USE OF ENTOMOPATHOGENIC FUNGI AND NEEM BIO-PESTICIDES FOR BRASSICA PESTs CONTROL AND CONSERVATION OF THEIR NATURAL ENEMIES

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Abstract

Field trials were conducted on-station at Kenya Agricultural Research Institute, Thika between 2005 and 2007, to evaluate the effect of an entomopathogenic fungus, *Beauveria bassiana* 1x 108cfo/gram (Biopower®) and neem (*Azadirachta indica* Juss) oil extracts, Azadiractin 0.15%w/w (Achook®) and Azadirachtin 0.03%w/w (Nimbecidine®) against diamondback moth, *Plutella xylostella* and aphids, *Brevicoryne brassicae L.,* and *Myzus persicae* (Kalt) and *Lipaphis erysimi* (Sulz) in cabbage. The trial design was randomized complete blocks with six treatments replicated four times. The treatments included three different rates of the entomopathogenic fungus, the two neem products and the untreated (control). Pest infestations showed significant (p=0.05) reduction of aphids and diamondback moth during the first and second cabbage crop season when the bio-pesticides were applied either at the higher or recommended rates. However, during the third crop season all the bio-pesticides treatments significantly reduced pest infestation. There was an increase in marketable weight of cabbage in all the bio-pesticide treatments over the control. Natural enemies were recorded in all the treatment plots. These included predators (ladybird beetles, syrphid flies, spiders) and parasitic wasps, *Diaeretiella rapae* and *Diadegma semiclausum*. Use of these bio-pesticides at either the recommended or higher rate would offer an important component of integrated pest management strategy for Brassica in Kenya.

Key words: Aphids, *Beauveria bassiana*, Diamondback moth

Introduction

Cabbage, *Brassica oleracea* var capitata is the most important local market vegetable in Kenya (KARI-Thika, 2005). The crop is grown mainly by smallholder farmers for cash and subsistence in all the arable areas above 800 m above sea level (KARI-Thika, 2005). Production of the crop is hampered by several abiotic and biotic constraints. Among the biotic agents, insect pests and diseases pose a major constraint. The diamondback moth, *Plutella xylostella* (L.) and aphids *Brevicoryne brassicae* L., *Lipaphis erysimi* (Sulz) and *Myzus persicae* (Kalt) are the most important insect pests of Brassica (Nyambo and Pekke, 1995). The pests infest the cabbage plant at all the growth stages causing defoliation, leaf curling and stunting of the plant. Severe infestation by these pests usually cause economic crop losses and may result in 100% yield loss. To control these pests, various methods are available (Arouiee and Karimzadeh, 2006; van Schoor and Stassen, 2006). Natural enemies of diamondback moth and aphids such as ladybird beetles, lacewings, syrphid flies and parasitic wasps have been reported to exert appreciable control where they occur in significant numbers (Gichini *et al*., 2008). However, in Kenya, chemical pesticides are predominantly used resulting in the well known adverse effects associated with pesticide. Indiscriminate application of broad spectrum chemical pesticides exterminates these susceptible natural enemies and leaves behind the pests that are more resistant to pesticides. Bio-pesticides are pest management tools derived from natural resources that could be an important component of integrated pest management strategy. Due to their environmental safety and specificity, bio-pesticides are gaining popularity in the management of pests of horticultural crops (Feng *et al*., 1994). In Kenya, entomopathogenic fungi *Metarhizium anisopliae* has been reported to control onion thrips (Maniania *et al*., 2003) while *Beauveria bassiana* has recently been tested for the control of banana weevil, *Cosmopolites sordidus* (Omukoko, 2010). Similarly, extracts of neem, *Azadirachta indica* have the advantage of having very little side effects on non-target organisms, including natural enemies. In Kenya, bio-pesticides contribute less than 2% of pesticides used in Kenya, while chemical pesticides account for the rest (Wabule *et al*., 2004). With the recently developed protocols for registration of bio-pesticides in Kenya their registration is expected to increase. This study assessed the effectiveness of a new entomopathogenic fungus and two registered neem bio-pesticides to control major brassica pests and conservation of their natural enemies with the aim of commercializing the bio-pesticides as components of an integrated pest management strategy for brassica in Kenya.

