Effect of two neem-derived pesticides on Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Coleoptera: Chrysomelidae) under laboratory conditions

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Abstract: Mortality and antifeedant activity of two different neem-derived pesticides were investigated on larvae of Colorado potato beetle (*Leptinotarsa decemlineata* Say). In no-choice tests, mortality of larvae increased with increase in time period, meanwhile the feeding damage decreased with the increase of neem leaf extract concentration in contrast to NeemAzal T/S (1% azadirachtin) in which neither there was any significant difference in mortality nor on feeding damage. In the choice test, none of the treatments were lethal to the larvae tested. The larvae fed on the leaves irrespective of the treatment.

Keywords: azadirachtin, neem leaf extract, biological control, Colorado potato beetle, potato

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Introduction

Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say, Coleoptera: Chyrsomelidae) is an important pest of potato causing significant economic losses world-wide. CPB destroys all the green vegetative parts of potato, sometimes resulting in 100% yield loss and is also a vector of bacterial potato ring rot disease (*Clavibacter michiganensis* subsp. *Sepedonicus* Smith 1910 Davis et al. 1984) (Alkan et al. 2015). CPB is a multivoltine insect and uncontrolled populations can destroy the whole yield during the growing season (Alkan et al. 2017). CPB feeds mostly on solanaceous crops as they contain high concentrations of toxic glycoalkoloids in their foliage which the beetle detoxifies and excrete them with the diet (Wimer et al. 2015). Management of CPB using chemical insecticides is a common control measure that is applied since many decades. (Alkan et al. 2017). As a result of regular chemical control, CPB is currently resistant to most classes of synthetic insecticides (Kutas and Nádasy 2005). This ability of detoxifying the active compounds can explain their ability to develop resistance to different insecticides (Wimer et al. 2015).

Combination of chemical insecticides is a simple approach to prevent the development of resistance (Trisyono and Whalon 1999), but the damage to the environment and the beneficial organisms dwelling in such environments is still inevitable. The growing challenges and concerns about the negative impacts on the environment and resistance to various insecticides lead researchers to look for alternative solutions to these. An alternative control method is biological control using entomopathogenic microbes such as *Bacillus thuringiensis* var. *tenebrionis* Berliner, 1915 (*Btt*). It is considered as a promising agent against CPB but frequent usage of *Btt* could result in resistance to it (Trisyono and Whalon 1999). Apart from microbes, several plant extracts have been screened for their toxic and/or antifeedant effects on CPB. Plant derived pesticides and insect feeding inhibitors for crop protection are gaining attention (Kutas and Nádasy 2005) but are still not exploited to their maximum potential. There could be several advantages of these plant-derived pesticides such as they are of natural origin, harmless to humans and non-target organisms and as such environmentally friendly. Combined application of *Btt* and plant derived insec-

ticides can prevent the development of resistance to either of them. They represent a sustainable control method permitted in organic farming (Skuhrovec et al. 2017).

Azadirachtin, one of the most active insecticidal compounds found in Neem (*Azadirachta indica* A. Juss.) has been studied previously for its effects on CPB. It is a tetranortriterpenoid and is known to possess strong antifeedant properties (Isman et al. 1990). Zabel et al. (2002) demonstrated the effect of neem extracts on CPB third instar larvae under laboratory and field conditions. They found a satisfying antifeedant activity of neem on CPB larvae under laboratory conditions and foliage protection under field conditions and suggested neem as a part of integrated pest management (IPM) programs in small orchards, private gardens and tree rows. Schmutterer (1985) found that there is a strong insecticidal effect of neem seed kernel extract on CPB larvae. In addition, there was a significant reduction in the feeding damage in the treated plots. In another study conducted by Moreau et al. (2006), the effect of companion planting along with different botanical extracts was evaluated. They found that 2% of neem extract sprayed on the potatoes on the field resulted in lower CPB densities, lower leaf damage and higher yields as compared to control plots as compared to other treatments Novodor, companion planting, garlic and capsaicin extracts. When Hiesaar et al. (2000) applied different water dilutions of NeemAzal-T/S (1% azadirachtin) on CPB eggs, they found that the embryonic development of the eggs was almost complete but only 47% eggs hatched, while the rest perished inside the eggshell. Additionally, they found a direct mortal effect on 2-day-old larvae of first instar, whereas fourth instar larvae showed varied effects along with potent antifeedant properties.

