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Effect of cow milk on sucking pests and insect predators on rose

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ABSTRACT

Cow milk was evaluated for toxicity bioassays against three pests of rose, i.e., aphids (*Macrosiphum roseiformis*), thrips (*Scirtothrips dorsalis*) and two spotted spider mites (*Tetranychus urticae*) and its influence on two predatory insects ladybird beetles (*Coccinella septempunctata*) and minute pirate bugs (*Orius laevigatus*). In addition, anti and pro effects of the milk on behavioural responses of all experimental insects/mites were determined by using choice-no-choice method. Results revealed that milk has a potential to be used as a insect / mite control agent on roses, as it gave remarkable control of all the tested sucking pests of rose and found completely safe towards two general insect predators. In addition, repellent effect of milk was also noticed on these pests.

Key words: Cow milk, thrips, aphids, spider mites, control, insecticide, predator.

Cow's milk has many bioactive compounds, proteins, carbohydrates, fatty acids, minerals and other nutrients, which facilitate healthy growth and development. Besides health benefits, milk is a good medium for bacterial culture. This property made it to be used in control of number of insect problems (Young, 1982). In ancient times, farmers used cow's milk as a medium for saprophytic bacteria and virus inhibitor (Nene, 1999). Virkshayurveda and Chavundaraya which deals with agriculture and botany, described the use of milk, which changed the flower colour and enhanced the fruit taste (Shenoy *et al*. 2000). There is a scarcity of information with respect to field application of milk for pest control. However, other cow products, such as: *panchgavya* (Natarajan, 2002; Vivekanandan 1999a,b), cow urine (Gupta and Yadav, 2010), cow dung (Sadawarte and Sarode, 1997; Boomathi *et al*. 2006) and cow butter milk (Gupta and Rai, 2006) had success stories in insect pest control. Synthetic insecticides are used frequently for the control of various sucking pests on ornamentals and in greenhouses. Reports elucidated the side effects of these chemicals on biocontrol agents (Smith and Krischik, 1999; Rebek and Sadof, 2003) and other pest related problems like, development of resistance in pests to different group of insecticides (Schreiber *et al*. 1990; Zhao *et al*. 1995) and outbreaks of secondary pests (Sclar *et al*. 1998; Gupta and Krischik, 2007). Recent pest management approaches are focusing more towards natural and safer control measures especially on ornamentals and green houses, where a lot of dependency prevailed on synthetic pesticides. Cow's milk, if as a curative measure could get incorporated in this system, would be a safe approach. In current research, cow milk was evaluated with toxicity bioassays against three pests of rose (aphids, thrips & spider mites) and for influence on two predatory insects (lady bird beetles & minute pirate bugs).

MATERIALS AND METHODS

Experimental material: Cow milk was brought from V.P.O Chunni Kalan, Fatehgarh Sahib, Punjab, India. Three concentrations were made and tested against test insects. One hundred ml of cow (whole) milk was taken as X and further two serial dilutions (0.5X & 0.25X) were made with distilled water. Fresh milk was used for further experiments.

Insects/ mite: Natural populations of rose aphids (*Macrosiphum roseiformis*), thrips (*Scirtothrips dorsalis*) and two spotted spider mites (*Tetranychus urticae*) were collected from rose garden of Panjab University, Chandigarh, India, as and when available and needed for the experimentation. In addition, generalist predators of these pests, ladybird beetles, *Coccinella septempunctata* (LBB) and minute pirate bugs, *Orius laevigatus* (MPB), were selected for the study. LBB were collected from different locations near Chandigarh, from mustard and wheat fields. These beetles were kept in plastic containers covered with muslin cloth and fed with thin slices of apple and diluted honey solution till they were used for bioassays and behavioral assays. MPB were procured from Bio Bee India Pvt Ltd., New Delhi, India (http://biobee.in/products-and-services/solutions/bioorius/).

