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EVALUATION OF NEMATOCIDAL ACTIVITY IN NATURAL HONEY

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Abstract

Nematode parasites cause serious diseases in humans and livestock animals. The present study was undertaken to explore the effect of honey on the model nematode *Caenorhabditis elegans*. *In vitro* immersion tests showed that honey samples exhibited strong paralyzing effects on different developmental stages of *C. elegans* with LD_{90} in the range of 0.75-1.5%. Among the honey tested, *Plactranthus* honey showed most potent activity with LD_{90} of 0.75% followed by *Acacia* honey (LD_{90} 0.75-1.5%). This study pointed out the potential of honey as an antinematodal agent.

Introduction

Human and livestock animals suffer from helminth infections caused by trematodes (flukes), nematodes (roundworms) and cestodes (tapeworms) (Kumar & Clark, 2005). They are of huge importance for human tropical medicine and for veterinary medicine. WHO estimates that nearly two billion people harbour parasitic worm infections (http://www.who.int/wormcontrol/statistics/). Nematode parasites (roundworms) cause serious morbidity to people mainly in the developing countries. In human, several nematodes produce diseases such as Filariasis, Strongyloidiasis, River blindness etc. (Hotez *et al*., 2004). Most of our knowledge on nematode anatomy, development, and genetics arise from studies on *Caenorhabditis elegans*, but are transferable to other nematodes (Kurz & Ewbank, 2003). The *C. elegans* is a free-living nonpathogenic nematode which lives in temperate soil environment where it survives on feeding microbes such as *E. coli*. The adult *C. elegans* is 1mm long consisting of nearly thousand somatic cells and variable number of germ cells in gonade. Due to the simple anatomy, short life cycle, easy and rapid culturing and low testing cost, *C. elegans* has been used as a model 'parasite' for identification of nematocidal activity in plant extracts, purified natural products and synthetic compounds (Kumaran et al., 2003; Kermanshai et al., 2001; Enwerem, 2001; McGaw et al., 2000).

Honey has long been used for treatment of various diseases in folk medicine around the world (Molan, 2001). Antimicrobial activities of honey against bacteria and fungi are extensively reported in the scientific literature (Al-Waili, 2004; French *et al*., 2005). However, its antinematodal potential has not been studied so far. In continuation of our research on medicinal benefits of honey (Azim *et al*., 2007; Mesaik & Azim, 2008), we have attempted to investigate the antinematodal effect of honey using *Caenorhabditis elegans* as experimental tool.

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Material and Methods

Caenorhabditis elegans (strain N2) and *Escherichia coli* (strain OP50) were received from Caenorhabditis Genetics Center, University of Minnesota, USA. A total of 7 natural untreated honey samples were collected from different locations of northern Pakistan. In addition to a multifloral wild honey sample from oriental hive bee (*Apis cerana*), 6 predominantly unifloral honey samples collected from colonies of European bee (*Apis mellifera)* foraging on *Acacia modesta* (Kh1, Ac4 and Aj4) and *Plactranthus* spp., (Sw2, Sw3, Sw4) were used for experimentation.

Caenorhabditis elegans (strain N2) was grown monoxinically with *Escherichia coli* strain OP50 as food source on NGM medium (Sulston & Hodgkin, 1988). The poured Petri plates of NGM medium were seeded with 0.1-0.2 ml of overnight cultures of *E. coli* grown in Lauriabroth and allowed to grow into a lawn before nematodes were added. Fifteen to 50 nematodes of different developmental stages (i.e. larvae, adult males and hermaphrodites) along with few eggs (Hall & Altun, 2008) were placed in 500µl of S medium (Sulston & Hodgkin, 1988) in microtitre wells containing 30µl of 5X concentrated overnight culture of *E. coli*. Each honey sample at 0.5, 0.75, 1.5, 3.0, 6.0 and 12.0% dissolved in sterilized water or in S medium was added directly to the wells. Survival of *C. elegans* was observed after 4-5 and 12-13 hours of incubation and the nematode viability was assessed by counting hatched eggs and motile worms using an inverted microscope**.**

