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RESEARCH ARTICLE

PESTICIDAL PROPERTIES OF CHIVE (*ALLIUM SCHOENOPRASUM*) AGAINST CABBAGE APHID (*BREVICORYNE BRASSICAE*) IN RAPE (*BRASSICA NAPUS*)

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ABSTRACT

The cabbage aphid is of agricultural concern vectoring at least 20 viral pathogens in crucifers. The aphids have demonstrated tolerance to a number of synthetic pesticides. Botanical pesticides are reasonably sustainable and effective in suppressing cabbage aphid populations in crucifers hence improved crop quality and yield per hectare. An experiment was run to test the efficacy of various concentrations of a botanical pesticide derived from chive (*Allium schoenoprasum*) fresh leaf extract in controlling cabbage aphid (*Brevicoryne brassicae*) in rape. The experiment was laid out in a Complete randomized design (CRD) with four treatments and four replicates as follows; 12g chive extract, 8g chive extract, 4g chive extract and 0g control (water spray). Analysis of variance to separate mean mortality was done using Gens tat version 18 and least significant difference at 0.05 probability level was used to separate means. SPSS version 20 was used in estimating LC₅₀ value and excel was used in calculating the regression equation. Significant differences ($p < 0.05$) were observed throughout the trial, where highest mortality rates were observed in 12g chive extract (94.5%) and lowest mortality in control (12.2%) was observed. Pesticide concentration at LC₅₀ for the chive extract was estimated as a concentration of 7g/l. There were no observed signs of phytotoxicity even though other studies have shown that chances are high when the dose is increased. The experiments revealed that chive leaf extracts had pesticidal effects hence can be used to effectively control cabbage aphid in rape by smallholder vegetable producers.

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INTRODUCTION

Over the past 50 years crop protection has relied heavily on synthetic chemical pesticides but their availability is now declining as a result of legislation and the evolution of pesticide tolerance in pest populations, therefore alternative pest management tactics are needed (Gerhandson, 2002). Rape (*Brassica napus L*) is a subtropical plant that belongs to the family Brassicaceae that includes covo (*Brassica carinata*), mustard (*Brassica juncea*), cauliflower (*Brassica oleracea*) varieties and other crucifers (Karban and Baldwin 2007). Rape is one of the most important and widely grown vegetable crops by smallholder farmers in Zimbabwe mainly for subsistence and as a source of income (Mudzingwa, 2013). Its leaves are rich in vitamin A, ascorbic acid and thiamine and have high levels of glucosinates (El- Beltagi and Mohamed 2010), which during preparation form compounds with anti-oxidants and have anti-cancer activities (Holland, 1991). Rape is a cold season crop (Decoteau, 2000) and its production is limited to periods of low temperature especially in the semi-arid region of

Zimbabwe, for example, Chiredzi, which mostly experiences high summer temperatures, unreliable and unpredictable rainfall. Some smallholder farmers make a living from the crop in most areas of Mashonaland Province of the country (Jackson, 1997). The farmers supply the vegetable crop to the urban markets while some grow the crop in home gardens exclusively for home consumption. Dobson, (2002) reported that pests and diseases are major constraints causing losses (up to 80% on yield) in quality and marketability. Crop yields have remained far below the crops' genetic potential in the smallholder sector due to diseases and pest attack. Being a non-indigenous vegetable, rape is vulnerable to attack by a number of insect pests that include aphids, bagrada bugs, diamond back moth, afrolis, white grubs, among others. Most farmers consider aphids as the most common pest of rape pest causing 70-80% yield losses in oilseed rape (Turner and Chivinge, 1999). They are of economic importance since they decrease plant vigour and growth causing low yields and reduced product quality. The most common species that attacks rape plants is the cabbage aphid (*Brevicoryne brassicae*:

