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Short Communication

Insecticidal effect of neem oil in the control of *Brevicoryne brassicae*

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ABSTRACT:

Cabbage is a vegetable sheet that is considered as an important crop in Niger and is commonly used to reduce malnutrition and food insecurity in rural areas. Unfortunately, cabbage is often damaged by caterpillar's attacks, especially the aphids. This study was conducted as part of the activities of ICBA's project for scaling up small scale irrigation technologies for improving food security in sub-saharan Africa. The objective of the study was to evaluate the effectiveness of neem oil in reducing the damages caused by aphids on cabbage field. In the laboratory tests, the insecticidal effects of pure neem oil of doses of 50, 100, 150 and 200 µL applied to aphids in petri dishes were compared. Laboratory test results showed that once neem's oil was sprayed on the leaves of the cabbage in plates, aphids' attacks were reduced or stopped. It was found that 100 µL can kill 50% of aphids in 24 h, while 100 µL was enough to eliminate 100% of aphids in 96 h. A block of Fischer randomized field trial was performed to compare the concentrations of 5, 10 and 15% of neem oil. Results showed that concentrations of 15% led to 50% reduction of aphids in three days, and 75% in seven days. The rural producers of Niger could extract neem's oil as they are in all villages and apply it directly in their fields. This would allow fighting aphids' and improve the quality of the production of the cabbage.

Keywords:
Insecticidal, Azadirachtin, Cabbage, Aphid, Niger.

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INTRODUCTION

Cabbage is the second most cultivated crop in Niger and occupies a strategic position as an important food for the population of Niger. Cabbage is mainly grown in the regions of Tahoua (3,264.98 ha), Tillabéry (1,889.12 ha), Zinder (1,768.01 ha), Agadez (1,614.11 ha), Dosso (1,601.18 ha), Maradi (1,146.83 ha), Niamey (779.35 ha), and Diffa (640.23 ha) in a total of 1,270,381 ha in 2017 (Mag, 2017). Its production was estimated as 346,686.34 tons in 2017, with an average yield of 24.01 tons/ha (Mag, 2015). It is cultivated in the cold dry season from October to March; however, adapted varieties (hybrids) that can grow in the hot humid season were promoted by the authorities of Niger.

Cabbage is attacked by many pests, some of which degrade the quality of the apple (e.g. caterpillars), while others prevent its development. Producers, therefore, use several insecticide products imported from Nigeria to treat their crops (Reca, 2017). Most of the insecticides used are poisonous to users, treated plants and animals. In fact, Africa uses less than 10% of global pesticide production but accounts for 75% of the deadly cases due to pesticides (Bambara and Tiemtoré, 2008). Alternatives for crop protection in West Africa include the use of insecticide-resistant plants or the use of biopesticides that have an advantage because of their low persistence, low toxicity to humans, and their mode of action on pests.

In Niger, neem is present in all regions of the country, both in the countryside and in the city where it is planted by local populations for its shade, the use of the wood in construction or heating and for its medicinal effects. Azadirachtin in neem was isolated for the first time by Butterworth and Morgan (1968).

In a study carried out in Niger on 15 neem trees, the almonds collected have an *azadirachtin* content averaging 3.4±0.67 g.kg^{-1}. The oil extraction process used in this work was artisanal because it used only hot water to extract the pure oil, with this process, the extraction rate obtained was 18%, which was much lower than that obtained by Koul *et al*. (1999) (30%). Schiffers *et al*. (1998) demonstrated that by HPLC azadirachtin content was about 18 g per liter of neem oil, while the extracts of the sheet contain 0.6 g per liter of solvent.

It has been established that the active substance "azadirachtin" contained in the oil of neem kernels can cause more than 90% of the effects of most conventional insecticides (Faye, 2010). The latter does not kill insects, at least not immediately, but acts as a repellent, disrupting their growth and reproduction. Works by Schmutterer (1990), De Groot (1996), Kumar *et al*. (2003), Lesueur (2006 and 2008) and Faye (2010) confirmed the toxic effect of *azadirachtin* on the biological activities of several insects.

