LARVICIDAL ACTIVITY OF THREE MEDICINAL PLANTS AGAINST CATTLE SCREWWORM (CHRYSOMYA BEZZIANA) IN EASTERN CAPE, SOUTH AFRICA

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Abstract

Infestations of cattle with myiasis caused by Chrysomya bezziana Villeneuve are one of the most prevalent parasitic conditions in the Eastern Cape. Most rural farmers in the province use alternative materials in the control of these parasites. This study was conducted to evaluate the efficacy of aqueous extracts of three commonly used plants: Aloe ferox, Ptaeroxylon obliquum and Calpurnia aurea against the larvae of C. bezziana. Ten larvae were placed in each Petri dish with plant extracts at concentrations of 2, 3, 4, 5 and 10 % (w/v). Distilled water and Wound Oil N.F. (Propetamphos 0.25% m/v) were the negative and positive control respectively. Larval mortality was assessed at 3h intervals for 24h and the experiment was carried out in triplicate. Sustained immobility of larvae, after exposure to extracts, was considered death. Generally, larval mortality was positively correlated with time of exposure and concentration, with the exception of A. ferox, which had lower mortality at 5 and 10%. Both C. aurea and P. obliquum caused 50% mortality at 9h and 21h post exposure to extracts respectively, whereas A. ferox did not within 24h. Highest larval mortality of 100% and 73% were recorded at 10% concentration for C. aurea and P. obliquum respectively while maximum mortality of 30% was recorded for A. ferox at 4% concentration. The plants exhibited variable efficacy against the larvae of C. bezziana. C. aurea showed very high efficacy, which makes it a potential natural larvicide against the cattle screwworm.

Keywords: Calpurnia Aurea, Cattle, Mortality, Plant Extract, Screwworm

Introduction

The cattle screwworm fly, Chrysomya bezziana Villeneuve is an obligate parasite, which poses a threat to most of the world’s livestock (Sutherst et al., 1989). Cattle myiasis caused by the larvae of C. bezziana also known as maggots is one of the most prevalent parasitic conditions in the Eastern Cape Province of South Africa. The control of these parasites for many years has relied heavily on the use of chemicals, organophosphates and subcutaneous ivermectin but these commercial drugs tend to be unaffordable for the rural farmers. Therefore, most of the resource-poor farmers use alternative remedies since they offer a low-cost intervention for the treatment of animal diseases and care of wounds. Researchers are now focused on finding newer remedies, which will be safe, effective and also easily available at low cost especially to the resource-poor farmers.

Some plant extracts and oils have already been tested for larvicidal activities on Chrysomya spp. (Kumarasinghe et al., 2002; Wardhana et al., 2007). In South Africa, the leaves of Calpurnia aurea (Aiton) Benth and powdered roots are used to destroy lice and to relieve itches. Unspecified part of the plant is used to destroy maggots and the leaves are used to treat allergic rashes, particularly those caused by caterpillars (Asres et al., 2001; Tadeg et al., 2005). Split or crushed fresh leaves of Aloe ferox Mill have an excellent reputation for the treatment of sores and injuries in livestock as well as healing of open wounds, burns, and ulcer in humans (Van Wyk and Gericke, 2003). The strong and durable wood of Ptaeroxylon obliquum (Thunb.) Radlk contains an aromatic resin known to cause violent sneezing. It is used as a moth repellent and the powdered bark is used to controls ticks on livestock (Lemmens, 2008).

The objective of this study was to evaluate the efficacy of A. ferox, P. obliquum and C. aurea against the larvae of C. bezziana. These plants have been reported (Soyelu and Masika, 2009) to be among the most commonly used plants for the treatment of cattle wounds and myiasis in parts of the Eastern Cape Province, South Africa.

Materials and Methods

Larvae collection and identification
Fly larvae were collected from cattle wounds at Amatola Basin (32° 41’ S and 26° 59’ E) of the Eastern Cape Province, South Africa following the procedure previously described by Adams and Hall (2003). Collected larvae were evaluated and confirmed to be the larvae of Chrysomya bezziana at the Department of Entomology, Onderstepoort Veterinary Institute (ARC-OVI), Pretoria. All animals used were
carefully and humanely handled in accordance with the guidelines approved by the ethics committee of the University of Fort Hare. The larvae used for the study were maintained on a crude meat diet in the laboratory (Sukarsih et al., 2000) and used for the experiment on the same day.

**Preparation of plant extracts**

Leaves of *A. ferox*, *P. obliquum* and *C. aurea* were collected from Amatola Basin and voucher specimens deposited at the Giffen herbarium of the University of Fort Hare. Fresh leaves of each plant were macerated and extracted separately with distilled water by shaking on an orbital shaker for 24 hours. The aqueous extracts were filtered first by using a muslin cloth and then Whatman’s No. 1 filter paper after which extracts were freeze-dried. Each plant extract was then stored in a sealed glass bottles and kept in the refrigerator (4°C) until use.

**In vitro larvicidal assay**

The *in vitro* assay was carried out as previously described by Wardhana et al. (2007) with a little modification. Aqueous leaf extracts of plant materials in concentrations of 2%, 3%, 4%, 5% and 10% (w/v) were prepared and poured into separate Petri dishes (90mm, Merck®). Then, ten larvae were placed in each Petri dish and larval mortality was assessed at 3-hour interval for 24 hours. Wound oil N.F. (Propetamphos 0.25% m/v, Dichlorophen 1.0% m/v; Bayer Pty Ltd., RSA), a known active agent for the treatment of wounds and maggot infestations on cattle and sheep, was used as the positive control while distilled water was used as negative control. The experiment was done in triplicate and sustained immobility of larvae after exposure to extracts was considered as death of the larvae.