Homoptera: Aphididae) that feed by sucking the sap from the plants and if in large numbers remove sufficient sap to kill the leaves and the growing tips. Infested seedlings become stunted and distorted. Continued feeding on mature plants causes wilting, yellowing and general plant stunting (Hill, 1983). Affected leaves wrap up or curl inwards (Dube *et al.*, 1999). The cabbage aphid is a vector of twenty-three (23) viral diseases of cruciferous plants (Kessing and Mau, 1991). One of the diseases transmitted is turnip mosaic virus. Other identified pests of brassicas include diamond back moth (larvae), *Lipaphis erysimi*, green peach aphid *Myzus persicae* (Grzywacz *et al.*, 2010; Kuntashula *et al.*, 2006; Kfir 2003; Sibanda *et al.*, 2000). Some smallholder farmers predominantly use synthetic pesticides to control aphids (Turner and Chivinge, 1999; Sibanda *et al.*, 2000; Obopile *et al.*, 2008) and dimethoate, an organophosphate being the most widely used chemical to control aphids in Zimbabwe locally known by the trade name Rogor. The decision to apply pesticide is predominantly based on noticing the presence of a pest on the plant not necessarily pest thresholds (Obopile *et al.*, 2008).

Besides high costs, synthetic chemicals pose environmental and health risks to the producer and consumer. The risks emanate from shortcomings in chemical handling practices, like large deviation from recommended chemical dose, and chemical drift to non-targets environments through run off into the soil and ground water (Sibanda *et al.*, 2000; Williamson *et al.*, 2008). Furthermore there are increasing challenges of build-up of resistance to some of the pesticides in the aphid populations (Gerhandson, 2002). It is within the challenge that it becomes very imperative to explore the possible utilization of relatively cheaper, accessible, safer and environmentally benign alternatives, to the presently dominating synthetic pesticides. Some biopesticides like botanical pesticides can be home made, are accessible, less expensive, easy to handle and use and not harmful. They have little impact on natural enemies of pests (Schmutterer, 1997) hence can be used in the development of integrated pest management systems (Charleston *et al.*, 2005). Bio pesticides extracted and derived from naturally occurring bioactive plant compounds are called botanical insecticides. They are based on a variety of plants that include garlic, onion, chillies, neem, tobacco, pyrethrum (Nayar *et al.*, 1990; Raguramam *et al.*, 1999; Defago *et al.*, 2006; Rahuman *et al.*, 2008) and some weeds like *Solanum pandiriforme* (Sodom apple), *Bidens pilosa* (black jack) and *Lippia javanicam* (fever tea) (Municipal development partnership in Eastern and Southern Africa, MDP, 2006).

All organs of *A. schoenoprasum* bulb, leaf and stalk exhibit antioxidant activity in studies performed on this plant, being high in the leaves (Bronet, 2006) containing numerous organosulphur compounds such as allyl sulphides flavonoids, carotenoids, phenolic compounds (Burdock, 1996). *A. schoenoprasum* does not contain allicin, but when the leaves or whole plant is crushed, two chemicals inside react to form allicin (Sarwar, 2015). *A. schoenoprasum* has insect repelling properties (Kaufmann *et al.*, 1999) and antimicrobial properties (Rattanachaiakun and Phumkhachorn, 2008) that can be used in gardens to control pests and diseases. Although *A. schoenoprasum* is repulsive in general, due to the sulphur compounds, the flowers attract bees, and they are at times kept to increase desired insect life. The other danger of using inorganic pesticides is the possibility of insects developing resistance to pesticides and emergence of new pests.

Silva *et al.* (2012) reported that the aphid has developed resistance to at least seventy different synthetic compounds, and have also developed a different insecticide resistance mechanism worldwide. Use of synthetic pesticides has become costly, limiting its use to commercial farmers who can afford. In horticultural crops like rape, aphids are among key pests of the crop causing major economic losses. Kapondo (2004) acknowledged the use of natural pesticides as the best option to address yield losses due to pest attack as well as addressing issues of degradations associated with the use of synthetic pesticides. Thus the need to provide alternatives to the use of conventional pesticides, that produce vegetables free of synthetic pesticide residues while maintaining high product and quality (Munyima *et al.*, 2004). The plant extracts have minimal toxicity to non-targeted organisms and degrade hastily in the environment (Munyima *et al.*, 2004). Chives can be affordably produced in backyard gardens or on flowerbeds and locally found as ornamental flowers in botanical gardens. Plant extracts provide a safe and viable alternative as compared to synthetic pesticides and research has shown that these are mostly compatible with beneficial insects, predators, parasitoids and pollinators). Organic pesticides have minimal environmental degradation, increased safety for farmers and improved product quantity and quality. Botanical pesticides are important alternatives to synthetic pesticides since they possess an array of beneficial properties including repellence, antiherbivory activity, growth regulatory activity and toxicity to insect and mite pests (Prakash *et al.*, 2008). This study therefore aimed at evaluating the use of various levels of aqueous extracts of fresh leaves of *A. schoenoprasum* in suppressing cabbage aphid populations in *B. napus* with the following specific objectives; i) determining cabbage aphid mortality when sprayed with various concentrations of *A. schoenoprasum* extracts ii) estimating LC₅₀ value of the *A. schoenoprasum* fresh leaf extract concentrate and iii) estimating the best concentration that gives best results. These objectives were based on the null hypothesis (H₀) that *A. schoenoprasum* leaf extract has no pesticidal effect on the management of cabbage aphid in rape.

