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The Efficacy of Bio-rational Compounds against the Diamondback Moth, Plutella xylostella (L.) (Lepidoptera: Plutellidae) in Nepal 【Research report】

應用生物合理性成分防治尼泊爾小菜蛾 Plutella xylostella (L.) (Lepidoptera: Plutellidae) 之效果評估【研究報告】

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Abstract

This study evaluated the efficacy of using the urine of four animals (cow, buffalo, goat, sheep), a urine-mix (cow urine and plant leaves) and Borerguard, a commercial entomopathogenic product containing Beuveria bassiana and NPV, against the diamondback moth (DBM), Plutella xylostella under field conditions in Nepal. The urine of these animals controlled the DBM significantly better than the control. Urine-mix had a better efficacy against the DBM. The plot treated by the urine-mix had the least number of cabbage leaves damaged, followed by the plot treated with cow urine and then the plot treated with goat urine. The use of Borerguard performed better than any of the other treatments. During the study, two commercial traps (Wotta T trap and Delta sticky trap) also were evaluated to monitor the DBM. The total number of DBMs trapped in the Wotta T trap was significantly higher than in the Delta sticky trap. This study revealed that nature-based products could be a viable option for organic farming.

摘要

評估四種動物 (乳牛、水牛、山羊及綿羊) 尿液、乳牛尿液混拌樹葉、一種結合昆蟲病原Beuveria bassiana及NPV的市售產品 (Borerguard) 對尼泊爾田間小菜蛾Plutella xylostella防治之功效。結果顯示，相對於控制組而言，以混拌動物尿液防治具顯著降低小菜蛾族群之效果，並較只使用單一動物尿液處理更能大幅降低小菜蛾在甘藍菜上的危害；然而在所有處理中又以Borerguard防治效果最為顯著。實驗亦測試兩種市售捕捉器 (Wotta T trap and Delta sticky trap) 在監測小菜蛾的效果，結果顯示Wotta T trap防治率顯著性高於Delta sticky trap。此研究顯示，使用天然成分能有效的害蟲防治，且有助於有機農業的發展。

Key words: Plutella xylostella, urine, cabbage, DBM, traps

關鍵詞: Plutella xylostella、尿液、甘藍菜、DMB、捕捉器。

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The Efficacy of Bio-rational Compounds against the Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Nepal

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ABSTRACT

This study evaluated the efficacy of using the urine of four animals (cow, buffalo, goat, sheep), a urine-mix (cow urine and plant leaves) and Borerguard, a commercial entomopathogenic product containing *Beuveria bassiana* and NPV, against the diamondback moth (DBM), *Plutella xylostella* under field conditions in Nepal. The urine of these animals controlled the DBM significantly better than the control. Urine-mix had a better efficacy against the DBM. The plot treated by the urine-mix had the least number of cabbage leaves damaged, followed by the plot treated with cow urine and then the plot treated with goat urine. The use of Borerguard performed better than any of the other treatments. During the study, two commercial traps (Wotta T trap and Delta sticky trap) also were evaluated to monitor the DBM. The total number of DBMs trapped in the Wotta T trap was significantly higher than in the Delta sticky trap. This study revealed that nature-based products could be a viable option for organic farming.

Key words: *Plutella xylostella*, urine, cabbage, DBM, traps

Introduction

Cabbage and cauliflower contribute 28% to the total vegetable production in Nepal. Cabbage alone is cultivated on 27,840 ha, which is almost 12% of the total land area of Nepal (Timila *et al.,* 2007; Anonymous, 2010). Cabbage can tolerate more extreme temperatures than cauliflower. As such, it is an important cash-generating vegetable crop in Nepal. In Nepal, farmers can get a higher return

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by growing off-season cabbage. However, in recent years the production and productivity has been declining because of several factors, including insect pest damage (Budhathoki *et al.,* 2004; Gurung, 2005).

