Research Article Open Access

Assessing and Enhancing the Impact of *Cotesia plutellae* in Management of Diamondback Moth *Plutella xylostella* on Kale *Brassica Oleracea* var. *acephala* in Semi-Arid Areas of Kenya

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Received
July 09, 2016

Accepted
April 25, 2016

Published Online
June 30, 2017

Abstract: Diamondback moth (DBM, *Plutella xylostella* L.) is a major pest of crucifers which can cause yield losses of up to 100% if left unmanaged. Classical biological control of DBM was adopted in Machakos and Kitui County through *Cotesia plutellae* imported from South Africa in 2002 and released in March 2004 in farmers' fields where kale *Brassica Oleracea* var. *acephala* is one of the major leafy vegetables. Preliminary studies in 2004-2006 showed that the parasitoid had not established in the release sites and augmentation releases were done in 2006. Monthly surveys were conducted (May 2012 to April 2013) to assess the spread and contribution of *C. plutellae* in the management of the DBM. *Brassica oleracea* var. *acephala* fields in twenty five randomly selected farms were sampled for number of DBM larvae and pupae, damage, parasitism and parasitoid guild. The data was recorded from 20 plants in each selected farm. The DBM larvae and pupae were placed in plastic containers and taken to the laboratory for DBM or parasitoid emergence. Results revealed that the mean number of DBM/plant in Kitui and Matuu ranged from 0.4 to 2.5 and 0.3 to 3.5, respectively. Mean damage score ranged from 1.2 to 1.6 in both regions, which was positively correlated to the number of DBM on *B. oleracea* var. *acephala*. Hymenopteran parasitoids recovered from DBM included *Cotesia plutellae*, *Diadegma semiclausum*, *D. mollipla*, *Apanteles* sp., *Chelonus* sp., *Oomyzus sokolowskii* and *Itoplectis* sp., while the hyper parasitoids included *Mesochorus* sp., *Pteromalus* sp., *Notanisomorphella* sp., *Eurytoma rosae* and *Eurytoma* sp., *Brachymeria* sp. and *Pediobius* sp. *Cotesia plutellae* was the most abundant parasitoid followed by *D. semiclausum*. Diversity of parasitoids varied between months and study sites. *Cotesia plutellae* had established and spread beyond the release sites contributing between 40 and 90% parasitism while the indigenous parasitoids *Itoplectis* sp., *Apanteles* sp., and *D. mollipla* contributed less than 5% parasitism. The results show the spread and establishment of *C. plutellae* in the study sites. In conclusion, there is need for continuous training and educating the farmers to conserve the parasitoids that have contributed in the reduction of the DBM and damage on the crop. This has contributed in the reduction on pesticides use, spraying regime and eventual reduction of cost of production and residues on the produce.

Keywords: *Plutella xylostella*, *Cotesia plutellae*, parasitoids, parasitism, kale

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Cite this article as: Kahuthia-Gathu, R., M. Mwangi and M.O. Fiaboe. 2017. **Assessing and enhancing the impact of *Cotesia plutellae* in management of diamondback moth *Plutella xylostella* on Kale *Brassica Oleracea* var. *acephala* in semi-arid areas of Kenya.** Journal of Environmental and Agricultural Sciences. 11: 01-08.

