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An evaluation of fresh extracts from locally available pesticidal plants in Central Uganda

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An evaluation of fresh extracts from three locally available pesticidal plants in Central Uganda was carried out against two important cabbage insect pests in the field in order to assess their potency for pesticidal application. This followed the farmers' view that these plants could have pesticidal features as they had been observed to protect leafy crops against pests. Results suggest that *Euphorbia tirucalli* latex could reduce infestation of *Brevicoryne brassicae* below economic threshold levels. Extracts from *Jatropha curcas* and *Phytolacca dodecandra* likewise reduced *B. brassicae* levels but could not do so to required threshold levels. The same extracts were evaluated against diamondback moth *Plutella xylostella* but none was able to cause reduction of the moth larvae to economic threshold level. Only *E. tirucalli* latex caused reasonable management. It was concluded that *E. tirucalli* latex could be used as a management measure against *B. brassicae* and a contributory factor for management of *P. xylostella* infestations.

Key words: Fresh extracts, *Euphorbia tirucalli*, pest management, *Plutella xylostella, Brevicoryne brassicae,* cabbages.

INTRODUCTION

Due to high costs of synthetic pesticides, concerns over environmental pollution associated with continuous use of these chemicals and campaigns to 'live organic', there is a renewed interest in the use of botanicals for crop protection. Over the last few decades, there has been increasing focus on plant derived products to fight and reduce losses caused by agricultural pests and diseases (Devi and Gupta, 2000; Facknath, 2006; Ssekyewa et al., 2008; Tewary et al., 2005).

Most plant derived products are presumed to be less toxic to non target organisms, easily biodegradable and therefore do not persist in the environment as opposed to synthetic products which often end up being pollutants. Plant products are also cheap especially if they are locally available (Isman, 2006).

During the search for plant derived biocides, one approach used involves the screening of plant extracts for deleterious effects against different organisms (Akhtar and Isman, 2004; Akhtar and Isman, 2003; El Atta and Ahmed, 2002; Erler et al., 2007; Guerra et al., 2007; Hostettmann and Wolfender, 1997; Kartal et al., 2006; Sadek, 2003; Ssekyewa et al., 2008; Tewary et al., 2005).

In most cases, research has focused on chemical composition of sap, latex or juices occurring in different plant parts. For example, the Euphorbiaceae family is known for potent latex available in leaves and barks of most of its species. The latex is particularly rich in diterpene and triterpene esters (Furstenberger and Hecker, 1977; Khan et al., 1989; Rasool et al., 1989)

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which are known to be pesticidal (Rahuman et al., 2008). Phytolaceae sap on the other hand is rich in triterpenoids (Spengel, 1996) and saponins (Dorsaz and Hostettmann, 1986; Ndamba et al., 1994; Slacanin et al., 1988) reported to be molluscidal (Ndamba et al., 1994). The term latex refers to the milky exudate of plants that coagulate upon air exposure.

On the other hand, sap extracts or teas are obtained from plant parts, mostly leaves, by squeezing or pounding plant parts or mixing them with water. Succulent plants easily yield copious quantities of sap when squeezed while non-succulent leaves have to be pounded.

The present study focused on extracts from *Phytolacca dodecandra* L. (Phytolaceae), commonly referred to as endod; *Jatropha curcas* L. (Euphorbiaceae), commonly known as the physic nut and *Euphorbia tirucalli* L. (Euphorbiaceae), also known as the pencil tree.

Extracts from these plants have been mentioned in literature to display pesticidal properties (Adebowale and Adedire, 2006; Devi and Gupta, 2000; Emeasor et al., 2005; Gebre-Amlak and Azerefegne, 1999; Rahuman et al., 2008). In addition, they have been identified by local farmers as being able to improve health of several crops when applied. For example, farmers at Nkozi organic demonstration field in central Uganda intimated that *P. dodecandra* is good for the growth of cabbage (K. Kalanzi, Masaka, Uganda, personal communication) while farmers in Western Uganda mentioned that latex of *E. tirucalli* promotes general health of leafy plants.

Although extracts of these plants are generally claimed to improve crop health and reduce leaf damage, it is not entirely clear what pests they are specifically used against. Available literature shows that E. tirucalli has larvicidal (Rahuman et al., 2008; Yadav et al., 2002); anti-fungal (Mohamed et al., 1996); Piscicidal (Neuwinger, 2004); anti-viral (Betancur-Galvis et al., 2002) as well as anti-bacterial properties (Lirio et al., 1998).