**Statistical analysis**

Obtained data were analysed using Analysis of Variance (ANOVA, Program GLM) to determine the effect of concentration and time of exposure on larval mortality. Average larval mortality was separated among treatments using Duncan’s MRT procedure (SAS, 1999).

**Results**

Larval mortality was positively correlated with concentration of plant extracts (Table 1) and time of exposure (Fig.1) except for *A. ferox*, which had lower mortality at 5 and 10% concentrations. The highest larval mortality of 100% and 73% were recorded at 10% concentration for *C. aurea* and *P. obliquum* respectively while maximum mortality of 30% was recorded for *A. ferox* at 4% concentration. In the positive control, larvae were weak right from the first observation period (1-3h) and 100% mortality was observed after 12h post exposure. At 10% concentration, *C. aurea* displayed higher larvicidal activity than the positive control (commercial product) however; there was no significant difference in percent mortality after 9h post exposure to maggots (Fig.1). The negative control showed no mortality throughout the 24h period of observation as larvae remained mobile and active.

<table>
<thead>
<tr>
<th>Conc. (%)</th>
<th>Percent larval mortality due to each plant extract</th>
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<tr>
<td></td>
<td><em>Aloe ferox</em></td>
</tr>
<tr>
<td>2</td>
<td>8.80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>9.20&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>4</td>
<td>17.90&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>5</td>
<td>1.30&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>10</td>
<td>3.30&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a-e</sup> Values in the same column with the same letter are not significantly different at *P* ≤0.05.
Discussion

It was observed from the results presented that efficacy of plant extracts in this study differs and depended on concentration as well as the time of exposure to larvae. Generally, larval mortality increased with concentration with the exception of A. ferox where larvae exposed to the plant extracts were actively trying to crawl out, such that the most efficient concentration (4%) was unable to kill 50% of larvae over a 24-hr period. Probably, the larvae of C. bezziana were repelled by the extract of A. ferox rather than being killed when exposed to it.

A. ferox is widespread in the Eastern Cape province of South Africa and is traditionally used for the treatment of various skin problems including burns, wounds, eczema, skin cancer, and so on (Loots et al., 2007; Jia et al., 2008). Previous phytochemical studies confirmed the presence of aloin, aloe-emodin, polysaccharides, mannose, acemannan, barbaloin, aloenin, and lectins (Hutchings et al., 1996; Loots et al., 2007; Wintola & Afolayan, 2011) in the whole-leaf of A. ferox. Emodin and the mucilaginous polysaccharides contained in the pulp of Aloe leaf are the main ingredient responsible for promoting wound healing (Tang et al., 2007). The plant has been reported to be an insect repellent and a study by Mawela (2008) documented the repellent activity of its crude acetone extract at 10% concentration against the livestock tick, Rhipicephalus appendiculatus. Although A. ferox seemed not to be very effective against the larvae of C. bezziana, farmers might have considered them useful because of its wound healing and repellent ability.

The aqueous leaf extract of P. obliquum showed considerable larvicidal activity with an average larval mortality of 73% at the highest concentration (10%) tested within 24 h. The bark of P. obliquum is used to control ticks in livestock and known to contain resins, pyrogall, saptaeroxylon (an acid saponin) and an alkaloid (Archer and Reynolds, 2001). The resins in the bark of P. obliquum also possess some repellent activities (Pontes et al., 2007). So far, there is no scientific evidence to corroborate the larvicidal efficacy of it leaves.

The larvicidal efficacy of C. aurea was higher than the two other leaves at all concentrations tested and found to be comparable to the positive control, which is a commercial product, used for the treatment of wounds and maggot infestations on cattle and sheep. Although the identity of its larval killing-compounds has not yet been determined, various researchers have undertaken the chemical analysis of C. aurea and a range of compounds have been identified. Phytochemical studies revealed the South African specie of C. aurea yielded the well-known alkaloids; virgiline, hydroxylupanine, calpurnine and their carboxylic esters (Vermin et al., 1979; Van Wyk et al., 1991) but the main pharmacologically active compound of C. aurea is the alkaloid calpurneen and its 13α-(2'-pyrrolocarboxylic acid) ester (Vermin et al.,
The plant has been reported to possess ectoparasiticidal properties by the Borana people of northern Kenya who use it for treating humans and cattle (Zorloni, 2007). Its antimicrobial properties have also been reported (Taged et al., 2005; Adedapo et al., 2008) and in Eritrea, (Waka et al., 2004) fresh leaves of C. aurea crushed and soaked in water are used to wash the body of animals with lice and the animals are reported to remain free of lice for about two weeks afterwards. Farmers in the study area reportedly crush and soak the leaves of C. aurea in cold water and the infusion is used as a wash for cattle wounds.

Conclusion

This study has revealed that A. ferox, P. obliquum and C. aurea exhibited variable efficacy against the larvae of C. bezziana. All the plants tested showed considerable larvicidal properties but it is noteworthy that C. aurea showed very high efficacy, which makes it a potential natural larvicide against the cattle screwworm. The traditional use of the three plants was therefore justified to some extent by the results obtained in this study. However, further studies have to be done to determine the active principles and potential toxic effect of these plants on the animal skin.

Acknowledgements

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