1 Introduction

The diamondback moth (DBM) *Plutella xylostella* (L.), (Lepidoptera: Plutellidae), is a cosmopolitan and one of the most important pest of cultivated Brassicas worldwide (Furlong et al., 2013). It is considered a major pest throughout eastern (Ayalew, 2006) and southern Africa (Kfir, 1997). In Kenya it has been established as the greatest impediment to cultivation of all Brassicas (Kahuthia-Gathu, 2011). The DBM is the most universally distributed of all Lepidoptera, and it can migrate over very long distances (Talekar and Shelton, 1993). Under unmanaged conditions, the pest can cause significant yield losses of up to 100%. The dependence on broad-spectrum insecticides and frequent spray regimes in the control of DBM by farmers in East Africa led to development of resistance to a wide range of synthetic (Kibata, 1996) and biological insecticides (Sarfraz, 2004). This prompted the search for alternative management tools such as better use of natural biocontrol. Parasitoids are known to play an important role in natural suppression of diamondback moth in cultivated and wild crucifer ecosystems. Mustata (1992) observed parasitic wasps of the genus *Diadegma* (Hymenoptera: Ichneumonidae) and *Cotesia* (Hymenoptera: Braconidae) as major mortality factors of DBM throughout the world. In the East African region, biocontrol-based Integrated Pest Management (IPM) strategies were introduced in 2001 specifically targeting the DBM (Nyambo and Löhr, 2005). After the successful introduction of *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae) into cabbage *B. oleracea* var. *capitata* L., production systems in the highlands of Kenya, Tanzania and Uganda was greatly improved (Löhr et al., 2007). There was a need to introduce a parasitoid that would be efficient under the semi-arid areas of Eastern Africa where *B. oleracea* var. *acephala* are grown in valley bottoms (Kahuthia-Gathu et al., 2009).

Cotesia plutellae (Kurdjumov) (Hymenoptera: Braconidae), a solitary larval parasitoid of DBM, was considered for introduction into East Africa. The species is known to be effective even in areas of heavy insecticide use (Ooi, 1992) which is a valuable attribute since farmers tend to spray against an array of crucifer pests like aphids and DBM. *Cotesia plutellae* was imported from South Africa into Kenya in March 2003 for release in the semi-arid areas of Eastern Africa. It is reported to be the most efficient parasitoid with up to 80% parasitism even in areas with hyperparasitism and it co-exists with a range of other parasitoids (Nofemela, 2013). In addition it is a good parasitoid for augmentative releases. In March 2004, one thousand laboratory reared *C. plutellae* were released in Yatta and Athi River delta in Machakos County. During the first post release survey in April 2005, the parasitoids *D. semiclaususm*, *D. mollipla*, *O. sokolowskii*, *Apanteles* sp., and *Brachymeria* sp. were recovered from DBM larvae and pupae sampled from *B. oleracea* var. *acephala* in Athi delta. However, the total parasitism rate was relatively low i.e., less than 15% (Kahuthia et al., 2009). *Cotesia plutellae* was recovered from DBM in Athi and Yatta delta between March and May 2006 with parasitism rates of 27% and 0.5%, respectively. Field releases were repeated between 2005 and 2006 in Kibwezi, Makindu, Mtito-Andei, Kitui and Loitokitok and Thika Districts. Field observations in Thika, Yatta and Kitui, between 2009 and 2011 indicated that *C. plutellae* was established and had spread beyond the release sites (Nyambo personal observations). However, its spread and impact on DBM populations in small holder *B. oleracea* var. *acephala* production systems and associated natural enemies remained undocumented. In the present study, establishment, spread and impact of *C. plutellae* on population densities of DBM, its distribution as well as parasitism rates in Kitui and Matuu are documented.

2. Materials and Methods

The research was conducted between May 2013 and April 2014 and aimed at assessing the impact of *C. plutellae* on the DBM in the *B. oleracea* growing areas of Machakos and Kitui County situated at 37° 34´ E and 1° 10´ S, and 38° 0´ E and 1° 24´ S, respectively. The farmers grow *B. oleracea* var. *acephala* throughout the year along river valleys or near water sources for supplementary irrigation. The study sites lies between 890 and 1,100 m above sea level and receives bimodal rainfall of between 500 and 900 mm per annum. The mean temperatures range from 26 to 35 °C and the areas comprises of clay to sandy soils. The studies were conducted in one year during the dry months of January to March, July to September and short rainy seasons from April to June, and long rains from October to December. However, most of the cropping is under irrigation since the rains are inadequate.