J. curcas and *P. dodecandra* are known for their molluscidal and larvicidal features (Abebe et al., 2005; Isharaza, 1997; Lemmich and Thiilborg, 1995; Rahuman et al., 2008) but also for being insecticidal in grain storage (Adebowale and Adedire, 2006; Emeasor et al., 2005). However, these attributes are not directly related to controlling insect pests in the field.

It is in this context that the present study was set up by screening three plant extracts for insecticidal features against major field cabbage pests in Uganda, including the diamondback moth *Plutella xylostella* L. (Plutellidae) and the cabbage aphid *Brevicoryne brassicae* L. (Aphididae). It should be noted that these pests have for some time now, been a serious problem to production of cruciferous crops not only in East Africa (Badenes-Perez and Shelton, 2006; Macharia et al., 2005) but also in the rest of the world (Furlong et al., 2008; Guilloux et al., 2003; Isayama et al., 2004). A research of this kind is therefore highly necessary in order to develop possible bio-controls for such pests.

MATERIALS AND METHODS

Experimental field

The experiment was carried out at the organic demonstration plot of the Uganda Martyrs University (GPS: 0º 00´ 49´´S 32º 00´ 32´´E) on the shores of Lake Victoria in Central Uganda. This took place between December 2008 and June 2009.

The experimental block was divided into 21 plots each 6 m² in size to accommodate six treatments and controls, which were all replicated three times.
Treatments were arranged in a randomized complete block design.

The cabbage experimental plants

Cabbage (*Brassica oleracea* L. Brassicaceae) plants used were a Copenhagen hybrid of the capitata type that makes heads and mature in 8 to 10 weeks. Seeds were bought from a local farmers' shop and germinated in a nursery bed nearby the experimental block. Transplanting took place three weeks after sowing. Fifteen cabbages were planted in each plot at a spacing of 0.5 m by 1 m.

Extracts

Three extracts were used: Latex from *E. tirucalli* and *J. curcas* and sap from *P. dodecandra.* Latex from *E. tirucalli* was obtained locally by making incisions on the mature branches of the tree. It was let to ooze into a small bottle with a cover wrapped with aluminum foil to stop solarization that would cause it to deteriorate (Oliveira-Filho and Paumgartten, 1997). The bottle was put in a cooler (icebox) and kept at about 4°C to prevent coagulation.

Likewise, *J. curcas* extract was obtained by making incisions on mature upper parts of the stem. The extract was collected in sample bottles and taken directly for use.

On the other hand, extract from *P. dodecandra* was obtained by pounding leaves and squeezing them. A clean white piece of linen was used to squeeze and sieve the extract out of the pounded mass of leaves, which was taken directly for use in the experiment.

Two concentrations of each extract were applied: 25 and 12.5% *E. tirucalli* latex (P2 and P1); 50 and 25% latex of *J. curcas* (J2 and J1) and 50 and 25% sap of *P. dodecandra* (E2 and E1) respectively. Control plots were sprayed with previously collected rain water. Extract dilutions were made by mixing pure extracts of the plants with appropriate quantities of rain water (v/v) to obtain required percentages.

Fresh extracts were obtained on actual spraying days except for *E. tirucalli* latex which was obtained once every fortnight from a source in Masaka, Uganda (about 40 km from experimental site) where pencil trees are in abundance. Maximum preservation was guaranteed through storage under ice cold and dark conditions in the icebox (Oliveira-Filho and Paumgartten, 1997). It should be noted that latex of *E. tirucalli* is toxic and irritating to eyes and skin. It should be handled with care and hands should be washed thoroughly with soap thereafter.

Spraying

Spraying was carried out with a 1 L Knapsack sprayer twice every week for the whole experimental period (Karagounis et al., 2006).

Each experimental plant in plots was sprayed to dripping. Field infestation was already visible by the time of first spraying, that is, three weeks after transplanting. This experimental set up was used to be able to observe the potential 'curative' effect of extracts (Karagounis et al., 2006).

Sampling

Sampling of pests was done by field counts on a weekly basis just before spraying. Counting of *B. brassicae* was done directly. All larvae on both sides of the first three leaves from the core were counted. This implies that every week, a new set of leaves was considered so as to assess potential preventive capacity of extracts. Infestation was ranked on a 0 to 3 scale, where 0 = absence; 1 = 1 to 5 colonies; 2 = 5 to 10 colonies; 3 = over 10 colonies (Karagounis et al., 2006).