Toxicity bioassays : Bioassays were conducted to evaluate the toxic effect of milk on pests and predators selected for study. In cse of aphids, thrips and spider mites toxicity bioassays, compound rose leaflets were collected and washed thoroughly with water. These were treated with 3 different

concentrations (X, 0.5X & 0.25X) of milk. After air drying, these treated leaflets were kept in petridish (90 x15mm) and 7-10 aphids, thrips and spider mites were released in each petridish. Leaflets, untreated but washed with water served as control. Data on number of insects/mites survived were recorded after 24 and 48 hrs after release. The same procedures were followed in case of LBB and MPB but here 4-5 adults were released in each petridish. In addition, immature stages of aphids were given to these predators as feed in each petridish and provided *ad-libitum*. Here also observations were recorded on survival of predators at 24 and 48hrs after release. Three replicates for each concentration were made and three biological repeats were done at different dates (n=3x3). Data were analysed by ANOVA and means were compared using Tukey's HSD test and analysed for homogeneity using Welch's tests (JMP SAS Institute, 2005).

Food choice test: Behavioural response (food choice) of aphids, thrips and spider mites was studied by giving them two choices (untreated & treated rose leaves with whole milk). Rose leaves were cut to square discs (4x4 cm) and four leaves were kept in a petridish (140x20mm) lined with a moistened filter paper (Whatman filter paper 125mm). Treated and untreated leaf discs were arranged alternately around the circumference of the petridish. These were placed in the petridish at diagonal position. In this way two choices were given to test organisms (treated or untreated). Six thrips or 10 aphids or 6 spider mites were released at the center of the petridish. Observations on number of aphids or thrips or spider mites, present on treated or untreated rose leaf discs were recorded at 30, 90 and 180 min after the release. Goodness of fit (χ^2) analysis using PROC FREQ to determine, if the choice responses deviated significantly from random choice (1:1, 50%) was done (JMP SAS Institute, 2005).

RESULTS AND DISCUSSION

Aphids: Milk as whole, i.e., the higher concentrations (0.5X & X) showed significantly reduced number of aphids at 24 hours. The percentage mortality was 51 and 67 per cent and found significantly different from control (12%). Similarly, at 48 hours, both the higher concentrations (X & 0.5X) showed reduced number of aphids and the percentage mortality was 88 and 70 per cent (Table 1). These results showed that milk had the potential to control aphids, when applied as whole. Behaviour response of aphids via choice–no choice test revealed that average percentage of aphids reached to cow's milk treated leaves at 30 and 90 min of observations were non-significantly different from untreated control, whereas at 180 minute 40 per cent aphids reached to untreated leaves (Fig. 1), which had significantly more value than treated

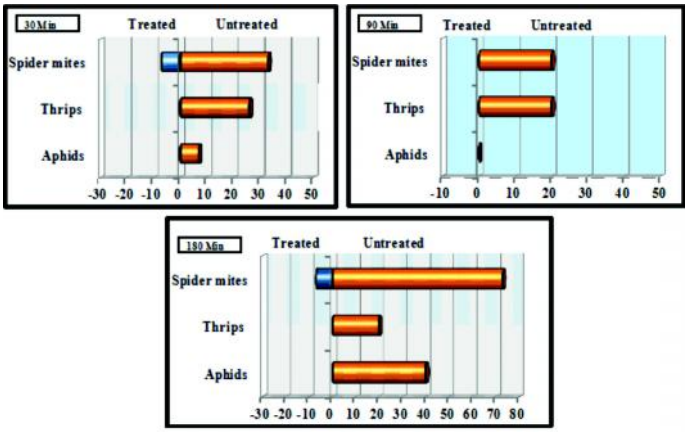

Fig. 1. Average percentage of rose pests reached to specific choices cow milk treated and untreated leaves (X concentration) at different time intervals.

Table 1. Per cent mortality of aphids, thrips and spider mites after 24 and 48 hrs on cow milk (3 concentrations) treated rose leaves.

Cow milk concentration	Mortality (%) after hrs					
	Aphid		Thrip		Spider	
	24	48	24	48	24	48
X (whole)	66.7±2.9a	87.8±4.0a	82.2±6.3a	100.0±0.0a	83.3±4.1a	94.4±3.6a
0.5X	51.1±5.3a	70.0±6.5ab	68.9±6.8a	88.9±4.8a	66.7±8.3a	80.5±6.9ab
0.25X	33.3±5.0b	50.0±6.9b	71.1±7.5a	84.4±5.6a	63.9±7.3a	75.0±4.1b
Control	12.5±3.9c	25.0±4.2c	28.3±6.2b	35.0±5.6b	4.1±2.8b	6.2±3.2c
F (df), P treatment	30.3 (3,38), <0.0001	26.9 (3,38), <0.0001	13.9 (3,38), <0.0001	27.4 (3,38), <0.0001	41.2 (3,38), <0.0001	84.1 (3,38), <0.0001
F (df), P treatment (Welch)	40.9 (3,18.5), <0.0001	38.1 (3,18.3), <0.0001	13.2 (3,18.9), <0.0001	-	87.9 (3,16.8), <0.0001	116.5 (3,18.2), <0.0001