Our aim of this study is to validate the effects of water extract of dried neem leaves, which has been used for centuries in the tropical and sub-tropical countries by the growers and farmers because of its easy availability and cheap costing; as compared to commercially available neem product (containing only 1% azadirachtin as the active ingredient) which is much more expensive, on CPB larvae under laboratory conditions in Hungary.

Materials and Methods

Preparation of neem leaf extracts (NLE)
The method was followed as per Doshi et al. (2018) and Petrikovszki et al. (2019) with modified working concentrations. Working concentrations of 1, 5, 10, 15 and 20% of NLE was prepared from a stock concentration of 20% using distilled water.

Preparation of azadirachtin (AZA)
A modified methodology of Doshi et al. (2018) and Petrikovszki et al. (2019) was used. The working concentrations used were 0.001, 0.003, 0.005, 0.01, 0.1% prepared from a stock concentration of 0.1% azadirachtin which was prepared by dissolving 10 mL of NeemAzal T/S (1% azadirachtin) in 100 mL distilled water.

Preparation of Bacillus thuringiensis var. tenebrionis (Btt)
Btt was prepared as a positive control. A 2% solution of commercially available Btt was made from Novodor (3.0% *Bacillus thuringiensis* var. *tenebrionis*) by mixing 2 mL of Novodor in 100 mL distilled water.

Collection of CPB larvae
Freshly hatched, first and second instar larvae from the untreated leaves of potato cv. 'Balatoni Rózsa' were collected in the experimental field of Szent István University, Gödöllő campus. Fresh non-infected potato leaves of the same potato variety were collected for different treatments and serve as a food source.

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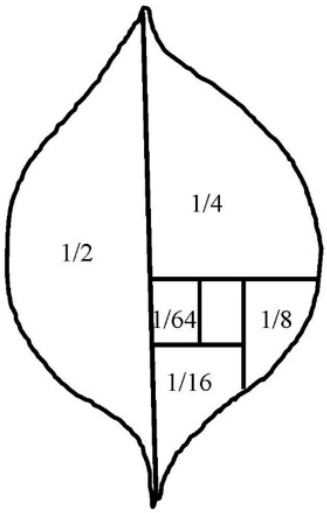

Figure 1. Diagrammatic representation of a potato leaf and used for assessing the feeding damage caused by Colorado potato beetle larvae.

a. No-choice test

The fresh undamaged potato leaves were dipped in the respective treatment solution for 10 seconds and kept outside for 1 min for drying at room temperature before placing them on moist filter paper in 9 cm glass Petri dishes. A total of 5 individuals, which included freshly collected mixed population of newly hatched and 1st instar larvae were placed on the top of the leaves using a fine brush. A negative control was performed by dipping the leaves in distilled water and positive control was by using 2% of Novodor. Each treatment was replicated 3 times. The Petri dishes were closed with the lid and kept at a temperature of 25±2°C, relative humidity of 60±5%, light intensity of 16L:8D conditions. Larval mortality and feeding damage (represented diagrammatically in Fig. 1) on the leaves was observed and recorded for a time period of 24, 48, 72, 96 hours. One-way ANOVA post-hoc Tukey's test was performed on the data using RStudio v 3.4.0 (2017) to compare the different treatments against each other and graphs were made in the excel.

b. Choice test

The setup for choice test was the same as the no-choice test except that it was performed in 15 cm diameter glass Petri dish with 2 fresh undamaged potato leaves, one treated with different concentrations of neem products and the other with distilled water and placed on the opposite side of Petri dishes on moist filter paper. Five individuals consisting random mixture of first, second and third instar larvae were placed in the centre of the Petri dish and the dish was closed with a glass lid. A negative control was performed by dipping both the leaves in distilled water and a positive control was performed by dipping one leaf in 2% Novodor (*Bacillus thuringiensis* var. *tenebrionis*) (*Btt*) solution and the other in distilled water. The conditions were the same as that in no-choice test. Larval mortality and feeding damage (Fig. 1) on the leaves was observed and recorded for a time period of 24, 48, 72, 96 hours. One-way ANOVA post-hoc Tukey's test was performed on the data using RStudio v 3.4.0 (2017) to compare the different treatments against each other and graphs and graphs were made in the excel.