To evaluate the DBM populations and damage on *B. oleracea* var. *acephala*, and the impact of the introduced parasitoid *C. plutellae* on DBM, twenty five to thirty five farmer managed fields were randomly selected and surveyed every month. The locations of the sampled farms were geo-referenced for ease of mapping distribution and spread of the parasitoid. Twenty *B. oleracea* var. *acephala* plants in each chosen field were randomly selected as recommended by Smith and Shepard (2004) and physically checked for presence of DBM larvae. To evaluate DBM population, the identified *B. oleracea* var. *acephala* plants were physically checked and the number of DBM larvae and pupae counted and recorded. The *B. oleracea* var. *acephala* leaves were also physically assessed for DBM damage using a visual damage score of 1 to 5 (1 = no damage, 2 = up to 25% damage, 3 = 25–50% damage, 4 = 50–75% damage and 5 = greater than 75% damage) following the procedures described by Momanyi et al. (2006).

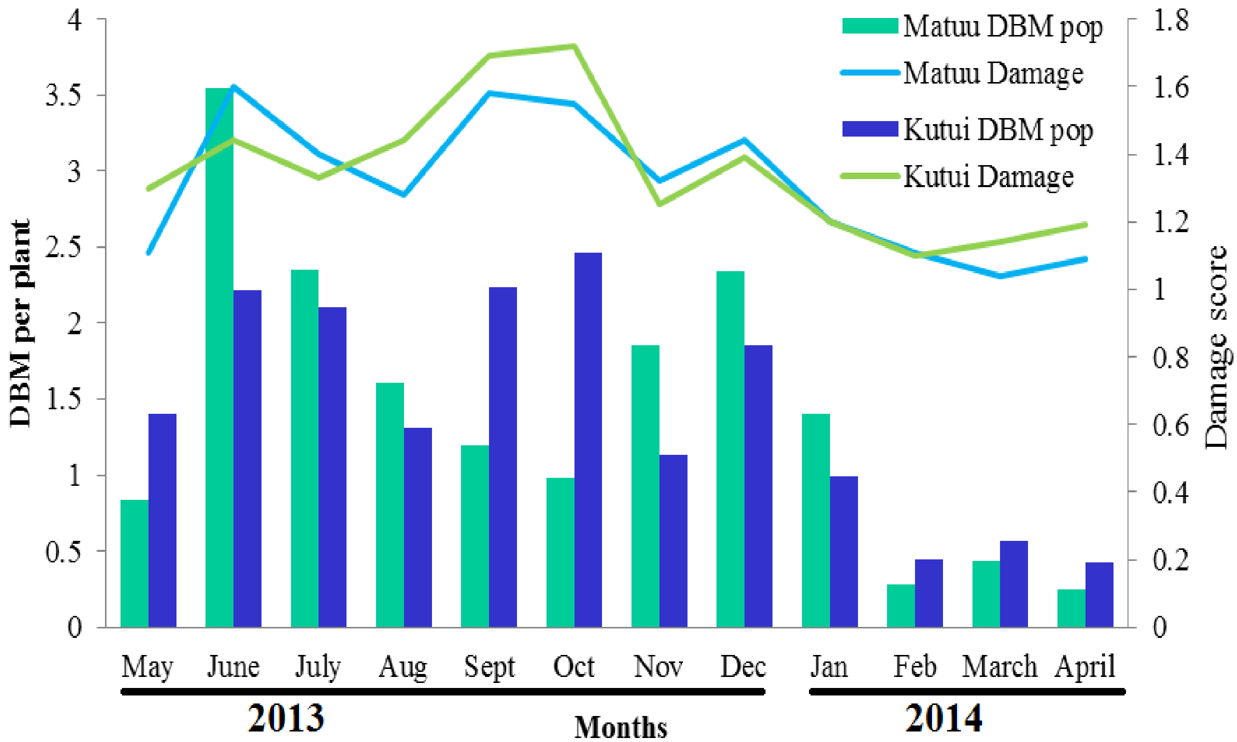

Fig. 1. Mean *Plutella xylostella* population per plant and damage score between May 2013 to April 2014 in Matuu and Kitui, Kenya