Azadirachtin is one of the most potent regulators of growth and food deterrence ever found against

Table 1. Average mortality of ash aphid in the laboratory

		Duration			
S. No	**Doses of neem oil (µL)**	**24 h**	**48 h**	**72 h**	**96 h**
1	0	0±00^{c}	0.28±0.7^{c}	2.1±2.4^{c}	2.4±3.4^{b}
2	50	38.3±0.7^{b}	61.6±1.1^{b}	78.3±1.4^{b}	98.3±0.4^{ab}
3	100	53.3±2.4^{ab}	81.6±1.4^{ab}	91.6±1.3^{ab}	100±0^{a}
4	150	51.6±2 ^{ab}	86.6±1.0^{ab}	93.3±1.0^{ab}	100±0^{a}
5	200	70±1.7^{ab}	91.6±0.7^{a}	96.6±0.5^{ab}	100±0^{a}
6	Pacha (Insecticide)	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}

Averages not sharing any letter were significantly different

Table 2. Binary logistic regression of aphid's number (dead)

S. No	LOGIT	24 h	48 h	72 h	96 h
1	Probability	P<0.001	P<0.001	P<0.001	P<0.001
2	Constant (β_o)	-1.09	-1.79	-0.84	-0.85
3	Regression coefficient (β_1)	0.32	0.35	0.36	0.37
4	Odds ratio	1.39	1.43	1.44	1.46
5	Wald's test (z)	14.08	12.49	12.04	11.95
6	Probability ratio (G)	281.82	224.5	227.51	235.5

several insect pest species, as well as some nematodes (Faye, 2010). It is such a powerful substance that the presence of a weak trace can prevent some insects from touching plants (Schmutterer, 1995). The present study aims to evaluate the effectiveness of neem oil in the control of a major pest of cabbage called aphid in Niger.

MATERIALS AND METHODS

Plant material

The variety of cabbage used is a F_1 hybrid, named Oxylus, manufactured by the company Séminis, headquartered in Saint Louis, Missouri, United States of America (USA). It has a cycle of 84 to 85 days after transplanting. The average weight of the apple is 1 to 3 kg, depending on the planting density. The average yield potential is between 100 to 120 tons/ha. It is resistant to *Fusarium* wilt and heat tolerant.

Preparation of the neem extract

Ripe neem fruits were collected between July and October 2017 in the city of Tahoua, Niger, and soaked in warm water for 24 h. The fruit pulps were removed by squeezing them to extract the seeds. These were sun-dried for a day and then for three days under the shade. They were shelled to obtain almonds that are pounded by hand to obtain the powder. Hot water (0.5 L) was added to the almond powder (5 kg), which was then pressed energetically to separate the oil from the dough and collect the oil that floats. This extraction technique was developed by Mahamadou (2009) in a technical sheet for the needs of producers in rural areas.

The oil emulsifier formulation extracted from neem fruit almonds were made using 50% oil, 30% pure ethanol, (stabilizer) and 20% gum arabic as an adjunct to fix active molecules on the leaves of cabbage (Zakari *et al.*, 2011). Samples with oil concentrations of 5, 10 and 15% were prepared for application on the cabbage field. They are compared to a white control and a reference insecticide called PACHA 25 EC (Acetamiprid

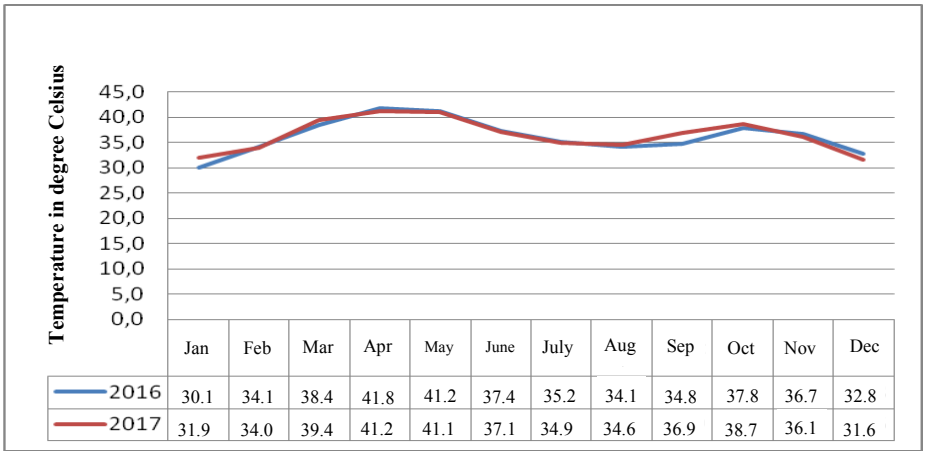

Figure 1. Evolution of temperature on 2016 and 2017 (National Directory of Meteorology, Niger)

Table 3. Yield of apple cabbage for neem's oil doses

S. No	Doses	Number of harvested healthy apple	Average weight of apple (kg)	Yield (kg.ha^{-1})
1	0%	17.66±1.52^{d}	0.533±0.10^{b}	18933±45^{c}
2	5%	27.66±4.50^{c}	0.793±0.05^{ab}	44187±10^{b}
3	10%	30.33±1.52^{bc}	0.763±0.13^{ab}	46153±69^{b}
4	15%	36±10^{ab}	0.733±0.12^{ab}	52867±98^{ab}
5	Pacha (Insecticide)	38±1.73^{a}	0.885±0.06^{a}	67120±14^{a}
6	probability	0.018	0.001	0.000

Averages not sharing any letter were significantly different

10g/l plus Lambda-Cyhalothrin 15g/l). PACHA 25 EC is a product registered in 2015 by Sahelian Committee of Pesticides (SCP).

