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# DETERRENT ACTIVITY OF NATURAL PRODUCTS ON TWO SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* KOCH

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**ABSTRACT**

**The feeding inhibition and oviposition deterrent activity of natural products were evaluated under laboratory conditions against *Tetranychus urticae* Koch. Mite feeding specks in the treatments varied from 61.3 to 428 at concentrations ranging from 0.625 to 40%. *Darekastra* and fermented buttermilk outperformed others to result in reduced number of feeding specks with increase in concentration. The feeding inhibition index for all the products was negative suggesting that these have varied level of inhibition. Cow urine, *Darekastra,* fermented buttermilk and *Tamarlassi* were found to effectively reduce the oviposition by mite. The oviposition deterrence index exhibited both attractiveness and repellent activity of natural products. Cow urine, *Darekastra* and fermented buttermilk proved as potent acaricides against *T. urticae* under both the deterrence parameters.**

**Key words:** *Tetranychus urticae*, natural products, parthenocarpic cucumber, bioassay, feeding specks, deterrence, cow urine, *Darekastra*, fermented butter milk, *Jeevamrit*, *Panchgavya*, *Tamarlassi*, vermiwash

Approximately 1,305 valid described species of two spotted spider mites have been recorded and among them 10% are the phytophagous (Ivana et al., 2018; Santamaria et al., 2020). Amongst these, *Tetranychus urticae* Koch has worldwide distribution feeding upon >1200 plant species and is known to infest agricultural, horticultural and ornamental crops (Migeon and Dorkeld, 2019; Mitra et al., 2020). In Himachal Pradesh, *T. urticae* has been recognized as a major pest of greenhouse crops especially cucumber (Ghongade and Sood, 2019). At present, management of *T. urticae* is mainly based upon intensive use of acaricides which has threatened the environment health and has increased risk to human health too. An alternative approach to chemical pesticides is the use of natural products (derived from cow byproducts).

Natural products have proved effective against *T. urticae* both under laboratory and field conditions such as *Darekastra* comprising *Melia* leaves (Carpinella et al., 2003; Su et al., 2011), vermicompost extract (Edwards et al., 2010; Arancon et al., 2007), *Tamarlassi* (Thakur and Sood, 2019) etc. Most of these have two components, cow urine and cow dung, both of which are effective against the insect pests (Karkar et al., 2014). *Melia azedarach* has excellent insecticidal activity, repellence, feeding inhibition as well as growth regulatory activities (Sharma et al., 2014) which are attributed to the limonoids (meliarachins A-K), steroids, triterpenoids in *M. azedarach* (Wang et al., 2020). Vermiwash is composed of growth regulating compounds such as auxins, micronutrients, macronutrients, actinomycetes but is also known to exhibit acaricidal activity (Aghamohammadi et al., 2016). The advantage of using these relies on their cheapness, and poor farmers can afford. Also, no harmful residues are present since these are biodegradable, pose no health hazards to humans and non-target organisms and hold toxicity against major pests of greenhouse crop (Saleem et al., 2019). The present study focused on the evaluation of deterrent activity of these products against *T. urticae*. This study will validate the acaricidal property of natural products by screening them on two major parameters i.e., feeding inhibition and oviposition deterrence.

## MATERIALS AND METHODS

Seven natural products namely; cow urine (fermented 15 days old from Indian cow), *Darekastra* (5 kg *Melia azedarach* leaves, 5 l cow urine, 2 kg cow dung, 100 l water), fermented butter milk (prepared from milk cultured for several days), *Jeevamrit* (1 kg cow dung, 1 l cow urine, 200 g jaggery, 200 g gram flour, 100 g local fertile soil, 20 l water), *Panchgavya* (5 kg fresh cow dung, 3 l cow urine, 2 l cow milk, 2 l curd, 1 kg desi ghee), *Tamarlassi* (fermenting butter milk in copper pot for minimum 15 days) and vermiwash (3 kg cow dung, 2 kg biomass, 200-300 adult earthworms layered into pitcher pot with water source. Earth worm secretion collected at bottom in a container