The presence of *P. xylostella* moth was determined by opening up the young fold of cabbage leaves where eggs are laid and hatch into caterpillars. The presence levels were determined by recording the number of larvae observed (Phillips, 1983). Six plants randomly chosen in each plot were considered in all treatments. This was considered a reasonable sample since the direct counting method, which was used, provides accurate results.

Leaf damage levels were obtained by estimating the damaged area of cabbage leaves by means of a squared paper grid. The third leaf from the cabbage core for six randomly chosen plants was considered per treatment. The third leaf was used since it was considered to have received reasonable exposure to pest encounters but has not shown signs of senescence.

The number of harvested cabbage heads was also counted, weighed and the number of marketable ones recorded to establish levels of protection (efficacy) of different extracts (Liu et al., 2003).

Data analysis

Data obtained from counts of *B. brassicae* and *P. xylostella* infestations were subjected to Kruskal-Wallis one way analysis of variance by ranks (H test) to establish the trend of infestation between treatments. Means were differentiated by Mann-Whitney U test where the H test was found significant. Results with P≤ 0.05% were considered statistically significant.

Data for leaf damage and number of marketable cabbages were presented as percentages. All calculations and graphic presentations were carried out using Microsoft office Excel 2007.

RESULTS

Levels of *B. brassicae* infestation in the field

Mean *B. brassicae* scores fluctuated between the first and fourth week of spraying for all treatments but experienced a fall from the fourth week onwards to the end of the growing season (Figure 1). This trend was observed for all treatments including the control. However, there was a significant difference in *B. brassicae* scores between different extracts (H = 27.31, df = 6, P = 0.00013). A comparison between treatment means and the control with Mann-Whitney test shows that a significant variation in population trend (infestation) were obtain in *E. tirucalli* (P1 and P2) and *J. curcas* (J2) extracts only (P1: U = 9.89, P = 0.0017; P2: U = 9.90, P=0.0165; J2: U = 6.91, P = 0.0086). However, closer inspection reveals that only *E. tirucalli* formulations (P1 and P2) reduced the *B. brassicae* levels to below one colony per 5 plants, that is, the economic threshold as estimated by PIP/PSM (2007).

Levels of *P. xylostella* infestation in treatments

Mean number of *P. xylostella* larvae decreased in all treatments after the third week of spraying (Week 5) but experienced a rise again starting from week 7 until the end of the growing season. There was a highly significant difference in larvae infestation between treatments (H = 32.45, df = 6, P = 0.000013). Mann-Whitney's post-hoc test revealed that both of *E. tirucalli* dilutions (P1 and P2) were more potent than other treatments but showed no difference between the two depicting the latex potency at even low dilutions (P1: U = 10.646, P = 0.0011; P2: U = 10.694, P = 0.0011).

Percentage reduction of larvae counts from the control was evident to the tune of: P2; 45%, P1; 40.2%, E2; 12.9%, E1; 7.24%, J2; 11.1% and J1; 9% (Figure 2). However, in all treatments, mean numbers of larvae counted remained significantly higher than one larva per plant, which is the recommended economic threshold for *P. xylostella* larvae in cabbages (Figure 2).

Cumulative leaf damage in the field

Leaf damage levels continuously increased in all treatments throughout the season. In some cases, damage levels in plants under treatment were as high as in plants from the control (Figure 3). Only extracts from *E. tirucalli* and *P. dodecandra* brought about reduction in leaf damage as compared to the control. Percentage reduction of leaf damage as calculated from the control was E1 -17%; E2 -15%; P1- 22.5%; P2 -25.6%; J1 -11.8% and J2 -11.8%. Mean leaf damage levels over the season ranged from 12.5% for P2 which was lowest to 20% for E1 which was highest.

Percentage of marketable cabbages per treatment

Marketable percentage of cabbages per plot was generally very low ranging from 6.7 to 33.3%. Only one formulation of *E. tirucalli* (P1) showed a slightly higher harvest marketable percentage at 33.3% as compared to the control at 20%. Some formulations of *P. dodecandra* (E1) and *J. curcas* (J1) even showed lower percentages than the control (Figure 4). All figures were below the usual range of 75 to 85% commonly observed in Uganda organic farms.