Table 1. Effect of different concentrations (%) of two different neem-derived pesticides on mortality of CPB larvae at different time interval under no-choice condition. Different letters represent significant difference at 95% confidence level. Data are mean of 3 replicates.

Treatment	Conc (in %)	24h mortality (mean ± SE)	48h mortality (mean ± SE)	72h mortality (mean ± SE)	96h mortality (mean ± SE)
Control 0	0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Neem Azal T/S (AZA)	0.001	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	0.003	0.0 ± 0.0 a	0.0 ± 0.0 a	6.66 ± 6.66 a	6.66 ± 6.66 a
	0.005	0.0 ± 0.0 a	7.00 ± 6.66 ab	6.66 ± 6.66 a	13.33 ± 13.33 a
	0.01	0.0 ± 0.0 a	7.00 ± 6.66 ab	13.33 ± 6.66 a	33.33 ± 6.66 a
	0.1	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
neem leaf extract (NLE)	1	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	5	0.0 ± 0.0 a	7.00 ± 6.66 ab	6.66 ± 6.66 a	6.66 ± 6.66 a
	10	0.0 ± 0.0 a	20.00 ± 20.00 ab	33.33 ± 13.33 a	40.00 ± 11.54 a
	15	0.0 ± 0.0 a	53.00 ± 24.03 b	66.66 ± 17.63 b	80.00 ± 11.54 bc
	20	0.0 ± 0.0 a	13.00 ± 13.33 ab	66.66 ± 13.33 b	93.00 ± 6.66 c
Btt	2	0.0 ± 0.0 a	0.0 ± 0.0 a	6.66 ± 6.66 a	26.66 ± 13.33 a

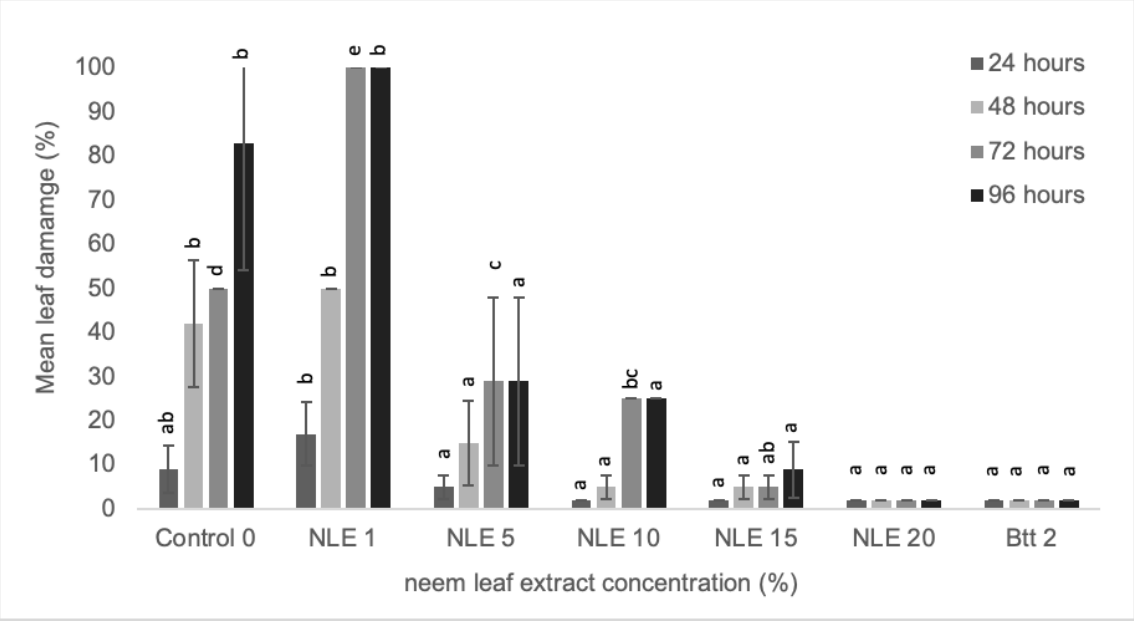

Figure 2. Effect of different neem leaf extract (NLE) concentrations (%) on mean leaf damage (%) caused by CPB larvae at different time interval under no-choice condition. Different letters indicate significant difference at 95% confidence level ($p<0.05$). Data are mean of 3 replicates.