MATERIALS AND METHODS

Experimental Site: The experiment was carried out in Budiriro Township in Harare, (17° 52'44"S; 30°55'24"E; 1383 m). The area is in natural ecological region IIa that has a subtropical highland climate. The average annual rainfall experienced in the area ranges between 750 and 1000 mm per annum with most of the rain falling between the months of November to March. The average annual temperature is 17, 95°C, low for the tropics and this is due to its high altitude position and the prevalence of a cool to south-easterly air flow (Surveyor General, 1995; Nyamapfene, 1991). The mean annual temperature ranged from 15 to 30 °C, during the same period (Muzemu *et al.*, 2011). According to Nyamapfene, (1991) the soils in Harare are predominantly paraferrallitic soils under the kaolinitic order with a coarse grained sand fraction, derived from granite. The kaolinitic soils are moderately to strongly leached soils; clay fractions mainly inert together with appreciable amounts of free sesquioxides of iron and aluminium (Hussein, 1981). The trial was conducted from June to September 2016.

Experimental Design and Treatments: The study was carried out using potted plants in which four treatments were

randomized with four replicates. The treatments evaluated were: 1:12g of fresh chive extract, 2:8g of fresh chive extract, 3:4g of fresh chive extract and 4:0g (zero/control - water sprayed). Rape seedlings (cv English Giant) were sourced from a reputable seedling dealer (Farm and City) and one seedling was planted in each 5 litre pot with containing soil mixed with ash from maize stover and watered to field capacity with a watering can. Plants were spaced at 50cm inter-row by 20cm in-row. Watering continued throughout the period of the trial. Application of a basal dressing fertilizer was done using compound D (7N; 14P₂O₅; 7K₂O) at an application rate of 350kg/ha per hectare whilst top dressing was done using Ammonium Nitrate (AN) (34.5%N) was applied at the rate of 100kg/ha. Top dressing was done at 2 weeks after transplanting.

Materials

Different materials were used at different phase of the project. A hoe and a shovel were used for preparing the soil by mixing it with ash material made from maize stover and in filling the planting pots. A watering can fitted with a can rose was used for watering the plants. Electronic balance (Pitbull[®]) was used for weighing fresh *A.schoenoprasum* leaves and a graduated transparent measuring cylinder was used for measuring the distilled water before making the extract. The fresh *A.schoenoprasum* leaves were ground in an electronic blender (Russell Hobbs[®]) before mixing the paste in 1000 ml of distilled water to make the extract. The extracts were obtained by passing the mixture through a muslin cloth. A soft brush was used for removing the aphids from the leaves for counting. A hand lens of magnification x 10 was used for identifying and counting of aphids whilst a hand sprayer was used for pesticide application.

Experimental Procedure: Loam soil was thoroughly prepared by mixing it with ash material made from maize stover using a shovel before filling in the pots of equal size (98.29cm² by 40cm). The pots were hand watered to field capacity using a watering can fitted with a can rose. One *B. napus* seedling was then transplanted in each of the pots in late afternoon to avoid transplanting stress (Mayana and Musiiwa, 1999).

Cabbage Aphid Infestation on Rape Plants: All the potted plants were infested with 10-15 *B. brassicae* collected from nearby infested covo (*B. carinata*). Infestation with the pest was done at 3 weeks after transplanting. A soft brush was used to safely remove aphids from *B. carinata* plants and placing them on the health leaves of *B. napus* plants in each of the 16 treatments.