was evaluated for feeding and oviposition deterrence against *T. urticae* under laboratory conditions in the Department of Entomology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur during 2016-17. These products were prepared freshly. *Tetranychus urticae* was cultured on French bean, *Phaseolus vulgaris* potted plants kept in insectary (25± $1^0$C, 70± 5% RH and 16 hr photoperiod -16 L: 8D). Adults were allowed to oviposit for 24 hr and thereafter removed. Eggs were reared till adult emergence to get uniform age samples. The leaf disc bioassay method elaborated by Erdogan et al. (2012) was used. Leaf discs of 3.5 cm dia were excised from French bean apical leaves. Excised discs were dipped into concentrations of natural products (prepared by serial dilution) for 30 sec. These were air dried and placed onto wet sponge in abaxial position. Control was also set up by dipping leaf discs into water. Treated leaf discs were placed under controlled conditions (25±$1^0$C and 70± 5% RH).

Feeding inhibition offered by natural products was evaluated in terms of number of feeding specks. Adult females (n=10) released onto treated and untreated leaf discs were allowed to feed for 24 hr and removed thereafter. Numbers of white speck were counted under stereozoom miniscope. Reduction in feeding was calculated taking into account number of feeding specks in treatment and control. Feeding inhibition index was worked out using the equation:

$$\text{Feeding inhibition index} = \frac{\text{Number of feeding specks in treatment} - \text{Number of feeding specks in control}}{\text{Number of feeding specks in treatment} + \text{Number of feeding specks in control}} \times 100$$

Values of index vary from +100 (very attractive) to -100 (complete deterrence). Oviposition deterrent activity of natural products was evaluated in terms of number of eggs laid by *T. urticae* up to 96 hr of exposure duration (HED) on to treated leaf surface. Female mites (n=10) were released on treated and untreated leaf discs. Change in oviposition was calculated taking into account number of eggs in control and treatment. Oviposition deterrence index (ODI) was calculated as per Hang et al. (1982).

$$\text{Oviposition deterrence index} = \frac{\text{Number of eggs laid in treatment} - \text{Number of eggs laid in control)}}{\text{Number of eggs laid in treatment} + \text{Number of eggs laid in control}} \times 100$$

The value of index varies from +100 (very attractive) to -100 (complete deterrence). Experimental data was subjected to statistical analysis as per Gomez and Gomez (1984). Square root transformation was used for normalising data. Transformed data were subjected to factorial ANOVA in CPCS programme. Least significant difference test (LSD) was used to determine significance of data.

## RESULTS AND DISCUSSION

Data pertaining on number of feeding specks produced on the leaf discs treated with varying concentrations of natural products was found to be significant (Table 1). Number of feeding specks varied significantly (p=0.05) for two products; cow urine and *Darekastra* at all the concentrations and were also least of all the products evaluated. Leaf discs treated with *Darekastra* and fermented buttermilk exhibited reduction in number of feeding specks with increase in concentration; values being significant only for *Darekastra*. An opposite trend was observed in for leaf discs treated with *Panchgavya* and *Tamarlassi* where feeding specks increased with increase in concentration. However, treatments did not differ significantly among each other. The remaining products such as cow urine, *Jeevamrit* and vermiwash did not show any definite trend in reduction of feeding specks with in concentration. The number of feeding specks in untreated check (UTC) remained significantly higher for all the treatments.