Field experiment

The test was carried out at the CERRA (regional center of agricultural research) of Tahoua (14, 51 'N and 5, 17' E). The land was plowed by a tractor with disc plows. After harrowing and crushing the clods, planks of 5 m^2 areas were prepared. Each board constituted a unitary parcel. The device was a split plot with three repetitions. two to three leaf seedlings that were viable after 25 days in the nursery were transplanted on November 15, 2017. The spacing used during transplanting was 40 cm x 40 cm.

Aphids were collected from the cabbages of Tadis Valley (14, 51 'N and 5, 28' E). Twenty specimens were deposited on the cabbage plant in the center of the experimental plot. Two treatments were performed on the 45th and 60th days after transplanting.

Aphids were counted on the leaves of three cabbage plants selected from the two plants in the board. Counting was performed before each treatment and also three and seven days after treatment.

Also, plastic cups with a capacity of 2 liters, green in color, were filled with water and placed in the center of each plot. The evolutions of other insects that visit the cabbage were also determined. Yields were estimated by harvesting healthy apples in each board (Zakari *et al.,* 2011).

Laboratory experiment

The laboratory test was performed to understand the behavior of aphids in direct contact with the product. Adult aphids were collected from the cabbage plants. A portion of the cabbage leaf was spread in petri dishes. Doses of 0, 50, 100, 150 and 200 µL were sprayed into the dishes. Ten adult aphids were introduced into each

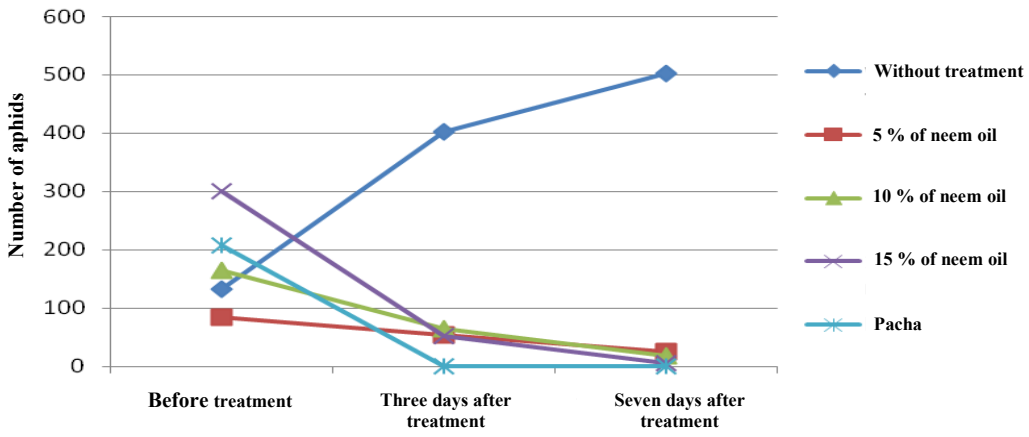

Figure 2. Evolution of the number of aphids before and after the treatment on the 45th day after the transplanting

petri dish (100 mm x 15 mm). Each dose was repeated six times. Aphids dead or alive were counted after 2, 4, 6 and 8 h. An aphid was considered dead when it does not react to touch by the brush (Zakari *et al.,* 2011). Mean aphid mortality (M_0) was expressed as corrected mortality (Mc), taking into account the natural mortality observed in the control (Mt) (Table 1) according to the Abbott formula (1925):

$$Mc = \frac{M_0 - M_t}{100 - M_t} \times 100$$

Data analysis

The data were subjected to an analysis of variance (ANOVA), on Minitab 16. The separation of the averages was performed by the Turkey test at the threshold of $\alpha = 5\%$.

RESULTS

Results of contact tests in the laboratory

Analysis of variance showed a highly significant ($P<0.001$) difference in ash aphid mortality over time. Aphid mortality increased with increasing neem oil dose and duration of treatment (Table 1). There is a toxic effect of neem oil on ashy cabbage aphids. Aphids in direct contact with *azadirachtin* can no longer feed. They gather on one side of the petri dish. There was a toxic effect of neem oil on ashy cabbage aphids.

The mortality also increased with the duration of the treatment. The number of dead aphids rises as time passes after treatment for all concentrations up to 48 h. It becomes stable between 72 - 96 h after treatment. The results of the binary logistic regression were given in Table 2.