Leaf Collection and Identification: *Allium schoenoprasum* fresh leaf specimens were collected from Ministry of Agriculture's botanical garden in Harare. The leaves were checked for any pathological disorders and contamination of other plants and were washed with distilled water.

Preparation of Extracts: The fresh leaves of *A.schoenoprasum* (12g, 8g, and 4g) were ground into paste and were each mixed with 1000ml of distilled water and were left to stand over 24 hours. Grinding of the leaves was conducted in a fume-wood compartment within the laboratories of Soil Chemistry Department under the Ministry of Agriculture (Zimbabwe) before mixing the paste material

with distilled water. The extracts obtained were filtered separately using a muslin cloth to separate the chaff material. 0.5ml of dish washing liquid was added in each extract including in the control (water sprayed) to break the surface tension of water and to the spread and penetration of the botanical pesticide into both the aphid's hydrophobic cuticle and leaf cuticle. The residual extracts were stored in refrigerator at 4 °C in sterile glass bottles for further use within a week. Percent extractive values were calculated equation 1.

$$\text{Percent Extracts (\%)} = 100 * \left(\frac{\text{Weight of dried extract}}{\text{Weight of leaf material}} \right) \quad (1)$$

The different concentrations were as follows: Treatment 1:12g, 2:8g, 3:4g and 4:0g (zero/control water-sprayed) each extracted with 1000ml of distilled water. Application of the pesticide using a hand sprayer started two weeks after aphid infestation (5 weeks after transplanting seedlings). Different hand sprayers were used for each concentration.

Sampling for Aphids: To determine the aphid population level, three leaves were randomly selected per plant. For each plant, three leaves were chosen randomly each from the top, middle and bottom of the plant (Reddy *et al.*, 2013). On the underside of each of these leaves, the numbers of aphids present were counted and scored using a lens magnification 10 x while the post treatment observations on population of aphids was also observed by the same procedure after 24 hours, 48 hours, 72 hours, 96 hours and 120 hours respectively.

Data Collection

Aphid Scouting: Stoll (2000) observed and set the Economic Threshold Level of aphids per plant as 50 aphids per plant, which was used as the basis for recommending a spray. Plants were scouted every week for signs of aphid infestation (Webb, 2010) after treatment. Three leaves were selected from each plant, one from the top, middle and bottom level of the plant (Reddy *et al.*, 2013) and the number of aphids present on the underside and top side of each leaf was counted using a lens magnification x 10. Newly born aphids and adult aphids as well as age sex classes were not separated but ignored throughout the experiment.

Aphid Mortality: The various concentrations of *A.schoenoprasum* extracts were applied in concentration series of 12g, 8g, 4g and 0g (zero/control water-sprayed) in 1000ml of distilled water to determine the biological efficacy of the extract on the mortality of *B. brassicae* adults and nymphs. The exact count of *B. brassicae* on the rape plants was determined immediately before treatment and after 24 hours, 48 hours, 72 hours, 96 hours and 120 hours of exposure respectively. Mortalities were determined after 24 hours, 48 hours, 72 hours, 96 hours and 120 hours of exposure respectively and the determined data became the foundation for estimating lethal concentration (LC₅₀). The acute toxicity measured as mortality after 24 hours of exposure was determined by the topical application to adults of *B. brassicae*. The experiment was repeated five times. Mortality rates with respect to each treatment were calculated using equation 2:

$$\text{Mortality \%} = 100 * \left(\frac{\text{No. of aphids before spraying} - \text{No. of aphids after spraying}}{\text{No. of aphids before spraying}} \right) \quad (2)$$

Test for 50% (LC₅₀) of the Pest Population: Percent mortality rates forms the foundation of 50% (LC₅₀) of the pest population. If mortality in the controls is between 5% - 20% results with the treated samples are corrected using Abbot's formula and if mortality exceeded 20% in the control treatment, the replicate is rejected. The observed mortalities were corrected by Abbott's formula (Abbot 1925). The efficiency was determined by the Abbot's formula, equation 3:

$$\text{Corrected Mortality \%} = 100 * \left(\frac{X - Y}{100 - Y} \right) \quad (3)$$

Where X = percentage mortality in the treated sample and Y = percentage mortality in the control.