DISCUSSION

A good pesticidal substance should protect a crop

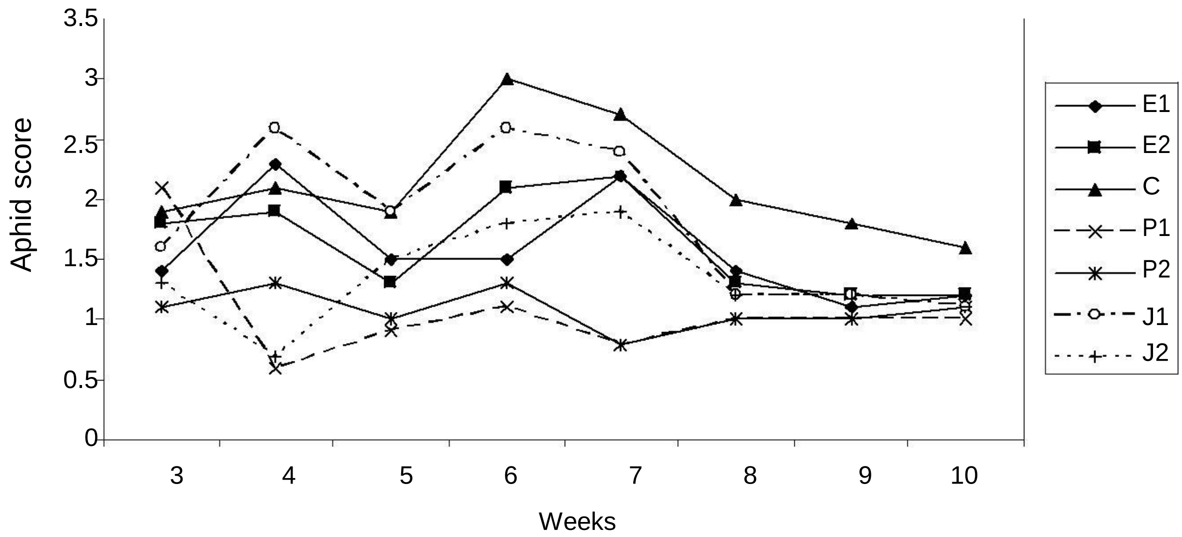

Figure 1. Mean weekly *B. brassicae* scores* among treatments. *Scores per treatment: 1 = 1 to 5 colonies, 2 = 5 to 10 colonies, 3 to over 10 colonies.

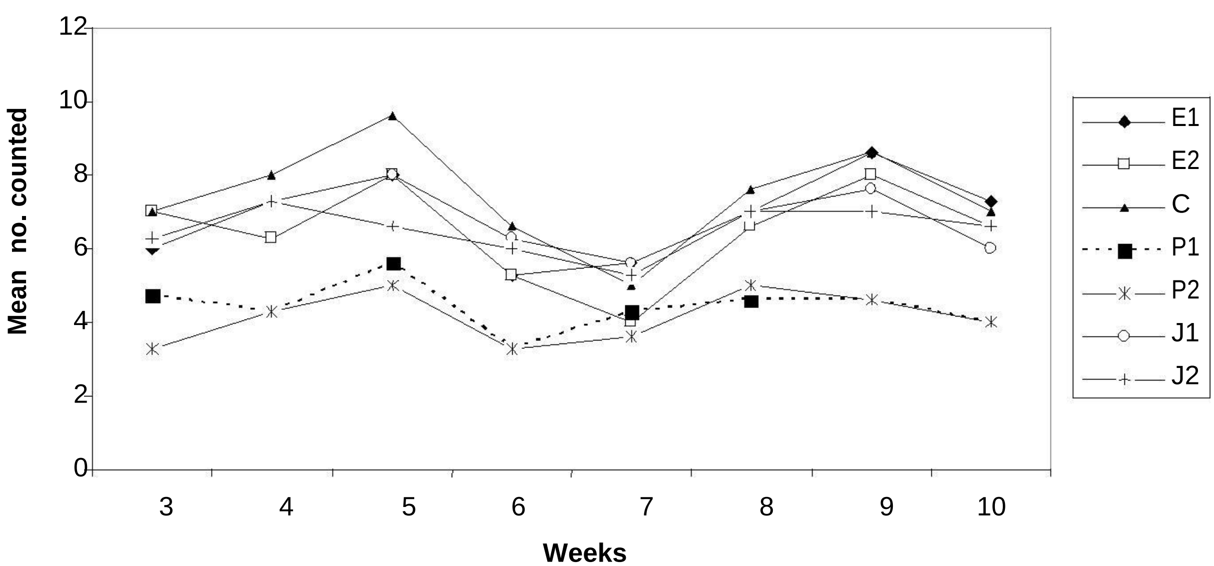

Figure 2. Mean number of *P. xylostella* larvae counted per treatment

against target pests to levels below economic threshold. Our results indicate that all extracts displayed considerable effectiveness to reduce *B. brassicae* infestation levels in the field but only *E. tirucalli* extracts (P1 and P2) were potent enough to reduce aphid levels below one aphid colony per 5 plants in a treatment.