At the 5% threshold required for Tukey's test, the tests are significant, regardless of the time of observation. The (positive) regression coefficient sign β_1 shows that aphid mortality increases according to the concentrations and observation times. The odds ratio showed that, if the neem oil is increased by one unit, aphid mortality increases by 200 µL for 24 h, 150 µL for 48 h, 100µL for 72 h and 50µL for 96 h after treatment.

Field test results

Condition of experiment on field test

The temperature at the research station varied between 36°C on November 2016 and 34°C on February 2017 (Figure 1). The relative e humidity varied from 34% in November 2016 to 17% in February 2017 Statistical analysis of the data showed that there was a significant difference ($P>0.05$) between neem oil doses compared to cabbage yield traits (Table 3).

Neem oil makes it possible to obtain a yield of apple that is not significantly different from that obtained with the reference insecticide. Figure 2 shows the evolution of the level of aphids as a function of the days of observation. Before treatment, the level of aphid infestation was estimated to be between 100 to 300 individuals per plant. Three days after the application of the product, a decrease in aphid infestation level was observed for all doses, while at the level of the control, the level of infestation increased.

DISCUSSION

According to the model obtained using the binary logistic regression, a 100 µL of neem's oil would be needed to kill 50% of aphids after 24 h and 200µL to eliminate 90%.

It appears from this work that neem oil can be used in the control of ashy cabbage aphids. In fact, the 100µL neem oil dose can reduce aphid infestation by 50% after three days of treatment. These results were similar to those obtained by Zakari *et al*. (2011) with *Neem* oil on cowpea aphid. Padi and Adu-Acheampong (1999) recommended a 3% concentrated Neem Azal as it was proven to be effective in laboratory conditions. Patricia and Eliane (2010) showed that, the neem oil, cause lower number of feeding sheaths per panicle and lower percentage of damaged grains. It can also cause significantly more damage on females of *Oebalus. poe-*

cilus than the males. Neem oil caused adult mortality higher than in the control at concentrations.

Neem oil has several biologically active compounds in its composition (Mulla and Su, 1999). but it owes its insecticidal power mainly to limonoid compounds such as salanin, nimbine and especially azadirachtin. Salanin and nimbine are antifeedants. Only azadirachtin acts as a growth inhibitor (lsman, 1997). Butterworth and Morgan (1968) were the first to isolate the active substance. Azadirachtin does not pose a significant risk when used at effective doses for non-target organisms such as bees, fish and aquatic insects (Scott and Kaushik, 1998).

Azadirachtin was tested on more than four hundred insect species among the Noctuidae, Cicadidae, *Chrysomelidae, Tortricidae* and Curculionidae. It was found to be active on more than 90% of them, which is accompanied by a great variability of LD_{50}, on more than 90 % of them (Koul, 1999). Azadirachtin acts as an antifeedant (Darazy-Choubaya, 2002) at concentrations ranging from ten to one hundred parts per million (ppm) depending on the species and mainly as a growth inhibitor or at concentrations ranging from one to ten ppm (Govindachari *et al*., 1996).

Other effects caused by azadirachtin have been reported, including a decrease in fertility. Azadirachtin treatment limits the ability of females to produce eggs. A decrease in oviposition is observed. According to Su and Mulla (1999), a treatment with azadirachtin, at a concentration of 5 to 10 ppm, on *Culex* populations, causes a significant change in the process of oviposition. Mondédji *et al.* (2014) revealed that the leaf extract of *A. indica* has a high insecticidal activity (mortality> 95%) and considerably reduces the fecundity of *M. persicae*.

According to them, the application of this extract (*A. indica*) on plants would significantly modify the feeding behavior of the aphid (probing by stylets, penetration of phloem, salivation and ingestion). Field experiments on the effectiveness of neem grain extracts by Gnago *et al*. (2010) showed that caterpillars and aphid populations were lower in treated plots than in untreated plots.

CONCLUSION

Laboratory experiments and field trials were conducted in Niger to evaluate the effectiveness of neem oil as an insecticidal agent. Laboratory test results showed that once neem oil was sprayed on the leaves of the cabbage in plates, aphids' attacks were reduced or stopped.

In laboratory, the neem oil dose 100 µL can kill 50% of aphids in 24 h, while 100 (µL) was enough to eliminate 100% of aphids in 96 h. The field trial results showed that concentrations of 15% neem oil led to 50% reduction of aphids in three days, and 75% in seven days.

This study showed that neem oil at 15% concentration can control the leafy cabbage aphid in the field as well as acetamiprid and Lambda-Cyhalothrin combined. Cabbage growers can therefore use neem oil to control cabbage aphid.

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