The LC₅₀ Value: Probit analysis of the mortality data of the various concentrations was conducted to estimate the LC₅₀ value (Finney, 1952).

Data Analysis: Analysis of variance of percent mortality rates were carried out using Genstat version 14 and the least significant differences at 0.05 probability level was used to separate means. Using SPSS 18 Software the significant differences were taken to Duncan multiple range test to compare the means.

RESULTS

Effect of *Allium schoenoprasum* Extract on *Brevicoryne brassicae* Mortality: There were significant differences ($p < 0.05$) among all the treatments observed from day 1 to day 5 after spraying rape with various concentrations of *A. schoenoprasum* extract. Day 1 showed a significant difference ($p < 0.05$) where 12g had the highest mortality rate (19%) and the lowest (2.3%) was recorded in 0g control (water sprayed). The pair wise comparison across the four treatments showed a significant difference ($p < 0.05$) in mean percent mortality rates amongst all treatments, in Day 2, 12g had highest mortality (38.29%) and the lowest in control (water spray) (4.82%). Pairwise comparison of mean percent mortality rates of Day 3 had a significant difference ($p < 0.05$), 12g *A. schoenoprasum* extract (57.38%) and the lowest of (7.2%) was in observed in water sprayed (control). Day 4, there were significant difference ($p < 0.05$) where 12g *A. schoenoprasum* extract had the highest (76.18%) and the lowest was observed in the control (9.52%). The pair comparison of Day 5 showed a significant difference ($p < 0.05$) in mean percent mortality rate in which 12g had a highest (94.5%) and the lowest was observed in 0g control (water sprayed) (Table 1). The LC₅₀ value was estimated at 7g of *A. schoenoprasum* fresh leaves (Table 2)

DISCUSSION

Results from this study showed that highest mortality rates were observed in *A. schoenoprasum* extract treatments and lowest mortality rates in control (water spray) agreeing with the works of Sarwar, 2015) who said *A. schoenoprasum* does not contain allicin itself, but when the leaves are crushed, two chemicals inside react to form allicin.

Effects of *A. schoenoprasum* Extract on *B. brassicae* Mortality Rate: The mortality of aphids in the test treatments was significantly higher than in the control.

The experiments revealed that *A. schoenoprasum* leaf extracts had both toxic and antifeedant deterrent effects. Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fibre to protect against diseases. They are nonnutritive compounds (secondary metabolites) that contribute to flavour colour (Johns, 1996; Craig 1999; Agbafor and Nwachukwu, 2011). Spraying *B. brassicae* with *A. schoenoprasum* extract had a pesticidal effect on the aphid population throughout the data collection period. The result are similar to the work of Singh *et al.*, (2001) which recorded 100% mortality rate in red spider mites to garlic extract. In addition to using garlic (*Allium sativum*) extract other similar works such as those by Prowse *et al.* (2006) recorded highest mortality rates against two species of Diptera. Control treatment was the lowest because there was no pesticidal effect and death observed could have resulted because of natural death. The pungency of *A. schoenoprasum* could have deterred the cabbage aphid from feeding (Dobson *et al.*, 2002) and it could be that allicin sulphur compound in *A. schoenoprasum* that excites allyl isothioyanale sensitive sensory neurons as well as activates the ion channels (Baustista *et al.*, 2005), which are present in pain-sensing neurons. Induction of pain could have significantly contributed to insect mortality by causing considerable stress to the cabbage aphid.

Allium schoenoprasum belongs to the same family with *A. sativum*, and allicin formed when *A. schoenoprasum* is crushed has been shown to be readily membrane permeable and thus able to rapidly penetrate cellular compartments in biological systems (Miron *et al.*, 2000). All organs of *A. schoenoprasum* bulb, leaf and stalk exhibited antioxidant activity in studies performed on this plant, being high in the leaves (Bronet, 2006) containing numerous organosulphur compounds such as allyl sulphides flavonoids, carotenoids, phenolic compounds (Burdock, 1996). This could have caused an increase in mortality observed in day 5 as the experiment.