This result might not be surprising in view of the chemical composition of *E. tirucalli* latex. The latex is known to be composed of a range of toxic substances including phenolic compounds, ellagic acid and tannins (Lin et al., 2001), diterpene esters (Khan and Malik, 1990), triterpenes (Furstenberger and Hecker, 1986; Khan et al., 1989; Khan et al., 1987; Rasool et al., 1989). All of these compounds may individually, additively or synergistically contribute to pesticidal action of the latex to *B. brassicae*.

Berger (1994) and Duke (1983) had earlier observed pesticidal properties of *E. tirucalli* against aphids but to the present time, it has never been experimentally demonstrated. Therefore, to the best of our knowledge, our study is the first to report on efficacy of E. *tirucalli* latex against field pests and particularly on agricultural insect pests.

On the other hand, several researchers have worked on and reported pesticidal properties of *J. curcas* (Adebowale and Adedire, 2006; Alyelaagbe et al., 2007;

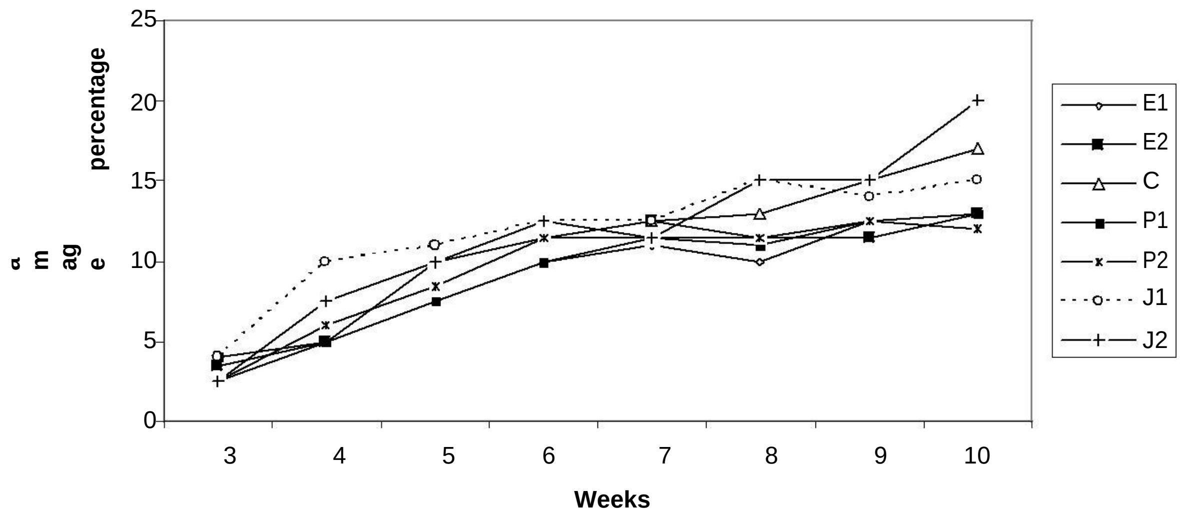

Figure 3. Cumulative percentage of leaf damage in treatments.