To evaluate the parasitism rates and parasitoid guild, the DBM larvae, pupae and parasitoid cocoons were collected, placed in well labeled plastic containers and taken to the laboratory for processing. The containers were lined with tissue paper to absorb excess moisture and closed with caps containing a fine muslin cloth to facilitate ventilation. The DBM larvae were kept in the Kenyatta University Agricultural laboratories at 23 ± 2 ºC, between 50-70 % relative humidity and a photoperiod of 12:12 hour (light:dark) cycle. The larvae were provided with fresh *B. oleracea* var. *acephala* leaves which were replaced after every two days until the larvae pupated or parasitoid cocoons formed. Samples of parasitoid cocoons and *P. xylostella* pupae were confined individually in glass vials (2.5×10cm) and observed daily for DBM, parasitoid or hyperparasitoid emergence. The parasitoid and hyperparasitoid species were identified, and their incidence was recorded. Parasitoid identification was based on wing morphology, using taxonomic keys as described by Azidah et al. (2000). All parasitoids and hyperparasitoids were preserved in ethanol (70%) for identification and confirmation. The incidence of parasitoids was used to calculate parasitism rates.

One-way ANOVA using general linear model (GLM) (SAS, 2004, SAS institute Inc.) was used to analyse the data on DBM infestations, damage scores, total primary parasitism, total hyperparasitism rates, and incidence per parasitoid species. The means were separated using the Student Newman Keuls test (SNK) test at P<0.05 (Sokal and Rohlf, 1995). Parasitism rates for solitary parasitoids were calculated as the sum of parasitoids divided by total number of adults (dead pupae+ DBM + parasitoids)*100.

3. Results

A mean population of less than 2 DBM per plant was recorded during the study period. Kitui recorded lower DBM numbers with a higher mean of 2.46 DBM per plant in October 2013 and lowest mean in April 2014. However, a higher mean of 3.5 DBM per plant was recorded in June 2013 and lowest of 0.25 DBM per plant noted in April 2014 at Matuu (Figure 1). Generally the damage on *B. oleracea* var. *acephala* was relatively low when the DBM populations were low. This was observed from January to April 2014 which was both dry and wet season. In Matuu, the DBM damage score ranged from 1.04 to 1.58 in March and October, respectively, while in Kitui it ranged around 1.1 in May 2013 and February 2014 with the highest mean of 1.72 DBM per plant recorded in October 2013 (Figure 1).