Materials and Methods

Field establishment

Field trials were conducted for three cabbage crop seasons between the year 2005 and 2007 at KARI-Thika. The cabbage crop was raised in nursery and transplanted after one month. Data collection commenced three

weeks after transplanting. First season trial data collection commenced from 15th August to 26th September 2005, while the second season data commenced from 10th February 2006 to 25th August 2006 and third season from 2nd May to 11th June 2007. The aim of the trials was to assess the effectiveness of entomopathogenic fungi, *Beauveria bassiana* 1x108cfo/gram (®Biopower) and neem oil extracts Azadirachta 0.03%w/w (®Nimbecidine) and Azadirachta 0.15%w/w (®Achook) against the control of diamondback moth and aphids. The entomopathogenic fungus, *Beauveria bassiana* causes white muscadine disease in insects (Groden, 1999). When spores of this fungus come in contact with the insect cuticle, they germinate and grow directly through the cuticle to the inner body of their host even without being consumed and proliferates throughout the host's body producing toxins, and eventually killing it. Once the fungus has killed its host, it grows back out through the softer portions of the cuticle, covering the insect with a layer of white mold that gives rise to its common name 'white muscadine disease'. Neem products work as insect growth regulators, anti-feedants, oviposition deterrents, sterilants, repellents and comprise residual insecticidal properties. Cabbage var. Copenhagen market, a popular variety reported to be susceptible to diamondback moth infestation (Waiganjo, 1998) was used. All the recommended agronomic practices for cabbage production were used as described by Anon., 1989. The experimental design was randomized complete blocks with six treatments replicated four times. The treatments included Biopower® at the higher rate (6g/l), recommended rate (5g/l) and a lower rate (4g/l) compared with neem products, Nimbecidine® at 2.5g/l, Achook® at 1.5ml/l and untreated control (water only). The plot sizes were 3x3m separated by a 1metre path between the plots and 2metre between the blocks.

Data collection and analysis

Pest monitoring commenced three weeks after transplanting and continued fortnightly thereafter until the crop matured. Treatment application commenced immediately after pre-treatment data collection. Pest infestation data collected included larvae and pupae counts of diamondback moth per plant in 5 randomly selected plants per plot and aphid population assessed through score rating from 0=no infestation to 5=severe infestation. Parasitoids were retrieved from cabbage leaves infested with mummified aphids or diamondback moth in each treatment plot. One cabbage leaf infested with either aphid or larvae of diamondback moth was placed in a plastic petridish containing a moist filter paper for parasitoid emergence. Predators (spiders, lacewings and ladybird beetles) observed in each plot were recorded during pest sampling. Marketable weight of cabbage was assessed during harvest by removing all infested leaves from each cabbage in the two inner rows of each plot, consisting 12 cabbage plants. The crop was subjected to natural pest infestation. Statistical data analysis was carried out using SAS (2000) software. Analysis of variance (ANOVA) was performed to compare pest infestation and yield among the treatment options and means separated using Student-Newman-Keuls (SNK) multiple range test at P=0.05.

Results

In all the three cabbage crop season trials, aphids and diamondback moth were recorded infesting the crop. In addition, natural enemies recorded included the braconid*, Diaeretiella rapae*, recovered from mummified aphids, hymenopteran wasps *Diadegma semiclausum,* recovered from diamondback moth and predators (ladybird beetles, syrphid fly and spiders). The natural enemies were observed in all the treatments and their population was not significantly different among the treatments (Table 1).

During the first cabbage crop season, the overall aphid infestation varied between 1.88 and 3.2 score in the higher rate Biopower® and untreated plots respectively (Table 2). Significantly higher aphid numbers were recorded in the untreated plots than all the bio-pesticide treatments except Biopower® at the lower rate (4g/l). However, when used at either higher rate or recommended rate, Biopower® showed the highest efficacy in aphid control during the first and second crop season trials. Aphid infestation during the second season was low varying between 0.8 and 2.1 in the recommended (5g/l) Biopower® and the untreated plots

Table 1: The effects of treatments on Beneficial insects and their population means ±s.e in three cabbage crop seasons at KARI-Thika 2005-2007.