leaves (χ^2 =8.32, Prob>ChiSq=0.0039). Choice- no-choice results indicates that when two choices (milk treated or untreated rose leaves) were given to aphids and these were left for some time with the choices, initially they did not make any choice up to 90 minutes but later at 180 minutes, about 40 per cent reached the untreated rose leaves.

Thrips: Milk at all the concentrations tested against thrips caused high mortality at 24 and 48 hours after application. At 24 hours, it caused 71, 69 and 82 per cent mortality at 0.25, 0.5 and X concentrations, which was significantly different from control (28%). The mortality after 48 hours of treatment with milk was 84, 88 and 100 per cent at 0.25X, 0.5X and X concentrations (Table 1). In choice-no-choice experiment average percentage of thrips reached on cow's milk treated rose leaves at 30, 90, 180 minutes was non-significantly different from those reached to untreated leaves**.** These results indicates that in presence of milk treated leaves along with untreated leaves thrips were unable to make any choice. This may be because of deterrent effect of milk and for that reason only, the mortality of thrips found very high as they were unable to perform normal actions (choice of food) in presence of milk.

Spider mites: All the tested concentrations of milk caused high mortality of mites at 24 and 48 hours after application. At 24 hours, it caused 64, 67 and 83 per cent mortality at 0.25, 0.5 and X concentrations and differed significantly from control (4%). The mortality after 48 hours of treatment was 75, 81 and 94 per cent at these concentrations. The results proved that milk was highly effective against spider mites (Table 1). In preference-non-preference test average percentage of spider mites on cow milk treated rose leaves at 180 minutes was 6.7 per cent as compared to 73.3 per cent on untreated leaves (χ^2=8.32, Prob>ChiSq=0.0039**)**. Choice-no-choice results indicates that when two choices (milk treated or untreated rose leaves) were given to mites at 30 and 90 minutes only 33 and 20 per cent, reached to untreated leaves, whereas at 180 minutes, 73 per cent spider mites were present on untreated leaves. This preference for untreated leaves indicates the high repellency towards milk by spider mites (Fig. 1).

Predators: Milk at all the concentrations had no influence on lady beetles, when observed at 24 and 48 hours after application. Survival of lady bird beetle was cent per cent at all the concentrations tested even after 48 hours of treatment (Fig. 2A). Similar results were obtained when milk treated leaves were given to minute pirate bugs (Fig. 2B). The results revealed that milk was almost safe for these predators and could be compatible for use along with other methods in pest control practices for rose.