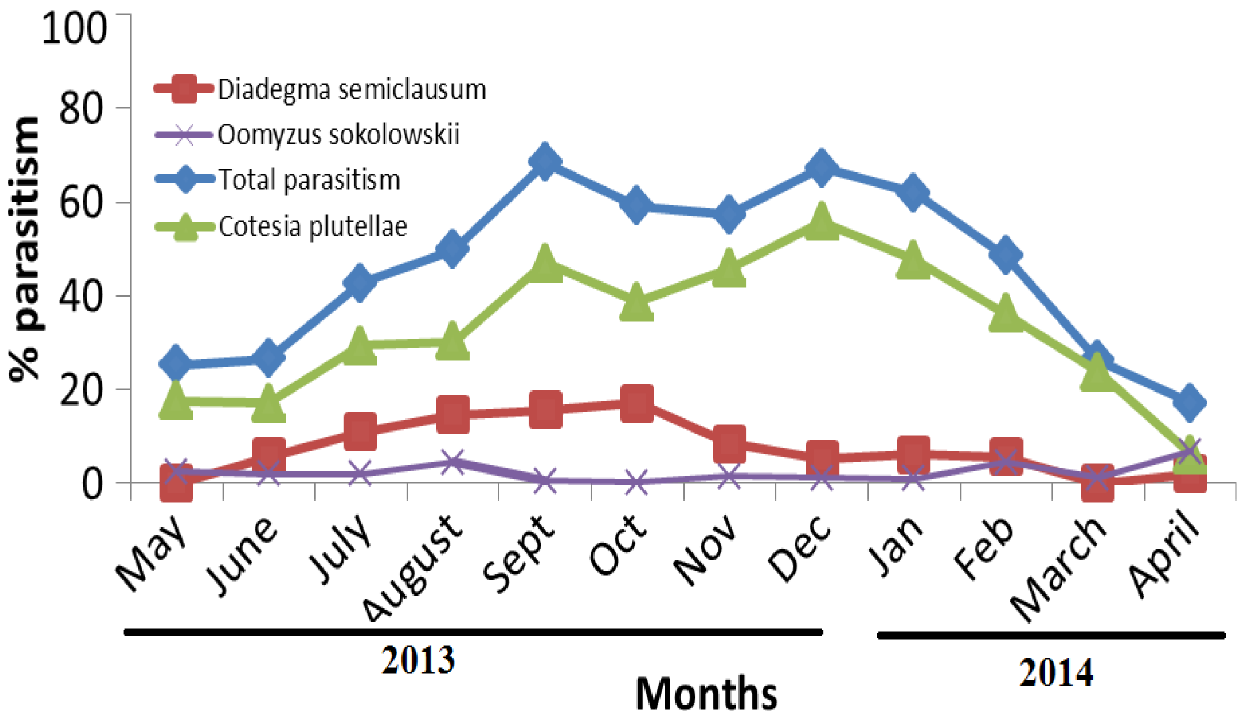

Fig. 2. Parasitism rates on *Plutella xylostella* found on *Brassica oleracea* var. *acephala* between May 2013 to April 2014 in Matuu, Kenya

Solitary and gregarious endo-parasitoids were recovered in both Matuu and Kitui throughout the study period from *B. oleracea* var. *acephala* which included the introduced parasitoids *C. plutellae* and *D. semiclausum*, the indigenous parasitoids *Itoplectis* sp., *O. sokolowskii* and *D. mollipla*. *Cotesia plutellae* were recovered throughout the study period and exhibited a wide seasonal variation in parasitism of *P. xylostella.*

However the numbers of *D. mollipla* specimens recovered from the sites were quite low. *Cotesia plutellae* was the most dominant parasitoid specie contributing to overall parasitism rates of 36 to 90%, and 55 to 92% in Matuu and Kitui, respectively (Fig. 2 and 3). Mean percentage parasitism by *C. plutellae* on DBM was significantly higher during the rainy season between November and December 2013 (50%) and least during the short rainy season between April and June (15%). There was significantly difference (*P* <0.001) between the two rainy seasons.

However, the parasitism rates were comparable during the dry seasons in the study sites. A number of hyperparasitoids such as *Eurytoma* sp., *Mesochorus* sp., *Pteromalus* sp., and *Mesochorus* species were recovered from the *C. plutellae and D. semiclausum* though their numbers were relatively low and did not contribute to overall parasitism rates.

4. Discussion

In the tropics and subtropical regions, the problem of DBM is usually most severe since Brassicas are cultivated throughout the year. The continuous cultivation and short life cycle which takes about 14 days can result in over 24 overlapping generations in a year. The rapid evolution of resistance to insecticides makes DBM particularly challenging to control in a sustainable fashion.

Unlike in Australia and the United States of America, where farmers use a threshold level of 1 DBM larva per plant (Furlong et al., 2004), no threshold levels for Brassica pests have been established in Kenya. The threshold of 1 DBM larva per plant is achievable with only biological control of DBM in some countries (Nofemela, 2013). The DBM counts from the present study were close to one larva/plant in all farms regardless of the season. In contrast to the previous results where Kahuthia-Gathu (2007), recorded between 0.6 to 5.2 DBM/plant in Athi River delta, and 0.6 to 2.9 DBM/plant in Yatta, Machakos County during the initial surveys before the release of *C. plutellae*. The reduction in DBM populations indicates that the introduction of *C. plutellae* has had a considerable impact on the reduction of DBM population density overtime.