Reduction in mite feeding was found to be significant for cow urine, *Darekastra*, *Panchgavya* and vermiwash (Fig. 1). For cow urine treated leaf discs, reduction in feeding varied from 4.80 to 56.30%. However, no definitive trend was noticed but the reduction was highest under 10 and 20% concentration. *Darekastra* and fermented buttermilk treated leaf discs resulted in increased feeding reduction with concentration i.e., 53.71 to 91.24% and 15.76 to 26.14%, respectively, with maximum reduction being at 20%. However, *Panchgvaya* and *Tamarlassi* showed an opposite trend i.e decrease in feeding reduction varying from 71.73 to 23.03% and 58.79 to 48.03%, respectively; with increase in concentration. *Jeevamrit* and vermiwash exhibited in no definite trend in feeding reduction. The feeding inhibition index was found to be negative for all the evaluated natural products namely; cow urine (-2.48 to -39.22), *Darekastra* (-36.77 to -83.93), fermented buttermilk (-8.56 to -15.03), *Jeevamrit* (-19.44 to -29.57), *Panchgavya* (-13.33 to -56.05), *Tamarlassi*

Table 1. Feeding inhibition and oviposition inhibition by natural products on *T. urticae* adult females

| Concen-tration (%) | No. of feeding specks produced on leaf disc after 24 hr of exposure duration (HED) | | | | | | | % change ( - /+) in oviposition after 96 HED compared to untreated check | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cow urine | *Darekastra* | Fermented butter milk | *Jeevamrit* | *Panchgavya* | *Tamarlassi* | Vermiwash | Cow urine | *Darekastra* | Fermented butter milk | *Jeevamrit* | *Panchgavya* | *Tamarlassi* | Vermiwash |
| 0.625 | -* | - | - | - | - | 146.6± 2.40 (12.1)[a] | - | - * | - | - | - | - | -34.05± 3.29 (35.64)[a] | - |
| 1.25 | 315.3± 7.42 (17.7)[c] | 245.0 + 2.89 (15.6)[e] | 370.6± 11.84 (19.2)[b] | 200.3± 5.49 (14.1)[bc] | - | 150.0± 5.77 (12.2)[a] | - | -32.22± 3.48 (34.51)[a] | -19.01± 0.94 (25.83)[a] | -22.70± 3.30 (28.33)[a] | -12.31± 1.76 (20.45)[a] | - | -43.61± 3.83 (41.9)[b] | - |
| 2.50 | 356.6± 9.61 (18.9)[cd] | 214.0± 6.93 (14.6)[d] | 350.0± 7.64 (18.7)[ab] | 207.6± 4.33 (14.4)[bc] | 157.6± 5.36 (12.5)[a] | 175.0± 2.89 (13.2)[b] | 250.0± 52.17 (15.8)[a] | -30.92± 5.45 (33.61)[a] | 38.46± 4.70 (38.25)[b] | -26.70± 2.62 (31.04)[a] | -14.82± 1.84 (22.56)[a] | +3.96± 3.12 (9.64)[a] | -44.12± 2.85 (41.59)[b] | -8.28± 1.19 (16.63)[a] |
| 5.00 | 379.3± 1.45 (19.5)[d] | 174.3± 13.86 (13.2)[c] | 347.0± 14.22 (18.6)[ab] | 177.0± 4.36 (13.3)[a] | 240.0 + 10 (15.5)[b] | 185.0± 2.89 (13.6)[b] | 351.3 + 53.99 (18.7)[bc] | -35.72± 0.78 (36.69)[a] | -39.30± 2.97 (38.78)[b] | -44.80± 2.46 (41.99)[b] | -22.06± 1.52 (27.98)[b] | +9.56± 5.24 (16.68)[a] | -48.94± 3.46 (44.38)[b] | -16.33± 1.03 (23.81)[b] |
| 10.00 | 254.0± 42.25 (15.7)[b] | 61.3± 1.86 (7.9)[b] | 335.0± 14.43 (18.3)[ab] | 191.3 + 4.67 (13.8)[ab] | 272.3± 2.85 (16.5)[b] | 185.0± 2.89 (13.6)[b] | 375.3± 37.53 (19.4)[c] | -53.99± 6.87 (47.32)[b] | -39.60± 2.24 (38.97)[b] | -47.87 + 3.97 (43.76)[b] | -24.45± 1.34 (29.60)[b] | +53.36± 10.84 (46.93)[b] | -51.83± 3.50 (46.03)[b] | -18.58± 1.69 (25.48)[b] |
| 20.00 | 174.0± 5.29 (13.2)[a] | 46.3± 2.73 (6.8)[a] | 325.0± 8.66 (18.1)[a] | 219.6± 8.21 (14.8)[c] | 409.0± 5.77 (20.2)[c] | - | 337.6± 20.53 (18.4)[bc] | -54.42± 2.88 (47.53)[b] | -44.65± 3.54 (41.90)[b] | -50.85± 3.43 (45.47)[b] | -33.60± 4.34 (35.32)[c] | +46.82± 7.42 (43.12)[b] | - | -35.78± 2.99 (36.69)[c] |
| 40.00 | - | - | - | - | 428.0± 32.62 (20.6)[c] | - | 330.0± 40.41 (18.1)[b] | - | - | - | - | +56.89± 5.49 (48.99)[b] | - | -38.17± 2.84 (38.12)[c] |
| Untreated check | 398.6 + 5.93 (19.9)[d] | 530.0± 11.55 (23.1)[f] | 440.0± 5.77 (21.0)[c] | 325.6± 15.88 (18.1)[d] | 559.6± 17.03 (23.6)[d] | 356.0± 3.46 (18.8)[c] | 433.0± 14.43 (20.8)[d] | - * | - | - | - | - | -34.05± 3.29 (35.64)[a] | - |
| CD (p=0.05) | (1.7) | (0.8) | (0.9) | (0.7) | (1.2) | (0.4) | (1.2) | (8.33) | (5.80) | (6.15) | (5.14) | (15.17) | (6.23) | (4.41) |