Results and Discussion

In vitro antinematodal activity of 7 natural honey samples was tested against the different developmental stages (i.e. larvae, adult males, hermaphrodites and eggs) of *Caenorhabditis elegans*. Honey samples displayed strong paralyzing effects on all developmental stages of *C. elegans* with the LD_{90} values ranging 0.75-1.5% of honey (Fig. 1). All honey samples totally inhibited the growth of worms at ≥3.0% concentrations. In the wells containing 12% honey, all worms were found dead within 4-5 hours of incubation whereas no viable *C. elegans* was observed in the wells containing 0.75-3% honey after 12 hours (Fig. 1). After 16 hours, no significant change could be seen. Three *Plactranthus* honey samples (Sw2, Sw3 and Sw4) and one *Acacia* honey sample (Kh1) exhibited most potent activity among the tested samples with LD_{90} 0.75%. However, *Acacia* honeys (Ac4 and Aj4) showed LD_{90} 1.5% followed by wild honey (Sw1) with LD_{90} 3.0%.

Detection of potent nematocidal activity in honey against different developmental stages of the worm is an indication of the arsenal honeybee produces for defense against helminth parasites. Even though the constituent(s) of honey responsible for this property yet to be identified; however we can speculate honey peptides as the probable candidate(s) since several antinematodal peptides from natural sources have been reported (Park *et al*., 2004; Jang *et al*., 2004). Recently, a 5.8-kDa bioactive peptide from manuka honey has been determined (Tonk *et al*., 2007). The present study supported the idea of honey's usefulness against helminth diseases in particular application to intestinal nematode infections including Ascariasis, hookworm infection etc.

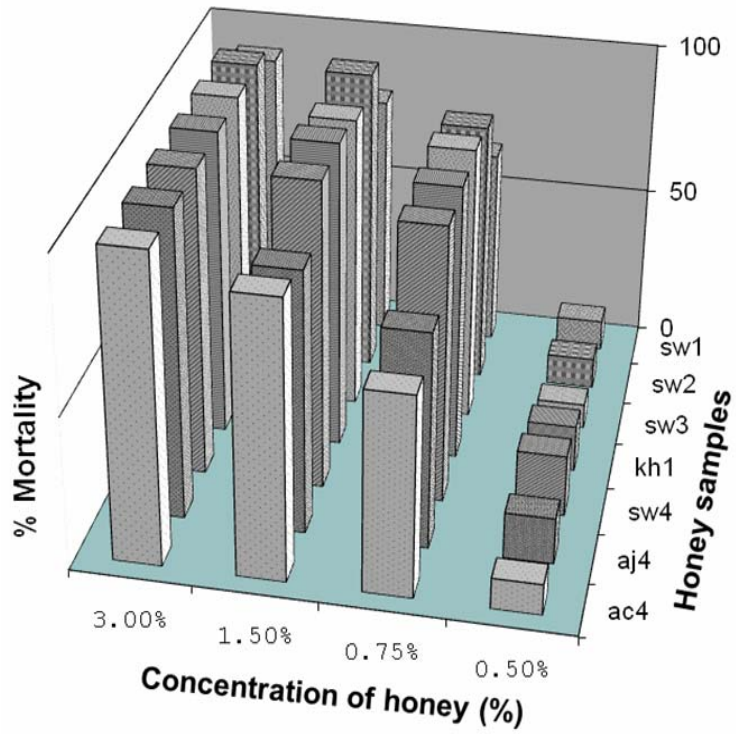

Fig. 1. Effect of a range of concentration of honey samples on mortality of *C. elegans* after exposure for 12 hours. The data are presented as percent mortality to the control. Acacia modesta honey sample codes, Kh1, Ac4 and Aj4; Plectranthus honey sample codes, Sw2, Sw3 and Sw4; wild honey sample code, Sw1.

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