Treatment	*Diadegma semiclausum*	*Diaeretiella rapae*	Predators (spiders, syrphid fly, ladybird beetle)
Biopower® 6g/l	1.15± 0.23	0.01 ±0.01	2.00 ±0.01
Biopower® 5g/l	1.09± 0.01	0.02 ±0.01	1.01± 0.01
Biopower®4g/l	1.11± 0.12	0.04 ±0.02	2.01± 0.00
Nimbecidine®	0.95± 0.13	0.04 ±0.00	1.92 ±0
Achook®	1.04± 0.41	0.01 ±0.01	1.01± 0.01
Control (water only)	1.19±0.21	0.02±0.00	2.4±0.01
P	0.891	0.264	0.733
c.v	28.68	34.19	22.13

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respectively. Aphid infestation during the third season varied from 2.2 and 3.8 recorded from Achook® and untreated plots respectively. During the third season, Achook®, Nimbecidine® and Biopower® at the recommended rate were significantly most effective bio-pesticides in that order of efficacy (Table 2).

Table 2: The effect of treatments on aphid infestation on cabbage crop in three crop seasons at KARI

Mean ±se aphid infestation scores on cabbage/treatment			
Treatment	Season1 (15/08/2005 to 26/09/2005)	Season2 (10/07/2006 to 25/08/2006)	Season3 (2/05/2007 to 11/06/2007)
Biopower® 6g/l	1.88±0.21b	0.89± 0.02a	3.28±0.21b
Biopower® 5g/l	2.08±0.11b	0.87 ± 0.09b	2.48±0.11bc
Biopower®4g/l	2.03±0.08ab	1.76 ± 0.67ab	2.65±0.10b
Nimbecidine®	2.09±0.125b	1.10 ± 0.65ab	2.54±0.13bc
Achook®	2.09±0.09b	1.15 ± 0.67ab	2.23±0.10c
Control (water only)	3.20±0.08a	2.11 ± 0.51a	3.80±0.09a
P	0.0001	0.012	<0.0001
c.v	41.92	23.99846	14.26

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Means within a column marked by the same letter were not significantly different at P=0.05

The effects of the treatments on diamondback moth infestation is shown below (Table 3). During the first crop season, Nimbecidine® treated plots recorded significantly least pest infestation (2.08) followed by Biopower® when applied at higher rate (3.12), Achook® and Biopower® at the recommended rate (3.19) while Biopower® at the lower rate recorded highest infestation (4.38) and did not significantly differ from the untreated plots during the first and second season. Similar trend in efficacy of the treatments was followed during the third season. However, ®Achook recorded the highest efficacy (0.03) followed by Nimbecidine® (0.77) and Biopower® at the recommended rate (0.93/plot) in that order but not significantly different from each other. Diamondback moth infestation ranged between 0.03 and 1.48 during the third crop season (Table 3).

The effects of the treatments on the marketable weight of cabbage during the three crop seasons (Table 4) showed that application of Biopower® at either the higher or recommended rate resulted in the highest marketable weight of cabbage, but not significantly different from all the other treatments. However, during the second season, significant difference was recorded with the highest marketable weight in plots treated with Biopower® at either the higher or recommended rate. In the third season, Achook® followed by Biopower® at the higher and recommended rate recorded the highest marketable weight in that order but not significantly different from the control plots. Marketable weight of cabbage per plot did not show significant difference among the treatments except during the second season.

Table 3: The effect of treatments on diamondback moth infestation on cabbage crop in three seasons at KARI

Mean ±se diamondback moth infestation numbers (larva and pupae) on cabbage/treatment			
Treatment	Season1 (15/08/2005 to 26/09/2005)	Season2 (10/07/2006-25/08/2006)	Season3 (2/05/2007 11/06/2007)
Biopower® 6g/l	3.12±0.188b	8.12 ± 0.12b	0.95±0.11b
Biopower® 5g/l	3.19±0.088b	8.06 ± 1.32b	0.93±0.09b

Biopower®4g/l	4.38±0.221ab	9.47 ± 1.26ab	1.05±0.11b
Nimbecidine®	2.08±0.25c	7.94 ± 0.95b	0.77±0.10b
Achook®	3.180±0.23b	9.00 ± 1.11b	0.03±0.11b
Control (water only)	5.81±0.29a	10.24 ± 1.65a	1.48±0.10a
P	0.0001	0.0316	<0.0001
c.v	42.53	50.63158	25.62

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Means within a column marked by the same letter were not significantly different at P=0.05.