Figures in parentheses $\sqrt{(x+1)}$ transformed values; *Concentration not evaluated; Figures in parentheses arc sine transformed values; No. of eggs laid by RSM adult females in UTC varied from 93 to 150; -ve : Reduction; +ve : Increase

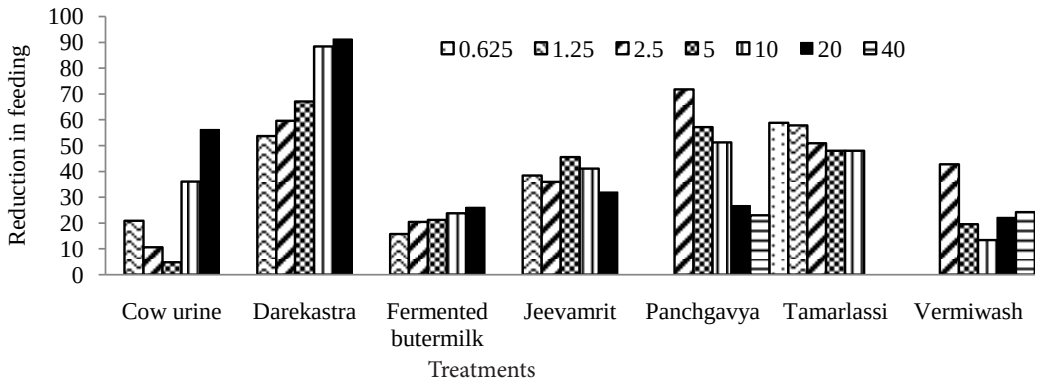


Fig. 1. Reduction in feeding of *T. urticae* adults due to natural products

(-31.61 to -41.66) and vermiwash (-7.14 to -26.79) which depicts that all the products offered varying degree of feeding inhibition. However, *Darekastra* proved to be highly effective and fermented buttermilk proved to be the least effective.