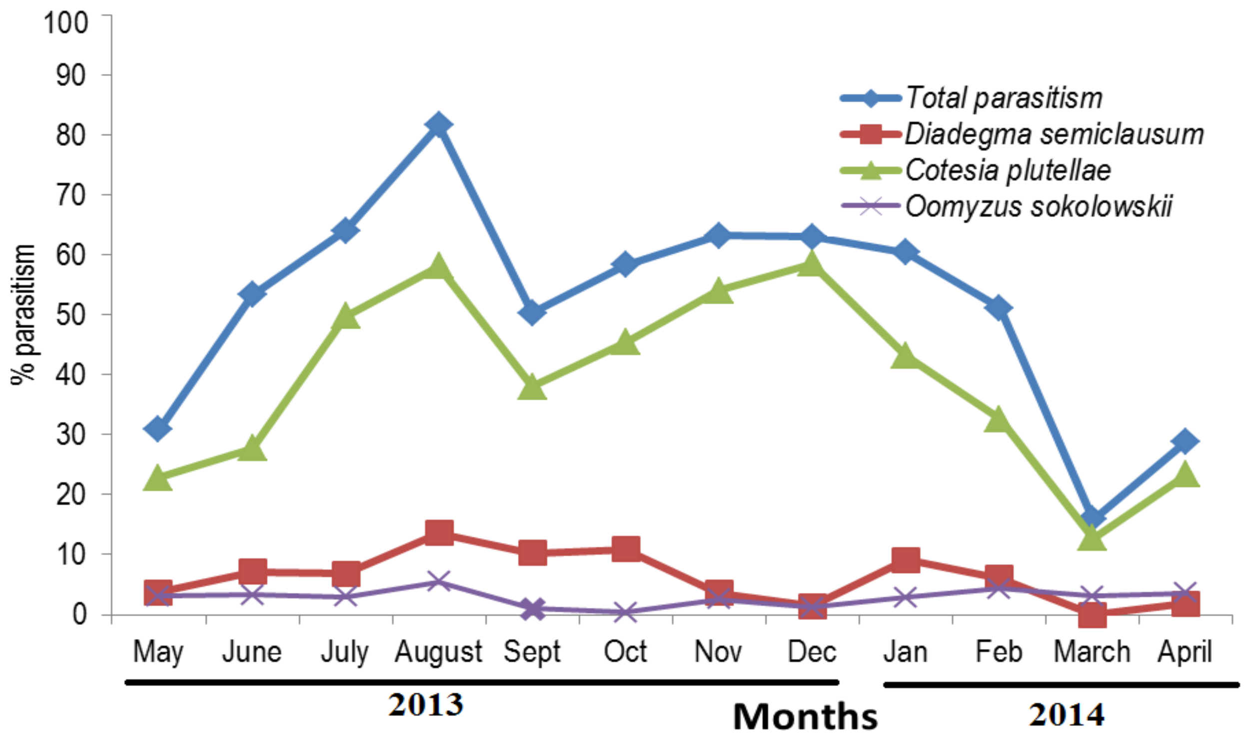

Figure 3: Parasitism rates on *Plutella xylostella* found on *Brassica oleracea* var *acephala* between May 2013 to April 2014 in Kitui, Kenya

There was a positive correlation between the mean number of DBM/plant and the level of damage with damage scores of around 1.2 associated with less than 1 DBM/plant. Similar results were observed by Kahuthia-Gathu et al. (2009) in the cabbage growing areas of Kenya where *D. semiclausum* had been released in 2002. A survey conducted before the release of *C. plutellae* in Yatta and Athi River delta in the semi-arid areas of eastern region indicated higher DBM population densities (5.2 DBM/plant in Athi River delta) and damage scores greater than 2. The number of *C. plutellae* in relation to the DBM on *B. oleracea* var. *acephala* was density dependent relationship. Similar observations were made on *B. oleracea* var. *capitata* where increase in diamondback moth numbers resulted in corresponding increase in parasitism (Goodwin, 1979; Cock, 1985; Alam, 1992). The ability of *C. plutellae* to increase with increasing DBM numbers proves its suitability for biological control and development of an IPM program for *P. xylostella* in *B. oleracea* var. *acephala* growing areas in Kenya. Similar observations were made Cobblar et al. (2012) on cabbages in Ghana.