The diamondback moth (DBM), *Plutella xylostella* is a serious pest of cruciferous crops throughout the world (Talekar and Shelton, 1993). Although the moth originated in the Mediterranean region, it has crossed all natural barriers and is believed to have become a global pest. It even has been reported on non-cruciferous crops like *Ameranthus viridis* (Vishakantaiah and Visweshwargowda, 1975). However, this pest exhibits a marked preference for cabbage and cauliflower as these crops have fleshy and succulent leaves and provide the necessary olfactory and gustatory stimuli for successful selection and colonization (Chand and Choudhary, 1977; Dubey and Chand, 1977). One of the main factors for low productivity as a result of insect pests in Nepal is due to the DBM. They cause heavy losses in the production of cole crops in Nepal (Anonymous, 1997; Anonymous, 1998). The attack by a large number of larvae hinders the growth of the plant and leads to a reduction in production of about 31-100% (Cardleron and Hare, 1986).

In Nepal, farmers routinely use several different chemical insecticides to combat this pest. These insecticides are hazardous to growers and consumers alike, and also create environmental problems (Anonymous, 1998; Kafle, 2002). In Nepal, the DBM has developed resistance to many of the commonly used insecticides, making it one of the most difficult pests to manage (Anonymous, 1998). Under these circumstances, alternative control measures such as the use of botanicals, nature based or microbial control might be a possible and viable option for the sustainable management of the DBM in Nepal. The adoption of organic practices is not only important but it also makes economic sense since it would utilize locally available resources (Anonymous, 2001).

Animal urine could be used to control the agricultural pests (Kafle, 2000, 2002; Kafle and Bhattarai, 2002). However, to-date there has not yet been a study report on the application of animal urine to control the DBM. Therefore, this study evaluated the efficacy of the urine of four animals (cow, buffalo, goat, sheep), and a urine-mix (cow urine and plant leaves) and compared them with Borerguard, a commercial entomopathogenic product containing *Beuveria bassiana* and NPV, against the DBM under field conditions in Nepal.

Materials and Methods

Monitoring the DBM

To monitor the DBM population dynamics during the study period, three Wotta T traps (Pest Control India, Bangalore, India) and three Delta Sticky Traps (Pest Control India, Bangalore, India) were placed randomly at 70 cm above the ground in the study area. The trapped DBM male moths were collected each week from each trap, counted and compared. The study site, Tathali, Bhaktapur, Nepal, was located at 27° 40.37' N latitude and 85° 24.01'E longitude at an elevation of 1,300 masl (meters above sea level) with a subtropical climate.

Efficacy of bio-rational products against the DBM

The urine of four animals (cow, buffalo, goat and sheep), a urine-mix (fermented cow urine and plant leaves) and borerguard (a commercial entomopathogenic product containing *Beuveria bassiana* and NPV) (Somphytopharma Limited, Andhra Pradesh, India) and an untreated control were evaluated (with three replications) as a treatment against the DBM.

Thirty days older "Green Coronet" variety of cabbage were transplanted into the field in plots measuring 3.15 × 2.4 m