LC₅₀ Value: The lethal concentration (LC₅₀) is the most frequently used measure of the acute toxicity of a substance. Expressing toxicity as LC₅₀ provides a relative measure that can be used to compare substances with different mechanisms based solely on their lethal effect. A smaller LC₅₀ value means relatively greater toxicity, indicating that a smaller amount of the substance is required for the death of the test organism. The extracts of *A. schoenoprasum* leaves that we prepared proved to have pesticidal activity as death was observed in all the concentrations except in control where lowest mortality was observed because the treatment had no pesticidal effect. The LC₅₀ value was estimated at 7g of *A. schoenoprasum* fresh leaves in 1 litre of distilled water to kill 50% of the cabbage aphids in rape indicating a relatively mild toxicity. This amount is a reasonably sustainable quantity that can be used by rape producers to effectively suppress cabbage aphid populations in their crop. The study demonstrated that *A. schoenoprasum* had pesticidal effects on aphids on rape. The efficacy of the plant extracts varied within the various concentrations levels as reported by (Ngowi *et al.*, (2007). The hypothetical example presented here demonstrate that the higher the concentration the greater the death rate and vice versa. The statistical predictions (Probit results) of the mortality responses vary continuously when plotted against the dose. However, it is possible that while increasing the chive concentration can result in high aphid mortality, it can also cause phytotoxicity. The LC₅₀ have limitations because they measure only one toxic effect that is death.

Table 1. Mean % Mortality of *Brevicoryne brassicae* Sprayed with Various Concentrations of *Allium schoenoprasum* Extracts

Treatments	Day 1	Day 2	Day 3	Day 4	Day 5
0g Control	2.321 ^a	4.82 ^a	7.20 ^a	9.52 ^a	12.209 ^a
4g chive extract	3.946 ^b	7.593 ^b	11.41 ^b	15.10 ^b	18.23 ^a
8g chive extract	7.429 ^c	14.40 ^c	21.60 ^c	29.05 ^c	35.91 ^b
12g chive extract	19.071 ^d	38.29 ^d	57.38 ^d	76.18 ^d	94.50 ^c
Grand mean	8.19	16.28	24.40	32.46	40.21
P Value	<.001	<.001	<.001	<.001	<.001
LSD	1.104	1.404	1.813	2.153	2.075
CV%	8.4	5.4	4.6	4.1	3.2

Means within a column are significantly different if they do not share a common superscript (p<0.05)

Table 2. The LC₅₀ value was estimated at 7g of *A. schoenoprasum* fresh leaves

		Coefficients ^a				
Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	.938	2.087		.449	.731
	log conc	4.820	2.362	.898	2.040	.290

Calculation of the LC₅₀ Value

concentration	log concentration (x)	% Mortality	Emperical
0	-	12.2	3.82
4	0.60206	18.23	4.08
8	0.90309	35.91	4.64
12	1.0791812	94.5	6.55

Y =ax+b

5=4.82x+0.938

X=((5-0.938)/4.82)

X=0.842739

LC₅₀=10^{0.842739}

LC₅₀=6.962073g

They do not give any indication of what dose/concentration may lead to other less serious, acute systemic effects or to other, possibly equally serious, contact effects or delayed systemic effects. According to Zhang and Zeltermann (1999), estimation of a safe exposure level to a known toxin is one of the most difficult problems that statisticians can face.

Conclusion

LC₅₀value study provides valuable information on acute toxicity, phytotoxicity and safety of an insecticide that will enable registration of any pesticide. The study showed that *A. schoenoprasum* fresh leaf extract has high efficacy against *B. brassicae* in *B. napus*. No pesticide is 100% safe and non-toxic. However the margin of safety for botanical pesticides is generally much higher than synthetic pesticides. (Ofuya 1997) showed that, as for many synthetic insecticides, the toxicity of botanochemical to biological control agents can be an important side effect in their use for pest control. Natural pesticides like *A. schoenoprasum* have potential for use in agriculture sector for plant protection. Use of organic pesticide can benefit smallholder vegetable producers to raise and improve their product quantity and quality since *A. schoenoprasum* can be produced with minimal cost, are accessible and eco-friendly with minimal damage on non targets organisms hence contribute to green technologies of crop protection.

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