In this season, the highest marketable weight of cabbage was recorded in plots where Biopower® was applied at either higher rate (20.90Kg/plot), or recommended rate (20.50Kg/plot). The untreated plots had the least marketable weight (15.93Kg/plot) among the treatments. Similarly, the untreated plots had lowest marketable weights of cabbage while plots treated to Achook® recorded the highest marketable weight (22.25kg) followed by Biopower® at the higher rate, Biopower® at the recommended rate, Nimbecidine® and lastly Biopower® at the lower rate. All the biopesticides treatments resulted in substantial marketable weight increase during this season. Plots treated with Achook® recorded the highest percent marketable weight increase (105.8%) followed by Biopower® (94.3%) applied at the higher rate, ®Biopower at the recommended rate (61.4%), Nimbecidine® (37%) and Biopower® at the lower rate (11%).

Table 4:The effect of pesticide treatments on the marketable weight of cabbage in three field trials at KARI-Thika

	Mean ±s.e marketable weight of cabbage/treatment in Kg.		
Treatment	Season1 (15/08/2005 to 6/09/2005)	Season2 (10/07/2006 to 5/08/2006)	Season3 (2/05/2007 to 11/06/2007)
Biopower® 6g/l	29.8±3.94	20.90 ± 0.83a	21.00±4.98
Biopower® 5g/l	29.0±3.80	20.50 ± 0.58a	17.45±5.06
Biopower®4g/l	25.87±3.13	17.83 ± 0.33b	12.00±2.70
Nimbecidine®	26.3±5.10	18.87 ± 0.32ab	14.81±5.22
Achook®	26.3±4.13	18.27 ± 0.48b	22.25±5.32
Control (water only)	22.3±3.82	15.93 ± 0.22c	10.81±0.81
P	0.083	0.0007	0.3382
c.v	28.36	10.81278	51.88

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Means within a column marked by the same letter were not significantly different at P=0.05.

Discussion

All the bio-pesticides applied reduced pests (aphid and diamondback moth) infestation in all the crop. The effects on the pest populations varied among the treatments compared to the untreated plots. The pesticides also appeared to be less harmful to the aphid and diamondback moth parasitoids which were recorded from all the plots. Bio-pesticides are known to have little effect on predators and parasitoids. Application of Bio-power® at the higher rate (6g/l water) or the recommended rate (5g/l water) significantly reduced aphid and diamondback moth infestation in all the crop seasons. The bio-pesticides were comparable to Achook® and Nimbecidine® that are registered in Kenya for the control of aphids and diamondback moth respectively. In the current study the third season was characterized with low diamondback moth infestation and high marketable weight increase compared to the untreated control. However, the analysis did not show significant difference among the treatments including the untreated control probably due to the low diamondback moth infestation. Observation of substantial numbers and species of natural enemies in the untreated and bio-pesticide treated plots is an indication of compatibility of the tested products with the non target organisms. The results are in agreement with earlier studies by Waiganjo *et al*. (2008).

Conclusions and Recommendations

The entomopathogenic fungus, *Beauveria bassiana*, Biopower® was effective in the control of aphids and diamondback moth in cabbage when applied at either higher (6g/l) or recommended rate (5g/l) but less effective when applied at the lower rate. The neem products (Nimbecidine® and Achook®) were effective against the aphids and diamondback moth infestation and compared reasonably well with the entomopathogenic fungi. All the bio-pesticides treated plots showed substantial weight increase from the untreated plots. Significant difference was recorded during the second crop season. The bio-pesticides were not harmful to the natural enemies as compared to the untreated plots. From the results of the three crop seasons, it is recommended that

Biopower® be incorporated in integrated management of the major brassica pests in Kenya at the recommended rate (5g/l). Bio-prospecting should be continued to explore available biocontrol agents in our rich biodiversity as alternatives to chemical pesticides for sustainable pest management.

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