Control of flea beetle, *Phyllotreta nemorum* L. (Coleoptera: Chrysomelidae) using locally available natural resources

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Aqueous extracts of six different plants (*Acorus calamus, Ageratum conyzoides, Azadirachta indica, Duranta repens, Spilanthes acmella* and *Urtica dioca*) and diluted animal urine (buffalo and cow) were tested for mortality rate of flea beetle (*Phyllotreta nemorum*) in the laboratory. Results were compared with the effects of commercial neem product (neem azal) on flea beetle mortality. The host plant taken for the study was radish (*Rhaphanus sativus*). Three concentrations of aqueous plant extracts (1kg/5 l, 1kg/10 l and 1kg/20 l of water), three concentrations of animal urine (20%, 15% and 10%) and two concentrations of neem azal (0.1% and 0.01%) were tested in three replications. Observations on the beetle mortality were made at 24 hrs and thereafter on alternate days for a week (168 hrs). All tested concentrations of *S. acmella,* buffalo urine and cow urine were effective in flea beetle control; *A. calamus, A. indica* and *U. dioca* were significantly better in controlling flea beetle ($P<0.05$), but only at the highest concentrations tested. The best treatments from *in-vitro* experimentation (the highest concentrations of *S. acmella*, buffalo urine and cow urine) were evaluated further *in vivo*. Results showed that all three treatments were effective in controlling the flea beetle ($P<0.05$).

Key words: Cattle urine, marati, neem, neem azal, radish

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Introduction

The flea beetle (*Phyllotreta nemorum*) is a widespread and common pest of cruciferous plants. Frequently it is serious pest in seedbeds and on newly transplanted vegetables. The adults feed on the cotyledons and leaves of young plants; feeding produces a shot hole effect. Occasionally seedlings may be completely destroyed. The larvae live in the soil and feed upon the roots of the host plants but do little damage.

Three species of flea beetles are reported from Nepal: *P. cruciferae, P. nemorum* and *Monolepta signata* (Vaidya 1995). Control of the flea beetle is a problem in many parts of the world. Fan and Huang (1991) included *Phyllotreta* species as serious pest in Taiwan. Various control measures, such as seed dressing with BHC or treatment with DDT, BHC or Derris dust, are in practice for the control of the flea beetle. Turnoc and Turnbill (1995) reported the development of resistance by the cruciferous flea beetle (*P. cruciferae*) towards insecticides including carbofuran, carbaryl, oxanyl, methamidofos and endosulfan. Fan and Huang (1991) also have noted the development of resistance by the insect. Along with resistance problems, there are many problems entailed in the application of chemical pesticides such as health hazards, environmental effects, adverse effects on non-target organisms, and destruction of natural enemies. Therefore, it is necessary to search for alternative methods to control the flea beetle in an eco-friendly manner. This paper reports on the use of natural agents such as plant- and animal-based products in controlling the flea beetle, *P. nemorum*.

Materials and methods

Experiments were first carried out in the laboratory using test cages and then repeated in the field using those treatments found to be successful in the laboratory. The field trials were carried out in Pokhara Valley, Kaski district, Nepal, from March to June 1999. Testing was performed on adult flea beetles (*P. nemorum*). Insects were collected from the cruciferous plants (especially radish) in the study area. Radish (*Rhaphanus sativus*) was chosen for testing because it can be cultivated easily and it allows effective assessment of flea beetles during the test. Transparent plastic bottles 7.5 cm high by 6 cm in diameter were used as test cages. The mouths of the bottles were covered with muslin to prevent the insects from escaping. Six pesticidal plants and 2 animal products were tested. Selection of the plants and animal products was based on information collected from local farmers; abundance and availability were taken into consideration. The selected plants were *Acorus calamus* (Bojho), *Ageratum conyzoides* (Ganmane ghans), *Azadirachta indica* (Neem), *Duranta repens* (Nil kanda), *Spilanthes acmella (*Marati) and *Urtica dioca* (Stinging nettle). Buffalo urine and cow urine were the selected animal products. The natural resources were collected from the experimental site in Pokhara Valley. Neem azal (Azadirachtin), a commercial neem product provided by Trifolio-m-Gmbh, Germany, was the only formulated compound tested.