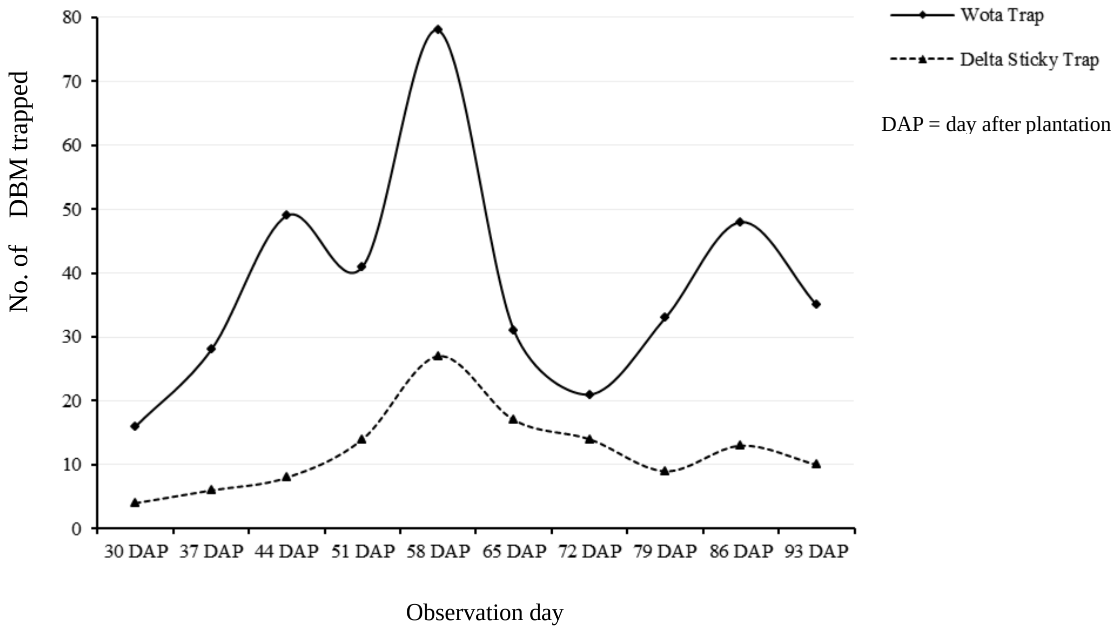

Fig. 1. Total no. of DBM male moths trapped by the Wotta T trap and Delta Sticky trap in Bhaktapur, Nepal.

using recommended cultural practices (Anonymous, 2011). Then each experimental plot was sprayed 4 times at 10 day intervals.

The animal urine (cow, buffalo, goat and sheep) was collected separately in a plastic container and was kept for 9 days to allow the urine to ferment. Prior to application the urine was diluted with water (1 urine: 4 water) and sprayed at the rate of 600 L/ha with a hand sprayer (Aspee, V-2007, Knapsack, Kolkata, India).

The urine-mix was a locally-made product using the leaves of local plants, *Urtica dioca*, *Artemisia indica*, *Azadirachta indica*, *Melia azadirach*, *Eupatorium adenophorum*, *Agave cantula* and *Sapium insigne*. Five-hundred grams of leaves from each plant were mixed and then chopped into small pieces (~1 cm length) and then mixed with 7 L of cow urine, packed in a black plastic container, and left for 21 days under room conditions allowing it to decompose and ferment. The urine-mix obtained after 21 days was then filtered and sprayed after being diluted with water (1 urine mix: 4 water) by hand sprayer.

The post-treatment observations were recorded for six plants that were randomly selected and tagged 3, 7 and 10 days after spraying (DAS). Although, the data were recorded at 3, 7 and 10 DAS, only the data of 10 DAS are used in this report.

A total of thirty plants per treatment (10 plants per plot) were used to estimate the final yield of cabbages for each treatment. The harvesting was done at 105 days after planting (DAP).

All studies were replicated three times. The means were compared using Tukey's HSD test (SAS, 2009).

Results

Monitoring the DBM

When the number of adult DBMs trapped by Wota T trap and Delta sticky

Table 1. Effects of different natural products on the control of DBM and the production of cabbages under field conditions in Nepal

Materials tested	Application (Mean ± SE)				No. damaged leaves/plants (Mean ± SE)	Production (kg/plant) (Mean ± SE)
	1st spray (40 DAP)*	2nd spray (50 DAP)	3rd spray (60 DAP)	4th spray (70 DAP)		
Cow urine	6.48 ± 1.64a	6.77 ± 1.82ab	6.84 ± 0.73ab	6.09 ± 0.74bc	3.50 ± 0.36bc	2.41 ± 0.03ab
Buffalo urine	6.95 ± 1.41a	8.00 ± 0.73ab	8.48 ± 0.81ab	7.44 ± 0.81bc	4.83 ± 0.59b	2.19 ± 0.01c
Goat urine	6.81 ± 0.79a	7.78 ± 0.68ab	8.19 ± 1.29ab	7.17 ± 0.99bc	3.87 ± 0.64bc	2.30 ± 0.02bc
Sheep urine	6.83 ± 0.73a	8.13 ± 0.71ab	8.91 ± 0.95ab	8.19 ± 0.96a	5.08 ± 0.48b	2.05 ± 0.03d
Urine-mix	5.79 ± 0.84a	5.76 ± 0.61ab	5.49 ± 1.26b	4.47 ± 0.68b	3.03 ± 0.40bc	2.53 ± 0.04a
Borerguard	4.89 ± 1.15a	5.13 ± 0.74b	4.86 ± 0.84b	4.02 ± 0.58b	2.30 ± 0.27c	2.48 ± 0.05ab
Control	8.40 ± 1.54a	9.97 ± 0.62a	11.31 ± 1.69a	10.20 ± 0.81a	6.51 ± 0.35a	1.80 ± 0.04e
p	0.58	0.04	0.02	0.001	0.0003	0.0001

Mean with the same letters in a column is not significantly different at 5% by Tukey test (SAS, 2010).