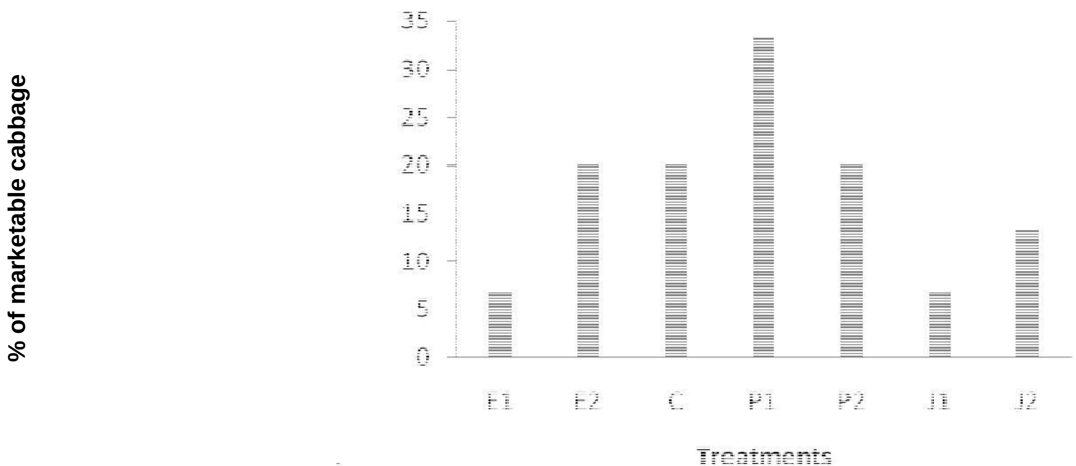

Figure 4. Percentage of marketable cabbages per plot in the treatments.

Emeasor et al., 2005; Rahuman et al., 2008) and *P. dodecandra* (Abebe et al., 2005; Gebre-Amlak and Azerefegne, 1999; Lemmich and Thiilborg, 1995). Also during the present study, extracts from both species caused a reduction in *B. brassicae* infestation levels albeit not to acceptable threshold levels.

The diamondback moth (*P. xylostella)* is known to be a very difficult pest to control (Sarfraz et al., 2005; Wang et al., 2007). Even against specialized synthetic pesticides, it easily develops resistance (Bautista et al., 2007; Sarfraz and Keddie, 2005; Wang et al., 2007; Zhao and Grafius, 1993; Zhao et al., 2006) or it conceals itself in a cabbage head (Macharia et al., 2005) thus making it elusive to management techniques.

In many cases, this pest is controlled using a combination of pest management techniques which may or may not include plant extracts (Attique et al., 2006; Facknath, 2006; Farrar and Shapir, 2005; Li et al., 2006; Raymond et al., 2006; Talekar and Shelton, 1993).

Our results show a reduction of *P. xylostella* larvae in the field ranging from 7% for J1 to 45% for P2. However, these percentage reductions may not mean much especially if the level of infestation at the moment of application of the treatment was already high.

According to our observation, larvae counts ranged from four (P2) to ten (E2) per plant. These counts are so high that even if a pesticide would bring about a reduction of 40% it would still not provide reasonable protection to the crop. However, combined with another technique such as biological control (Schroer and Ehlers, 2005) or trap crops (Badenes-Perez and Shelton, 2006) the pest load could be brought down to economic threshold levels.

Therefore, extracts from *E. tirucalli* whose potency resulted into an infestation reduction amounting to 45 and 40.2% (P2 and P1) respectively, could be used as contributory and/or supplementary therapy against the *P.*

xylostella moth. However, more research is needed in order to substantiate this hypothesis.

As an additional indicator to the failure or success of these extracts to manage pests in cabbage fields, we used leaf damage levels in the field and percentage of marketable cabbages among treatments. Results suggest that *E. tirucalli* extracts protected cabbage plants better than other extracts to the extent of causing a 22.5 and 25.6% reduction in leaf damage for P1 and P2 respectively. Although this was a fair result, it does not necessarily mean that the protection was satisfactory because marketable cabbages harvested fell short of at least 75% of the harvest which would be reasonable for an organic farmer. This shortfall is, however, more attributed to *P. xylostella* than *B. brassicae* infestation.

These results are in agreement with what was observed in our earlier laboratory trials where *E. tirucalli* latex was observed to provide protection to sweet paper *Capsicum annum* L. (Solanaceae) against green peach aphid *Myzus persicae* Sulzer (Aphididae; Homoptera) (Mwine, unpubished.).

In summary, the present experiment has demonstrated the possibility of using extracts from *E. tirucalli* latex to manage *B. brassicae* in cabbages. Where the extracts are used in presence of *P. xylostella* (and they usually infest together!), another contributory/complementary therapy should be co-opted to bring down infestation levels below economic threshold. An integrated pest management approach is recommended.

Results of this experiment have also helped to validate local farmer's observations that the extracts are beneficial for protection of leafy crops.

Although it does not give total protection (and really no pesticide does), in our opinion, *E. tirucalli* is a welcome pest management measure especially for organic farmers who must not use synthetic pesticides and may not have many alternatives. This is particularly so for African farmers, whose income is low and may not afford other expensive pesticides.

After all, *E. tirucalli* grows wildly and abundantly in many arid and semi-arid areas in Africa and is available at no cost.

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