For the preparation of an aqueous extract, a fixed amount of chopped plant parts was ground and soaked in water in polythene bags. The soaked materials were allowed to settle in the shade. After 48 hrs, the materials were squeezed and then filtered. The residue was again mixed with water and squeezed and filtered. This process was repeated three times. The filtrate was collected and diluted to make the required solution **(Table 1)**.

Laboratory tests were carried out by spraying radish leaves with the various extracts, urine and neem azal, and placing them inside the experimental cages separately. Ten beetles were

placed in each cage bottle. The mouths of the bottles were covered with muslin cloth for aeration and to prevent insect from escaping. The leaves inside the cage were replaced daily with leaves to which the same treatment had been applied at the beginning of the experiment. The experiment was continued for 168 hrs of spray application.

For the field experiment, three blocks of equal size (4.5 m x 1 m) were prepared. Each block consisted of four plots. A distance of 50cm was maintained between blocks and between plots. Each plot was of size 100 cm by 75 cm. Twenty plants were planted in each plot. Treatments were randomly arranged.

In the laboratory, the treatment was applied using a syringe. The volume of spray solution per leaf was about 3 to 5 ml. In the field, a hand sprayer was used for spraying. The rate of treatment application was controlled by adjusting walking speed. The distance between the nozzles and the plant tips was about 40-50 cm during application. The applied spray volume corresponds to 500 ml/plot. The time of application of test materials was between 3 pm to 4 pm. All the applications were made under natural weather conditions.

For assessment of mortality in the laboratory, three replications were used for each treatment. The effect of treatments on the flea beetle was recorded at 24 hrs, 72 hrs, 120 hrs and 168 hrs of treatment application. The three most effective treatments, as assessed in the laboratory study, were used in the field tests. Five plants in each plot were selected randomly for observation. The number of live flea beetles on these five plants was noted before treatment and 24 hrs after treatment application and then on alternate days for a period of one week.

The mortality coefficient (MC) value was estimated following Abbott (1925):

$$MC = [(T-C)/ (100-C)] 100$$

Where, T= Percentage mortality in control
C= Percentage mortality in treatment

In the laboratory, the percentage mortality of the flea beetle for the various treatments at varying concentrations 24 hrs and 168 hrs after treatment was analyzed by two-way ANOVA. In the field, the number of flea beetles per plant was used to estimate the mortality variance.

TABLE 1. Experimental materials and concentration of preparation used in the study

Experimental material	Concentrations		
	C_1	C_2	C_3
Fresh leaves of *Ageratum conyzoides, Azadirachta indica, Urtica dioca*	1Kg/5l	1Kg/10l	1Kg/20l
Fresh rhizome of *Acorus calamus*	1Kg/5l	1Kg/10l	1Kg/20l
Fresh fruits of *Duranta repens*	1Kg/5l	1Kg/10l	1Kg/20l
Fresh flower heads of *Spilanthes acmella*	1Kg/5l	1Kg/10l	1Kg/20l
Buffalo urine	20%	15%	10%
Cow urine	20%	15%	10%
Neem azal	0.1%	0.01%	

Results

Laboratory experiment

Variation in percentage mortality with time

In all treatments mortality occurred in the flea beetles. The percentage of mortality was higher in various treatments than in control and highest mortality occurred with Neem azal **(Figure 1, 2, 3)**. The mortality value gradually increased from the beginning of the treatment, and after 168 hrs, the values reached 76.7% for *S. acmella*, 73.3% for buffalo urine and 66.7% for cow urine at C_1 concentration. At C_2 concentration, it was 70%, 66.7% and 60% for *S. acmella*, buffalo urine and cow urine respectively. The percentage mortality data when analyzed for treatment effect showed a significant difference ($p<0.05$) between treatment concentrations and among treatments.