The introduced exotic parasitoids *C. plutellae* and *D. semiclausum* were the most dominant species accounting for more than 90% of the primary parasitism levels in the study area. This is contrary to the previous observations where indigenous parasitoid *D. mollipla* was dominant (Löhr and Kfir, 2004) in the East African countries. Nofemela and Kfir (2005) and Manyangarirwa et al. (2009) reported similar dominance of *C. plutellae* in South Africa and Zimbabwe. Generally parasitism by the indigenous parasitoid species was low and this could have been attributed to competition from the two exotic parasitoids (Wang and Keller, 2002). This observation was previously confirmed by Kahuthia-Gathu et al. (2009) and Löhr et al. (2007) who showed that *D. semiclausum* is highly competitive and displaced the indigenous parasitoids from cabbage fields in the highlands of Kenya. *Oomyzus sokolowskii* was the only gregarious parasitoid recorded during the study although the parasitism rates were low (Figure 4 and 6). Similar observations on *Oomyzus* sp., were made by Nofemela and Kfir (2005) in South Africa in areas where *C. plutellae* was the dominant parasitoid. This is contrary to earlier studies that showed *Oomyzus* sp., parasitism rates of up to 21.2% in Yatta in 2005. The decline could be due to both competition and displacement of the indigenous parasitoids by the exotic introduced parasitoids. Few specimens of *Apanteles* species were recovered unlike in earlier studies where their parasitism rates ranged between 0.4 and 6.8 % in Yatta (Kahuthia-Gathu, 2011). The diverse parasitoid fauna attacking *P. xylostella* has also been recorded and varies widely between geographical locations (Shelton et al., 2002; Nofemela, 2013; Machekano et al., 2017). Biological control success has been recorded in a number of countries through introduction of *C. plutellae* (Talekar and Shelton, 1993) confirming the potential of this species in management of DBM.

Several hyperparasitoid species were recovered from *C. plutellae* and *D. semiclausum* with similar observations made in South Africa by Ullyett (1947) and Kfir (1997). However, their numbers were relatively low. Similar observations were made by Liu et al. (2000) and Mahmood et al., (2004), who observed that hyperparasitoids were of little significance and of no economic impact. Therefore, the hyperparasitoids recorded had not negatively impact of the overall parasitism of *C. plutellae* in regulating *P. xylostella* populations on cabbage, as their occurrence was low, and they were facultative species. This is contrary to the findings by Morallo-Rejesus and Sayaboc (1992), who noted that presence of hyperparasitoids could reduce effectiveness of *C. plutellae*.

5. Conclusion

The study confirmed presence and establishment of *C. plutellae* in all the sites in Kitui and Machakos Counties. The parasitoids had also spread beyond the release sites. The high parasitism rates of *C. plutellae* contributed to high quality products due to less damage by *P. xylostella*, a reduction on pesticides use, frequent spraying regime, cost of production and residues on the produce. There is need for further research on the impact of *C. plutellae*, spread and establishment in other semi-arid regions not covered in the current study where Brassicas are grown under irrigation. Therefore the farmer's savings can be redirected to other economic uses.

Acknowledgements: Authors wish to thank the National Commission for Science, Technology and Innovation (NACOSTI) for funding the project. Our sincere thanks go to the farmers from Machakos and Kitui Counties in Kenya for allowing us to conduct the research in their farms. We are grateful to the Vice chancellor, Kenyatta University for providing us with the infrastructure and laboratory space. Our sincere gratitude goes to Kenyatta University technical staff that assisted in the processing of the materials.

Conflict of Interest: The authors declare that they have no conflict of interest.

Author Contribution: R.K. initiated and designed the research, R.K. and M.W. performed the experiment and wrote the manuscripts; MOF assisted in performing the experiment. All the authors discussed the result and assisted in manuscript preparation and revision.

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