* DAP = days after plantation

trap during the entire study period were compared, the total number of DBMs (380) trapped by the Wota T trap was significantly higher than the total numbers of DBMs (122) trapped by the Delta sticky trap ($F = 1029.34$, $p = 0.0001$). Although, the average number of adults trapped by both traps differed, the trend of the population dynamics was almost the same. In both traps, the number of adults increased in an increasing trend, then reached their peak at 62 DAP and then declined gradually in small increments at 86 DAP. The Delta sticky trap captured 122 adults per observation, which was 3.1 times higher than that of the Wota T trap (Fig. 1)

Efficacy of bio rational products against the DBM

After the first spray (40 DAP), the number of DBM larvae observed in each of the treatments were not significantly different ($F = 0.8$, $p = 0.58$). The percentages of DBM larvae controlled by cow urine, buffalo urine, goat urine, sheep urine, urine-mix and Borerguard in the control plot were 22.9, 17.3, 18.9, 18.7, 31.1 and 41.8%, respectively (Table 1).

After the second spray (50 DAP), a significantly smaller number of larvae were observed in the Borerguard treated plot compared to the control plot ($F = 3.04$, $p = 0.04$). The percentages of DBM larvae controlled by cow urine, buffalo urine, goat urine, sheep urine, urine-mix and Borerguard in the control plot were 32.1, 19.8, 22, 18.5, 42.2 and 51.54%, respectively (Table 1).

After the third spray (60 DAP) and the fourth spray (70 DAP), significantly less numbers of larvae were observed in the Borerguard treated plot followed by the urine-mix and cow urine treated plots compared to the control plot (third spray: $F = 3.8$, $p = 0.02$; fourth spray: $F = 7.08$, $p = 0.001$). After the third spray, the percentages of DBM larvae controlled by the cow urine, buffalo urine, goat urine, sheep urine, urine-mix and Borerguard in the control treatments were 39.5, 25, 27.6, 21.2, 51.5 and 57%, respectively, and after the fourth spray they were 40.3, 27.1, 29.7, 19.7, 56.2 and 60.6%, respectively (Table 1).

Effect of animal urines on the yield of cabbage

At the end of the study (96 DAP), after counting the number of cabbage leaves damaged by DBM larvae the highest number of damaged leaves were found in the control plot followed by the plot treated by sheep urine, buffalo urine, goat urine, cow urine, urine-mix and then the one treated with Borerguard. The percentages of leaves damaged by DBM larvae in the plots treated by cow urine, buffalo urine, goat urine, sheep urine, urine-mix and Borerguard over the control treatment were 46.2, 25.8, 40.6, 22, 53.5 and 64.7%, respectively (Table 1).

When the cabbages were harvested and the average weights of the cabbage yield for each treatment were compared, the cabbages in the urine-mix-treated plot were the heaviest followed by the plots treated by Borerguard, cow urine, goat urine, and buffalo urine. The percentages of cabbage weight gained in the plots treated by cow urine, buffalo urine, goat urine, sheep urine, urine-mix and Borerguard over those in the control treatment plot were 33.9, 21.7, 27.8, 13.9, 40.6 and 37.8%, respectively (Table 1).

Discussion

Borerguard and urine-mix resulted in the highest mortality of DBM larvae during this study followed by cow urine, goat urine, buffalo urine and sheep urine. Sheep urine caused the lowest mortality and urine-mix had the highest performance among all the treatments. Anonymous (2007) also reported similar results for the urine-mix. The efficacy of Borerguard was always better than the rests of the treatments. Borerguard contains *B. bassiana* and NPV, and *B. bassiana* has been reported as an efficient control agent against the DBM (Nath, 2001; Sood *et al.*, 2001). Similarly, Padmavathamma and Veeresh (1991) revealed an 84% control of DBM by NPV. Sood (2001) also reported that the percentage mortality increased with the increased amount of Borerguard.