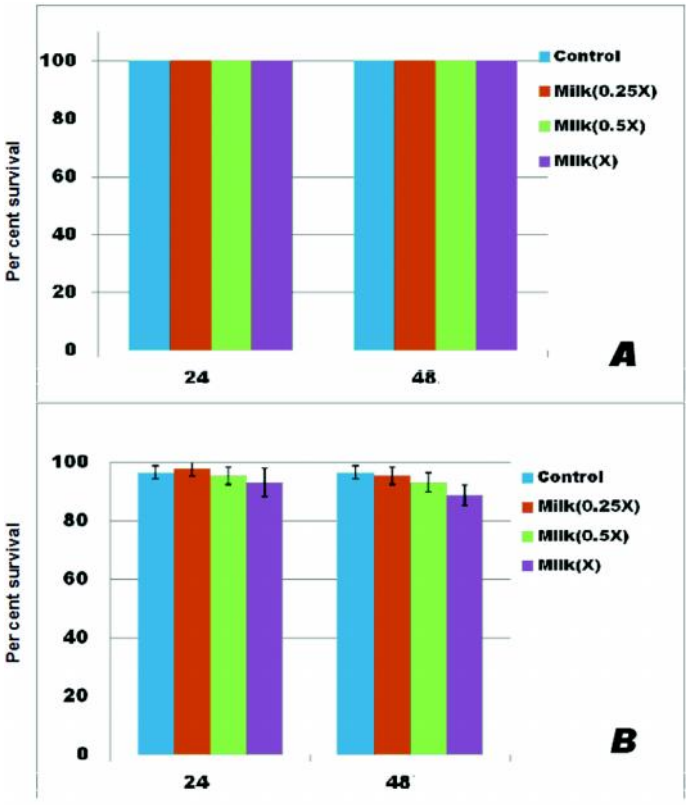

Fig. 2. After treatment in hrs percentage survival of Lady bird beetle (A) and minute pirate bugs (B) on milk treated rose leaves at 24 and 48 hrs after treatment

Results of above experiments revealed that milk has a potential to be used as a insect / mite control agent on roses, as it gave remarkable control of all the sucking pests of rose tested complete safe towards two generalist insect predators. In addition, repellent and deterrent effect of milk was also noticed with these pests. As very less information available showing insecticidal effect of milk in agriculture, results can not be compared with earlier findings. Although, Young (1982) documented that infestation of loopers that attack cabbage, cauliflower etc., can be controlled with the application of milk on plants as milk is a good medium for bacterial culture and these bacteria can paralyse the larvae's intestinal tract and brings death in 2 to 4 days. Besides milk, other products from cow had significant evidences of controlling insect pests. *Panchgavya*- a combination of five products (milk, ghee urine, curd & dung) reported effective in Jasmine, when sprayed at 3.0 per cent, two times during the crop season, doubled the fruit yield besides increasing the resistance to pest and diseases (Vivekanandan, 1999a). *Panchgavya* (1.0%) spray reduced the flower drop, increased fruit size, retained freahness and enhance taste in peach trees and also prevented fruit drops from green worms attack

(Vivekanandan, 1999b). A plethora of literature is available depicting role of cow urine against various insect pests (Nankinga *et al*. 1999; Ukey and Sarode, 2003) and plant diseases (Surender Kumar and Sehgal, 1998). Cow urine is recommended as an insecticide by mixing it with soil and applying on plants at much diluted concentrations (Anonymous, 2005). Field trials were conducted in *kharif* season for evaluating cow urine efficacy against stem borers and cost benefit in soybean production in comparison to conventional insecticide (chlorpyrifos) and biopesticide (Dipel). Highest cost benefit ratio (1: 18.9) was obtained from 5 per cent cow urine (Gupta and Yadav, 2006). Gupta and Yadav (2010) had shown the efficacy of cow urine against different lepidopterous pests of soybean. Different concentrations of cow urine with one conventional, pesticide were sprayed three times during crop season and results revealed that different concentrations of cow urine significantly reduced the number of *Spilosoma obliqua* and *Spodoptera litura* larvae in all plots. Higher concentration of cow urine caused significant reduction in whitefly, *B. tabaci* at seven days after first spray. Sadwarte and Sarode (1997) reported that the combination of cow dung and cow urine with half dose of insecticides was observed to have moderate impact on *H. armigera* on pigeonpea, while NSKE + half dose of insecticide was most effective treatment, but sole application of cow dung and cow urine were found ineffective. The efficacy of neem (*Azadirachta indica*) kernel extract in cow urine (NSKE 30 ml l^{-1}), neem oil (1.0%), dimethoate (0.045%), NSKE + dimethoate (0.03%), NSKE (3.0%) + dimethoate (0.03%), neem oil (1.0%) + dimethoate (0.03%) and neem kernel extract in cow butter milk (NCBM 20 ml l^{-1}) against *L. erysimi* on *Brassica juncea* has been noticed. The highest benefit cost ratio (30.1) was obtained with 2 % NCBM (Gupta and Rai, 2006).

It emanates from the study that the milk has potential to be considered as a highly effective product, which could use in sustainable agriculture as it does not have any toxic effect for the insect predators. In addition, milk showed high toxicity towards spider mites and thrips by reducing a significant percentage on treated rose leaves. It was moderately toxic to aphids and could be recommended only at higher concentration, i.e., milk as whole. Overall milk has a good possibility for use in control of sucking pest complex of rose in greenhouse IPM with totally safe effect towards its predators.

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