Results

a. No-choice test

Two different neem-derived pesticide products were used for this experiment with different concentrations to check their efficacy against CPB larvae (Table 1). In case of AZA, there is no significant difference in the mortality after 96 hours post-treatment even at the highest concentration of 0.1%. The

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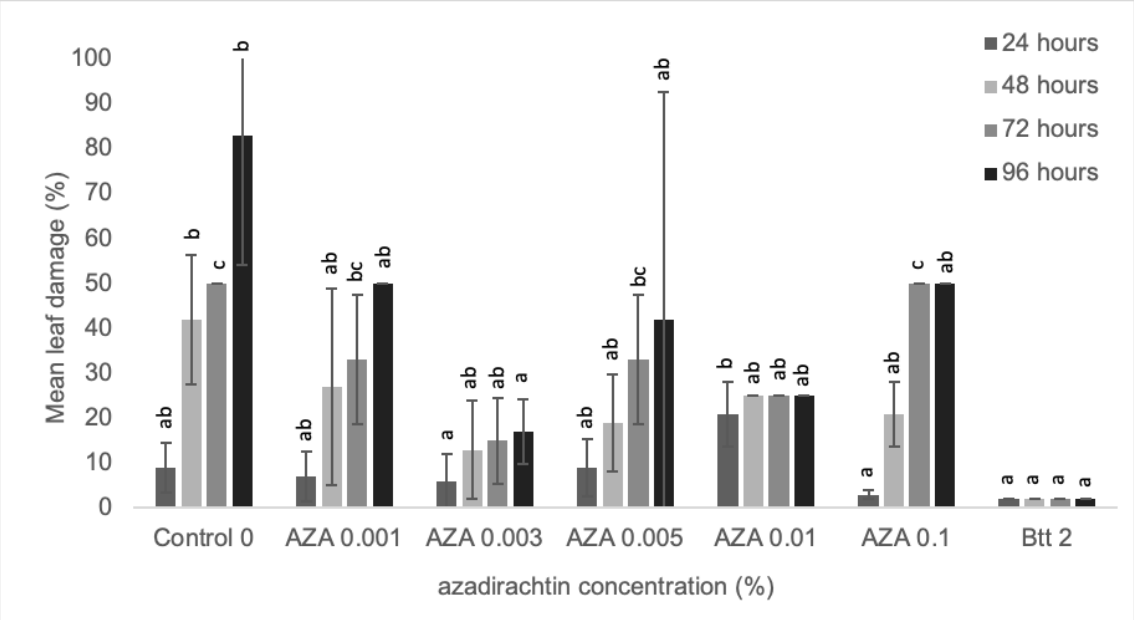

Figure 3. Effect of different azadirachtin concentrations (AZA) (%) on the mean leaf damage (%) at different time interval caused by CPB larvae under no choice condition. Different letters indicate significant difference at 95% confidence level ($p<0.05$). Data are mean of 3 replicates.

NLE was much more lethal as compared to AZA for CPB larvae. There was a significant difference ($p<0.05$) in mortality of CPB larvae with the increase in concentration as the time progressed. NLE 15% and 20% showed the highest mortality of 80 and 93% at 72h and 96h respectively and were significantly different from the rest of the treatments. *Btt* did not show any significant difference in the mortality of the larvae at the given working concentration.

CPB feeds mainly on potato leaves which is why the different concentrations of neem leaf extract and azadirachtin were tested on the feeding of CPB and leaf damage (%) was assessed (Fig 2, 3 respectively) at different time interval under no-choice condition. After 24 hours post-treatment, there was no significant difference between the feeding damage caused by the CPB larvae throughout the different NLE concentrations (Fig 2) compared to negative control. After 48 hours post-treatment, significant reduction in feeding damage was observed in the case of NLE 5-20% and *Btt* whereas NLE 1% did not show any difference as compared to Control 0. At 72h post-treatment, all NLE concentrations showed significant difference in feeding damage compared to negative control and for 96h post-treatment, NLE 5-20% and *Btt* showed significant difference compared to negative control, which coincides with the high mortality as seen in Table 1 after 72 and 96h post-treatment respectively.