TABLE 2. Mortality coefficient of flea beetle by treatment and concentration in laboratory

Treatment	Mortality coefficient of flea beetle					
	24 hrs after treatment			168 hrs after treatment		
	1Kg/5l (C_1)	1Kg/10l (C_2)	1Kg/20l (C_3)	1Kg/5l (C_1)	1Kg/10l (C_2)	1Kg/20l (C_3)
Acorus calamus	12.3	1.8	1.8	41.7	33.3	29.2
Ageratum conyzoides	12.3	1.8	1.8	33.3	25	20.8
Azadirachta indica	8.8	5.3	1.8	45.8	27.5	25
Duranta repens	12.3	5.3	1.8	33.3	29.2	20.8
Spilanthes acmella	19.3	8.8	15.8	70.8	62.5	45.8
Urtica dioca	1.8	5.3	1.8	37.5	29.1	25
Buffalo urine	22.8	19.3	12.3	66.7	58.3	50
Cow urine	19.3	15.8	5.3	58.3	50	45.8
Neem azal	57.9	5.3		79.2	12.5	
Control	5%			20%		

Mortality coefficient of flea beetle

Mortality coefficients of flea beetles for each treatment at 24 hrs and 168 hrs after treatment application were calculated. The mortality coefficient increased with increase in concentration in all cases except in the case of *S. acmella* and *U. dioca* at 24 hrs of treatment application.

Mortality coefficients for all treatments after 168 hrs of treatments application were found to be greater than MC values at 24 hrs of treatment application **(Table 2)**. All concentrations of *S. acmella*, buffalo urine and cow urine showed significant effects. C_1 concentration of *S. acmella*, buffalo urine and cow urine showed MC values of 70.8, 66.7 and 58.3 respectively, which are close to the value for neem azal (79.2).

Field experiment

Percentage reduction in flea beetle population

At 24 hrs of treatment application, the number of flea beetles per plant decreased

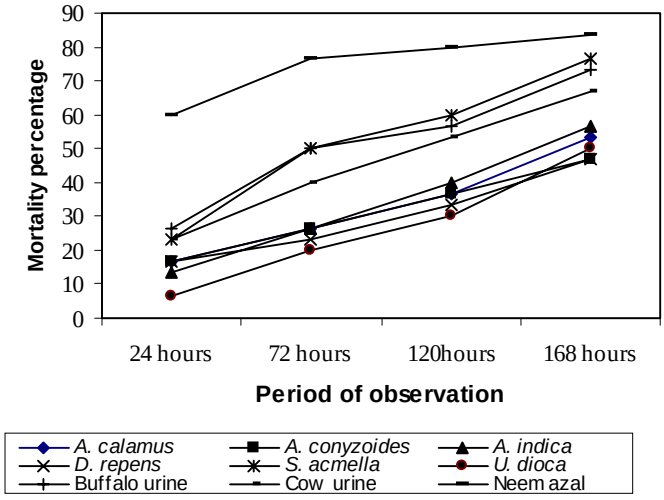

FIGURE 1. Percentage mortality of flea beetle for different treatments with respect to duration of treatment at C_1 concentration (1 kg/5 l) in laboratory

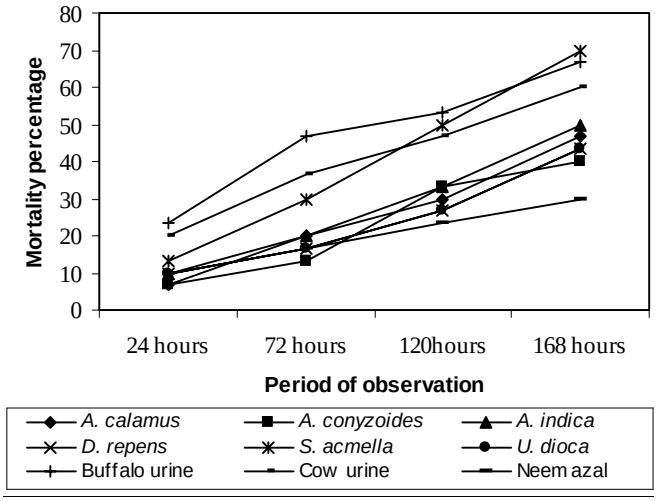

FIGURE 2. Percentage mortality of flea beetle for different treatments with respect to duration of treatment at C_2 concentration (1 kg/10 l) in laboratory

