

#### 5. Conclusion

The present study focused upon protection of major cereal grain wheat during storage. Higher concentrations (2% and 1%) of leaf powder of *Withania somnifera*, *Pongamia pinnata* and *Azadirachta indica* in wheat grains caused 100 percent mortality in *T. putrescentiae* after 45 days post treatment in present study. *Withania somnifera* was most potent against *T. putrescentiae* at low concentrations (0.5%, 0.6% and 0.7%) followed by *Pongamia pinnata* and *Azadirachta indica* was least potent. More studies are required in this aspect and *Withania somnifera*, *Pongamia pinnata* and *Azadirachta indica* leaf powder can be used for protection of wheat against *T. putrescentiae* during storage.

#### 6. Acknowledgement

Financial help and necessary facilities provided by CCS Haryana Agricultural University, Hisar are fully acknowledged.

#### 7. References

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