In the case of azadirachtin (Fig 3), the feeding damage was not consistent. At 24h post-treatment, no significant feeding damage was observed. At 48h post-treatment, only *Btt* showed significant reduction in feeding damage while in the case of 72h AZA 0.003 and 0.01% and *Btt* significantly reduced the feeding damage. In the case of 96hpost-treatment, only AZA 0.003% an *Btt* showed significant reduction in feeding damage.

b. Choice test

In this test, the effect of different neem de-

Table 2. Effect of different concentrations (%) of two different neem-derived pesticides on mortality of CPB larvae at different time intervals under choice condition. Different letters represent significant difference at 95% confidence level. Data are mean of 3 replicates.

Treatment	Conc (in %)	24h mortality (mean ± SE)	48h mortality (mean ± SE)	72h mortality (mean ± SE)	96h mortality (mean ± SE)
Control 0	0	0.0 ± 0.0 a	13.33 ± 6.66 a	13.33 ± 6.66 a	13.33 ± 6.66 a
Neem Azal T/S (AZA)	0.001	0.0 ± 0.0 a	0.0 ± 0.0 a	20.00 ± 11.547 a	20.00 ± 11.547 a
	0.003	0.0 ± 0.0 a	0.0 ± 0.0 a	13.33 ± 13.33 a	13.33 ± 13.33 a
	0.005	6.66 ± 6.66 a	6.66 ± 6.66 a	13.33 ± 13.33 a	26.66 ± 17.64 a
	0.01	13.33 ± 6.66 a	13.33 ± 6.66 a	40.00 ± 0.00 a	40.00 ± 0.00 a
	0.1	6.66 ± 6.66 a	20.00 ± 11.547 a	26.66 ± 6.66 a	33.33 ± 6.66 a
Neem leaf extract (NLE)	1	6.66 ± 6.66 a	13.33 ± 13.33 a	20.00 ± 11.547 a	33.33 ± 6.66 a
	5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	10	0.0 ± 0.0 a	6.66 ± 6.66 a	13.33 ± 6.66 a	13.33 ± 6.66 a
	15	0.0 ± 0.0 a	6.66 ± 6.66 a	13.33 ± 13.33 a	13.33 ± 13.33 a
	20	0.0 ± 0.0 a	6.66 ± 6.66 a	6.66 ± 6.66 a	13.33 ± 13.33 a
Btt	2	0.0 ± 0.0 a	6.66 ± 6.66 a	13.33 ± 6.66 a	26.66 ± 6.66 a