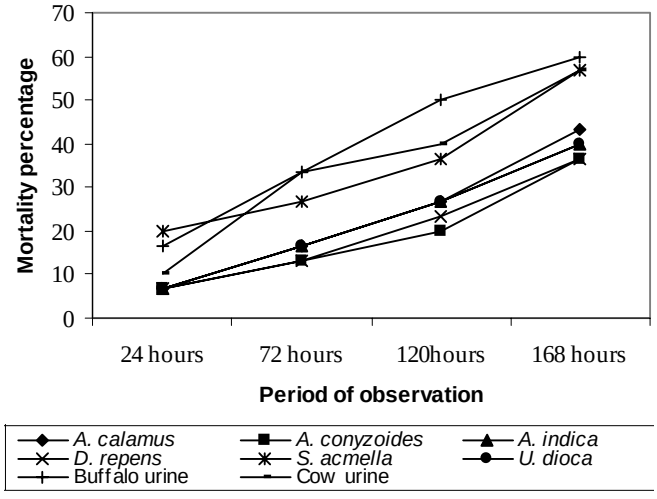

FIGURE 3. Percentage mortality of flea beetle for different treatments with respect to duration of treatment at C_3 concentration (1 kg/20 l) in laboratory

by 76% with cow urine, 74.5% with *S. acmella* and 55.7% with buffalo urine whereas in the control plot the value corresponds to 10.1% **(Figure 4)**. The highest reduction in flea beetle population was recorded in plots treated with cow urine. One week after treatment, there was a significant reduction in flea beetle populations (buffalo urine 75.4%, cow urine 75% and *S. acmella* 70.9%), while in the control plot the number of flea beetle per plant remained more or less stable throughout the study period **(Figure 4)**.

Variation in population per plant with respect to time

In the field, the population of flea beetles was greatly reduced in all treated plots compared to those in control plots. In the control plot, there was a slight fluctuation in the number of live flea beetle per plant. Flea beetle population per plant at the end of experiment was found to be the least on plants treated with cow urine (1.8 insects/plant). Buffalo urine (2.0) and *S. acmella* (2.1) were the second and third most effective treatments. However, in the control plot, there was only a slight change in populations, from an average of 9.2 before treatment to 8.9 one week after treatment **(Figure 5)**. The differences among the treatments were statistically significant

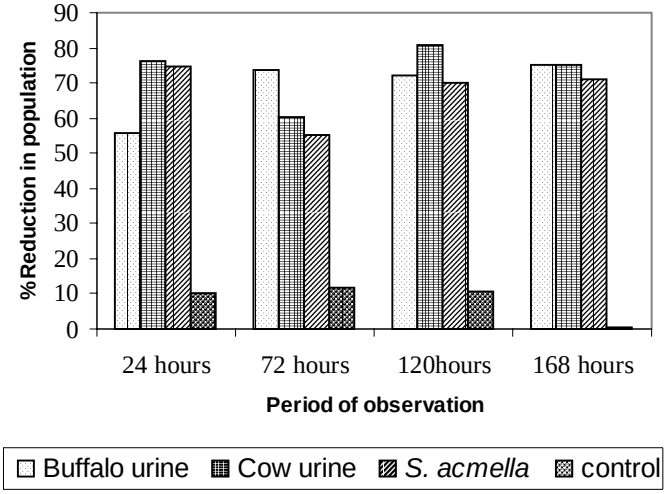

FIGURE 4. Percentage reduction in flea beetle population after treatment at C_1 concentration (1 kg/5 l) in field