The feeding inhibition property of *Darekastra* can be attributed to the presence of components of *Melia* which are pesticidal in nature. The chinaberry tree *Melia azedarach* is prevalant in Himachal Pradesh and is known to contain triterpenoids (like meliacarpins) and limonoids (meliartenin) which act as antifeedant, repellent and growth regulator against insect herbivores (El-Wakeil, 2013). The mode of action of *M. azedarach* as suggested by Breuer et al. (2003) is that it acts upon NADPH-cytochrome c reductase and cholinesterase in insects. Also, the limonoids and triterpenoids compounds are known to induce apoptosis/ programmed cell death (Akihisa et al., 2013). Exposure to *Darekastra* treated leaves results in inappropriate feeding of insect resulting into increased mortality. Hammad et al. (2017) recorded *T. urticae* mortality of 45 and 34% when exposed to leaf and fruit aqueous extract of *M. azedarach*, respectively. About 70% mortality of *T. urticae* eggs, nymphs and adults was recorded at 72 hr of treatment (Mwandila et al., 2013).

Oviposition deterrence activity of natural products against *T. urticae* presented as % change in oviposition was found significant (p=0.05) for all treatments (Table 1). However, treatments did not differ significantly from each other. The negative values represent reduction in oviposition while positive indicates increase. Change in oviposition was found negative for products viz. cow urine, *Darekastra*, fermented buttermilk, *Jeevamrit*, *Tamarlassi* and vermiwash which depict their efficacy in reducing the egg laying capacity of *T. urticae*. Reduction in oviposition ranged from 30 to 60% but cow urine, *Darekastra*, fermented buttermilk and *Tamarlassi* led to maximum reduction. *Panchgavya* resulted in positive values ranging from +3.96 to +56.89% depicting it as an oviposition inducer. Thus, cow urine, *Darekastra*, fermented buttermilk and *Tamarlassi* outperformed others to reduce oviposition. Oviposition deterrence index ranged- for cow urine (-25.46 to +3.17), *Darekastra* (-4.10 to +19.70), *Panchgavya* (-27.45 to +23.61), vermiwash (-8.19 to +13.88) exhibiting both attractiveness and deterrence activity. Cow urine exhibited deterrence at maximum concentration (10%), *Darekastra* and vermiwash were the least attractive and *Panchgavya* exhibited attractiveness to mites at maximum concentration. Fermented buttermilk (-7.01 to -28.89), *Jeevamrit* (-1.28 to -16.80) and *Tamarlassi* (-22.27 to -34.97) offered oviposition deterrence at all the concentrations.

Cow urine is known to be effective against insect pests. The oviposition deterrence of cow urine is not known but insecticidal activity against Bihar hairy caterpillar *Spilarctia oblique* sprayed @10% (Geetanjaly and Tiwari, 2014), *Spodoptera litura* @20% (Naik and Tiwari, 2018), sorghum shoot fly, *Atherigona soccata* @5% (Mudigoudra et al., 2009) is documented. *Darekastra* also possess the oviposition deterrence that can be attributed to its composition and mode of action of *M. azedarach.* Reduction of > 50% in oviposition activity and an increased premature period of *T. urticae* were recorded by Ashrafju et al. (2014). Outcomes of our study will act as reference for the oviposition deterrent activity of fermented buttermilk, *Tamarlassi* and vermiwash against *T. urticae* as this has not been documented earlier.

For both the parameters (feeding inhibition and oviposition deterrence, *Panchgavya* exhibited opposite trend i.e. treated leaf discs resulted in more feeding specks and induced egg laying by *T. urticae*. The possible reason is that *Panchgavya* is used as nutrient

rich soil amendment containing higher quantity of macro and micronutrients like zinc, copper, manganese resulting into good soil health, increased microflora, plant growth, improved seed germination when used for seed treatment (Jain et al., 2013) and hence did not offer deterrence to the spider mite adults. This study clearly concludes that *Darekastra* offered excellent feeding inhibition against *T. urticae* but at the same time it resulted in oviposition deterrence along with cow urine and fermented buttermilk.

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