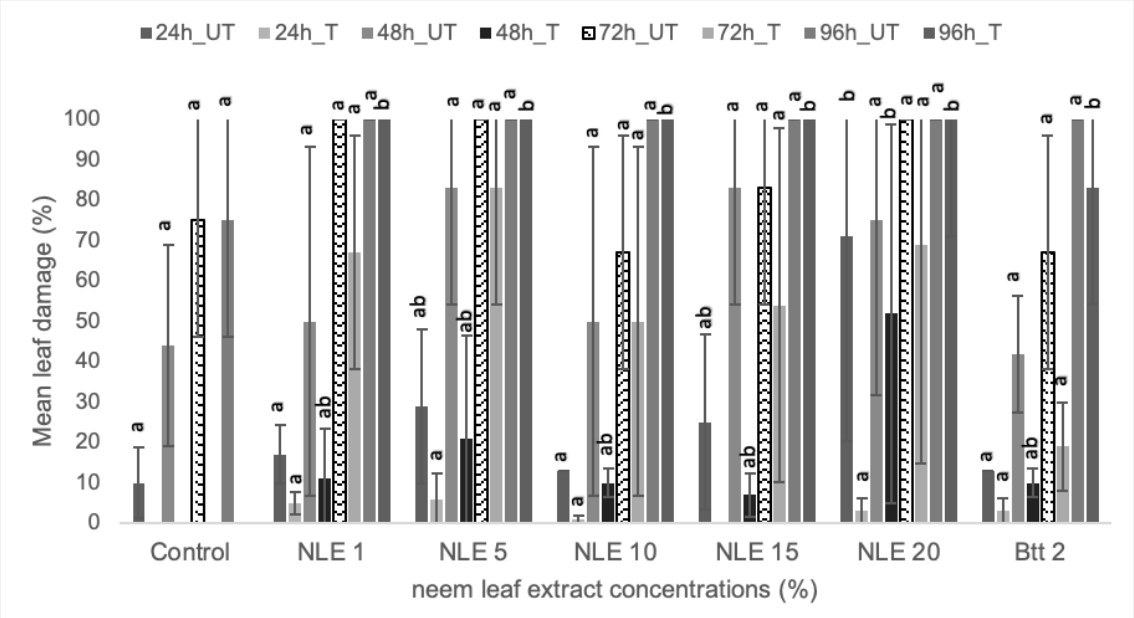

Figure 4. Effect of different neem leaf extract (NLE) concentrations (%) on the mean leaf damage (%) at different time intervals caused by CPB larvae under choice condition. Different letters represent significant difference at 95% confidence level ($p<0.05$). Data are mean of 3 replicates.

rived pesticide products on the mortality of CPB larvae and the feeding damage can be investigated better (Table 2). There is no significant difference between different treatments for the entire time period throughout the experiment. NLE 5% showed no mortal-

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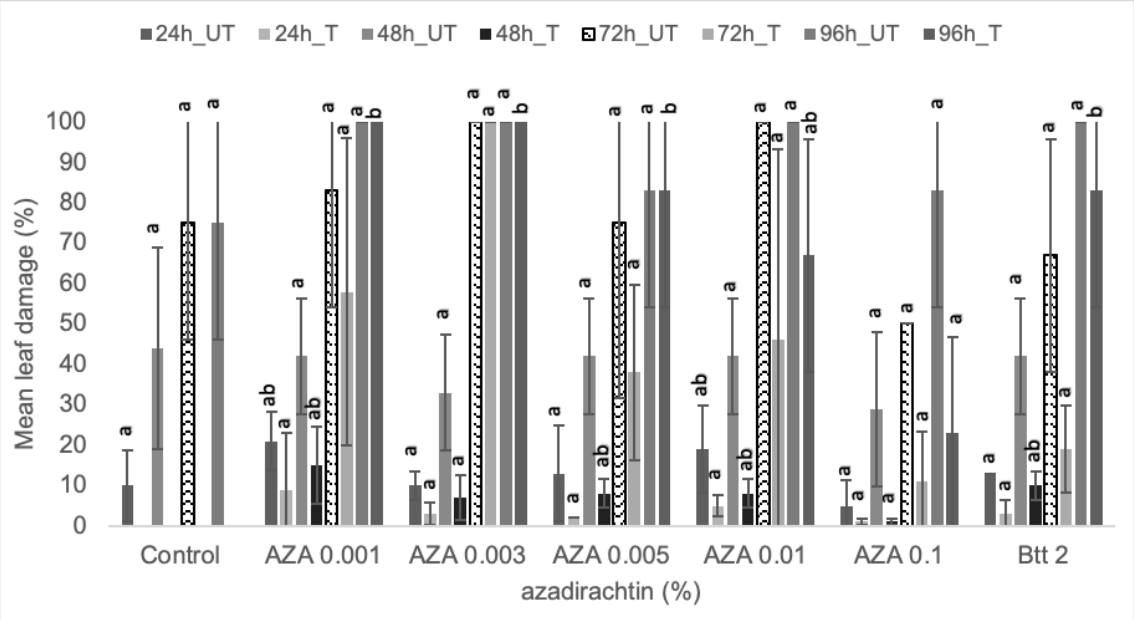

Figure 5. Effect of different azadirachtin (AZA) concentrations (%) on the mean leaf damage (%) at different time intervals caused by CPB larvae under choice condition. Different letters represent significant difference at 95% confidence level ($p<0.05$). Data are mean of 3 replicates.

ity even after 96 h post-treatment. The maximum mortality (%) was seen for AZA 0.01% after 96 hr post-treatment followed by AZA 0.1% yet the difference was not significant.
In the case of neem leaf extract, leaves treated with NLE 20% showed a significant difference in the leaf damage after 48h. In addition, it is also evident that all treatments had a significant reduction in the mean leaf damage at 96 h when compared to their respective untreated leaves (Fig. 4). Similarly, in the case of azadirachtin, all treatments had a significant reduction in the mean leaf damage at 96 h when compared to their respective untreated leaves (Fig. 5).