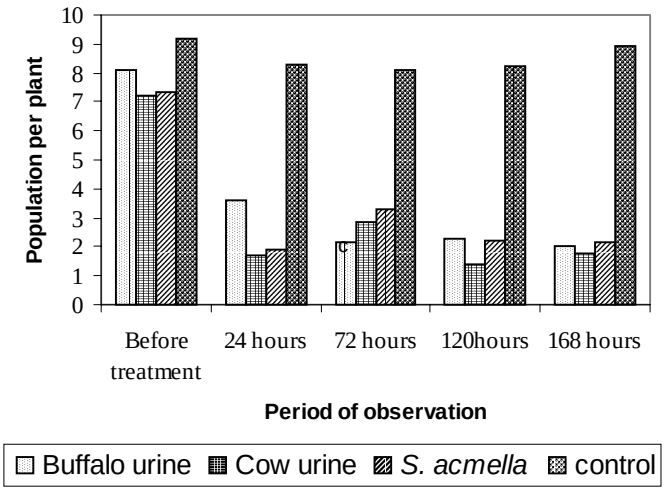

FIGURE 5. Flea beetle population per plant with respect to duration of treatment at C_1 concentration (1 kg/5 l) in field

($p<0.05$). However, treatments were not significant at 1% level at 24 hrs of treatment application. The effect was highly significant ($p<0.01$) after 168 hrs of treatment application.

Discussion

The study shows that all the tested natural resources possess pesticidal properties to some degree or other. *A. calamus, A. indica, S. acmella, U. dioca*, buffalo urine and cow urine are proved effective agents for flea beetle control; the effect of *A. conyzoides* and *D. repens* was not significant.

All tested concentrations of *S. acmella* showed significant results. Kadir et al. (1989) also showed that extracts of *S. acmella* were toxic against adult American cockroach (*Periplaneta americana*). The pesticidal property of *S. acmella* is due to its active component Spilanthol (Kadir et al. 1989). The N-isobutyl amides from flower buds of *S. acmella* were effective against *Aedes aegypti* larvae and *Helicoverpa zea* neonates at 12.5 and 250 µg/ml concentration respectively (Ramsewak et al. 1999).

Regmi and Karna (1998) have shown that *A. calamus* and *A. indica* have pesticidal value. Powdered rootstock of *A. calamus* has been reported effective as an insecticide, repellent and contact poison, and *A. indica* as a plant of multifarious pesticidal values (Regmi and Karna 1998). Joshi and Paneru (1999) described *A. calamus, A. conyzoides, A. indica* and *U. dioca* as plants with potent insecticidal properties and *A. indica* is effective against the flea beetle. Palaniswamy and Wise (1994) reported that neem-based products are effective with high mortality or repellency against the crucifer flea beetle (*P. cruciferae*). The pesticidal property of *A. indica* is due to the active principle, the limnoid azadirachtin. Azadirachtin is the most potent natural insect antifeedant, which suppresses insect feeding at concentration of less than 1 ppm (Ishman et al. 1991).

Cow urine and buffalo urine both showed significant results at all concentrations. Cow urine is traditionally widely used in Nepal for various purposes, including religious, ritual and medical applications, and insect control. According to Vaidya (1993), cow urine is the most effective solution for the control of *Lipaphis erysimi, Myzus persicae* and *Dorylus orientalis*. Budhathoki (1992) reported that diluted cow urine applied on broad leaf mustard significantly reduces powdery mildew. Farmers use cow urine in various concentrations (1:2 to 1:5) as curative plant protection measures against aphids of cowpea and bean and late blight of potato and tomato (Gyawali et al. 1994).

In the laboratory, no tested natural resources showed significant results at 24 hrs of treatment application. However, in the field, there was marked population reduction at 24 hrs of treatment application. It may be due to the repellent effect of different treatments. The effects persist up to one week and there was remarkable population reduction in the field even 168 hrs after treatment application. ■

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