Borerguard was found to be more effective than other treatments due to its toxic effects to the larvae. This effect was seen shortly after application because of the quick action of NPV and also because it lasts longer than the other treatments, which is due to the self-multiplication of *B. bassiana* and NPV and their slow action against host. The urine-mix was also found to be effective for controlling the larvae. The urine-mix is not only toxic to the DBM larvae but it also repels both larvae and adults, making it an efficient method to control the DBM larvae.

The 21 days fermented urine-mix might have a number of chemical compounds that may control the DBM, but their identity are still unknown. A detailed study and analysis to determine the effective bioactive chemical compounds of urine-mix and other materials used in this study and their efficacy need to be carried out. Sheep urine and buffalo urine were less effective than the other treatments against the DBM. This might be due to the different chemical composition of the urine of these various animals. Subedi and Vaidya (2003) stated that cow urine and buffalo urine provided significant control against the flea beetle, *Phyllotreta nemorum*.

The numbers of wrapper leaves damaged in the cow-urine treated plots were less than in the rests of the plots. The cabbages in the urine-mix and cow urine treated plots were vigorous with less damaged leaves, and their yield was closest to that of the Borerguard treatment. Cow urine contains high levels of nitrogen thereby increasing the plant growth (Katsvairo *et al.,* 2003).

Burubai and Eribo (2012) reported that cow urine contains urea, ammonia, growth hormones, uric acid, and minerals like iron, potassium, magnesium, calcium, copper and nitrogen. However, the applicability and potency of cow urine as a bio-pesticide depends on the fermenting time and dosage. Melon aphids and pickleworms can be controlled using 14 days fermented cow urine. Another study in East Africa also reported that cow urine was effective to control *Ootheca bennigseni* in *Phaseolus vulgaris* (Paul *et al*., 2007). Four compounds (3- and 4-methyl phenol, 3-ethylphenol, 3-n-propylphenol and 2-methoxyphenol) have been identified from fresh cow urine (Spath, 1995; Bernier *et al*., 2000; Kweka *et al*., 2011). The toxic and/or repellent effect of cow urine to DBM during our studies could be due to the activities of those compounds.

The higher numbers of DBM adults caught by the Wotta T trap were mainly due to the larger entrance compared to the Delta sticky trap. In addition, although the reasons for it are unknown, more non-DBM insects were also trapped in the Delta sticky trap compared to the Wotta T trap.

The highest DBM population was recorded at 62 DAP and then declined suddenly due to the rainfall right after the observation. The DBM population is negatively correlated with rainfall (Rao and Lal, 2005). The Wotta T trap was found to be effective for monitoring the DBM and could be an efficient monitoring tool for making a decision regarding DBM management in Nepal.

In conclusion, the urine-mix resulted in the highest net yield followed by Borerguard and then cow urine. Therefore, the application of urine-mix and cow urine could be efficient and sustainable techniques for Nepalese farmers due to the cost effectiveness and pest control efficacy.

The Wotta T trap was found to be effective for monitoring the DBM and could be a good tool in the management of the DBM in Nepal.

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應用生物合理性成分防治尼泊爾小菜蛾 *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) 之效果評估

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摘　　要

評估四種動物（乳牛、水牛、山羊及綿羊）尿液、乳牛尿液混拌樹葉、一種結合昆蟲病原 *Beuveria bassiana* 及 NPV 的市售產品 (Borerguard) 對尼泊爾田間小菜蛾 *Plutella xylostella* 防治之功效。結果顯示，相對於控制組而言，以混拌動物尿液防治具顯著降低小菜蛾族群之效果，並較只使用單一動物尿液處理更能大幅降低小菜蛾在甘藍菜上的危害；然而在所有處理中又以 Borerguard 防治效果最為顯著。實驗亦測試兩種市售捕捉器 (Wotta T trap and Delta sticky trap) 在監測小菜蛾的效果，結果顯示 Wotta T trap 防治率顯著性高於 Delta sticky trap。此研究顯示，使用天然成分能有效的害蟲防治，且有助於有機農業的發展。

關鍵詞：*Plutella xylostella*、尿液、甘藍菜、DMB、捕捉器。

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