Discussion

It is evident that neem leaf extract is toxic to the newly hatched and first instar larvae. Intoxication of CPB larvae when treated with different but higher neem leaf extract concentrations showed delayed but high mortality as seen from the no choice test as compared to azadirachtin. Delayed larval mortality in the case of neem leaf extract might be due to the antifeedant activity of different compounds found in NLE and larvae as seen from the results. Another possible reason could be that the various compounds present in the NLE are slow in their action (Trisyono and Whalon 1999) or the accumulation of lower concentrations of neem compounds in the gut system and then acting on the hormonal system as suggested by Zehnder and Warthen (1988) and Trisyono and Whalon (1999).

On the contrary, weak mortality results were obtained in the case of azadirachtin in the no choice test for both the products in choice test. This might be because of the mixed population of the larvae and there is a possibility that the second and third instar larvae have more evolved gut system to digest neem and excrete out the toxic compounds Wimer et al (2015) and sparing the untreated leaf for the first instar larvae with weaker gut system.

Another possibility can be the uneven distribution of different compounds on the leaf extract. Perhaps there was not enough of concentration of different compounds found in neem leaves on the leaf surface which in turn was not enough for larval mortality. Another reason can be the slow toxic effect of the different neem compounds.

With respect to antifeedant properties, a strong antifeedant activity was observed in the case of neem leaf extract in the no choice experiment which might be due to different compounds present in the leaf extracts acting either alone or in combinations. Similar results were obtained by Alford et al. (1987) when they tested antifeedant activity of Limonin against Colorado potato beetle larvae. Also, Zabel et al, (2002) found that neem extract had a strong antifeedant activity against Colorado potato beetle larvae under laboratory conditions which is like our results from the no choice test but contradicts the results from choice test. In the case of azadirachtin the antifeedant activity was weak in our experiment-, Our results contradict the work done by Hiiseer et al. (2000) where the azadirachtin from the same commercial product showed only 12% consumption is Howver, our findings are consonant with the results reported by Klocke and Barnby (1989). and with the work done by Hiiseer et al. (2009) where they could not find any significant effect on feeding activity. Kutas and Nadasy (2005) experienced similar results of low antifeedant activity in the case of azadirachtin (NeemAzal T/S) and they argued that this can be possible due to the low concentration of azadirachtin used for the experiment while the recommended dose is 0.3-0.5%.

Conclusion

In our experiments, we found mixed results according to the antifeedant and lethal effects of commercial azadirachtin and neem leaf extract, respectively. We found that in these aspects traditional neem leaf extract was superior to the commercial product. The reason for it could be that it contains not only azadirachtin but many other biologically active different compounds which exhibit different plant protection properties. Field trials are necessary to validate our hypothesis. In addition, detailed analysis of different compounds present in the neem leaf extract should be done to estimate their concentration.

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References

Alford, A.R., Cullen, J.A., Storch., R.H., Bentley, M.D. (1987). Antifeedant Activity of Limonin Against the Colorado Potato Beetle (Coleoptera: Chrysomelidae). Journal of Economic Entomology. 80: 575-578. DOI: https://doi.org/10.1093/jee/80.3.575

Alkan, I., Gökçe, A., Kara, K. (2015). Antifeedant activity and growth inhibition effects of some plant extracts against larvae of Colorado potato beetle [*Leptinotarsa decemlineata* Say (Col: Chyrsomelidae)] under laboratory conditions. Turkish Journal of Entomology. 39: 4. 345-353. DOI: http://dx.doi.org/10.16970/ted.35600

Alkan, I., Gökçe, A., Kara, K. (2017). Stomach poison activity of some plant extracts on Colorado potato beetle (Coleoptera: Chrysomelidae). Bitki Koruma Bülteni. 57: 3. 305 – 315. DOI: http://dx.doi.org/10.16955/bitkorb.297213

Doshi, P., Póss, A.M., Tóth, F., Szalai, M., Turóczi, G. (2018) Effect of neem-derived plant protection products on the isopod species *Porcellionides pruinosus* (Brandt, 1833). ZooKeys. 801: 415–425. DOI: http://dx.doi.org/10.3897/zookeys.801.25510

Hiiesaar, K., Metspalu, L., Jõudu, J., Kuusik, A. (2000). Diverse effects of Neemazal-T/S revealed by preimaginal stages of Colorado Potato Beetles, *Leptinotarsa decemlineata* say. In: Dr. H. Kleeberg and C. P. W. Zebitz (Eds.) Proceedings of the 9th Workshop; Hohensolms, Germany, March 13. – 15. 2000 Druck Graphic, 35396 Giessen, 79-83

Hiiesaar, K., et al., (2009). Influence of Neem-Azal T/S on feeding activity of Colorado Potato Beetles (*Leptinotarsa decemlineata* Say). Agronomy Research. 7: (Special issue I), 251–256

Isman, M., Koul, O., Luczynski, A., Kaminski, A. (1990). Insecticidal and Antifeedant Bioactivities of Neem Oils and Their Relationship to Azadirachtin Content. Journal of. Agriculture and Food Chemistry. 38: 6. 1406-1411.

Klocke, J. A., and M. A. Barnby. 1989. Plant allelochemicals as sources and models of insect control agents, 455- 465. In C. H. Chou and G. R. Waller [eds.], Phytochemicals ecology: allelochemicals, mycotoxins, and insect pheromones and allomones. Academia Sinica Monograph Series No. 9. Institute of Botany, Taipei, Taiwan

Kutas, J., Nádasy, M. (2005). Antifeedant Effects of Several Plant Extracts on Colorado Potato Beetle Larvae. Acta Phytopathologica et Entomologica. Hungarica. 40: (3–4). 355–365.

Moreau, T. L., Warman, P. R. & Hoyle, J. (2006). An evaluation of companion planting and botanical extracts as alternative pest controls for the Colorado potato beetle. Biological Agriculture and Horticulture. 23: 351–370. DOI: https://doi.org/10.1080/01448765.2006.9755336

Schmutterer H. (1985). Which insect pests can be controlled by application of neem seed kernel extracts under field condition? Zeitschrift für angewandte Entomologie. 100: 468–475

Skuhrovec, J., Douda, O., Pavela, R., Klouček, P., Božik, M., Zouhar, M. (2017). The Effects of *Pimpinella anisum* Essential Oils on Young Larvae *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). American Journal of Potato Research. 94: 64–69. DOI: http://dx.doi.org/10.1007/s12230-016-9549-x

Trisyono, A., Whalon, M. (1999). Toxicity of Neem Applied Alone and in Combinations with *Bacillus thuringiensis* to Colorado Potato Beetle (Coleoptera: Chrysomelidae). Journal of Economic Entomology. 92: 6. 1281—1288. DOI: https://doi.org/10.1093/jee/92.6.1281

Wimer, A., Philips, C.R., Kuhar, T.P., Adams, J.C., Szendrei, Z. (2015). A New Tool for Resistance Management: Baseline Toxicity, Ovicidal Activity, and Field Efficacy of the Novel Insecticide Tolfenpyrad on Colorado Potato Beetle, *Leptinotarsa decemlineata*. Advances in Entomology. 3: 139-147. DOI: http://dx.doi.org/10.4236/ae.2015.34017

Zabel, A., Manojlovic, B., Rajkovic, S., Stankovic, S., Kostic, M. (2002) Effect of neem extract on *Lymantria dispar* L (Lepidoptera: Lymantriidae) and *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Journal of Pest Science. 75: 19-25. DOI: https://doi.org/10.1046/j.1439-0280.2002.02006.x

Zehnder, G., Warthen, J.D. (1988). Feeding Inhibition and Mortality Effects of Neem-Seed Extract on the Colorado Potato Beetle (Coleoptera: Chrysomelidae). Journal of Economic Entomology. 81: 4. 1040-1044. DOI: https://doi.org/10.1093/jee/81.4.1040