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Efficacy of flavonoids in controlling *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae), a post-harvest pest of grain legumes

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Abstract

The effects of partially purified flavonoids obtained from *Calotropis procera* (Ait.) R. Br. and six standard flavonoids on the adults and eggs of *Callosobruchus chinensis* (L.), reared on mung beans (*Vigna radiata* L.), were studied. All flavonoids were toxic to adults and eggs depending on dose and exposure period. Flavonoids obtained from *C. procera* showed the highest contact toxicity followed by standard quercetin, rutin and quercitrin at 10 mg mL^{-1} doses in filter paper diffusion assay. Significant reduction in oviposition was found for all flavonoids at the doses of 5 and 10 mg mL^{-1} on grains in plastic jars. Flavonoids also showed an ovicidal effect on bruchid eggs as well as affecting the number and weight of the emerging adults as a function of concentration.

Keywords: Flavonoids; *Calotropis procera*; *Callosobruchus chinensis*; Grain protection; Grain legumes

1. Introduction

Grain legumes are important and economical sources of proteins in Asian and African vegetarian diets (Stanton et al., 1966). India is the largest producer of pulses in the world accounting for 28% of the global production. The annual production of pulses in India has fluctuated between 12 million metric tons (MMT) and 14 MMT within the last four decades, coming from around 24 million hectares (nearly 35%) of land under their cultivation (Anonymous, 1998). Grain legumes suffer heavy quantitative and qualitative losses during storage from the attack of stored grain pests, especially *Callosobruchus* species. Insects often cause extensive damage to stored grains and grain products, and this may amount to 5–10% in the temperate and 20–30% in the tropical zones (Nakakita, 1998). Caswell (1981) reported a loss of approximately 50% of cowpeas in storage for 3 or 4 months is due to infestation by *C. maculatus* (F.). The adzuki bean weevil, *C. chinensis* (L.), the most widespread and destructive primary insect pest of stored legumes, is prevalent in India. One of the most practical means to increase availability of grain legumes is to minimize pest-associated losses. It is, therefore, necessary to reduce storage losses by controlling pests on stored grains.

Control of these insect populations in stored food, feedstuffs and other agricultural commodities around the world is primarily dependent upon continued applications of organophosphorus and pyrethroid insecticides and the fumigants methyl bromide and phosphine. Although effective, their repeated use for decades has disrupted biological control by natural enemies and led to outbreaks of other insect species and sometimes resulted in the development of resistance to pesticides. It has had undesirable effects on non-target organisms, and fostered environmental and human health concerns (Champ and Dyte, 1976; Subramanyam and Hagstrum, 1995; White and Leesch, 1995). According to the April 2000 decision of the Montreal protocol, however, methyl bromide, a proven ozone depleter in

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the atmosphere, will be phased out by 2005 in advanced countries and by 2015 in developing countries (TEAP, 2000). Furthermore, phosphine resistance is becoming more common (Bell and Wilson, 1995; Chaudhry, 1995) and is a matter of considerable concern. These problems have highlighted the need for the development of selective insect-control alternatives of biological origin. Many plant extracts and essential oils may serve as alternative sources of stored-product insect-control agents because they constitute a rich source of bioactive chemicals (Hill and Schoonhoven, 1981; Konstantopoulou et al., 1992; Desmarchelier, 1994; Shaaya et al., 1997). Much effort has, therefore, been focused on plant-derived materials as potential sources of commercial insect-control agents.

Calotropis procera (Ait) R. Br. (Asclepiadaceae) commonly known as ‘Arka’ in India is a popular medicinal plant found throughout the tropics of Asia and Africa. Various parts of this plant have been widely used in traditional systems of medicine. An ethanolic extract of the flower of this plant is reported to have antimicrobial, anti-inflammatory, antipyretic, analgesic (Mascolo et al., 1988), anticancer (Smit et al., 1995) and antimalarial activities (Sharma and Sharma, 1999, 2001). Likewise water, ethanol, acetone and some other organic solvent extracts of this plant have insecticidal (Moursy, 1997), larvicidal (Markouk et al., 2000), antibacterial and anti-parasitic (Larhsini et al., 1999) activities. Flavonoids, cardenolides, triterpenoids, alkaloids, resins, anthocyanins, tannins, saponins and proteolytic enzymes are the main constituents in *C. procera* latex. (Deepak, 1995).

Flavonoids are a major class of secondary metabolites constituting about 5-10% of the known secondary products in plants ranging from bryophytes to angiosperms. To date there are about 5000 flavonoids documented in plants and the list is steadily increasing (Madhuri and Reddy, 1999). Flavonoids from leaves of *Annona squamosa* (Kotkar et al., 2002) and *Ricinus communis* (Upasani et al., 2003) were found to arrest the population growth of adzuki bean weevil, *C. chinensis* L in green gram (*Vigna radiata* L.) during storage. Thin layer chromatography and HPTLC studies have indicated the presence of 5 flavonoids in the alcoholic foliar extract of *C. procera*. Of the five, the major band was characterized and identified to be quercetin (Mendki, 2002).

This paper describes a laboratory study to assess the potential of partially purified flavonoids from *C. procera* and six standard flavonoids for the control of a stored grain pest. Effect of these flavonoids on behaviour of adults of adzuki bean weevil, *C. chinensis* (L.) and their possible mode of action was also investigated.

2. Materials and methods

2.1. Test insects

A culture of *C. chinensis* has been maintained on mung beans in our laboratory since 1999. Mung beans (*Vigna radiata* L.) were used to culture *C. chinensis* for all the experiments. The legume seeds were obtained from a supermarket, washed in tap water to ensure the removal of pesticide residues, dried and then heated at 65 °C for 6 h to kill any developing larvae. These seeds were stored in airtight tins until required for experiments, which were carried out at a constant temperature of 30 ± 2 °C, relative humidity 70 ± 5% at 16: 8 light: dark photoperiod (Mendki et al., 2001). Unmated adults were obtained from the culture by collecting mung beans with obvious emergence ‘windows’ indicating the presence of fully developed beetles. These beans were placed individually in glass test tubes, sealed with lids with holes for aeration, until the adults emerged.

2.2. Extraction and isolation of partially purified flavonoids from plant material

Partially purified flavonoids from *C. procera* were extracted as per the procedure of Agarwal (1997) slightly modified by Kotkar et al. (2002). The standard flavonoids, (flavone, rutin, quercetin, myricetin, fisetin and quercitrin) were purchased from Sigma Chemicals Co., St. Louis, MO, USA.

2.3. Insecticidal assay of flavonoids

The insecticidal activity of flavonoids against adults of *C. chinensis* was determined by direct contact application. Different concentrations (0.1, 1, 5 and 10 mg mL^{-1}) in 500 μL aliquots in methanol of each test compound was sprayed on filter papers (Whatman No. 2, 5 cm diameter). Controls received 500 μL methanol. After drying under a fume hood for 5 min, each filter paper was placed in the bottom of a glass Petri plate (5.5 cm diameter × 1.2 cm). Ten adult *C. chinensis* (0–24 h old) were placed on the filter paper in each dish and dish lids replaced. The treated insects were maintained at 30 ± 2 °C and 70 ± 5% relative humidity with 16: 8 light: dark photoperiod. Adult mortality was observed 1, 2, 3 and 4 days after treatment. Knocked-down beetles were regarded as dead, if they did not show any response after gentle touch with a fine paintbrush.

2.4. Effect of flavonoids on oviposition of C. chinensis

A 500 μL dose each of 0.1, 1, 5 and 10 mg mL^{-1} (in methanol) of flavonoids was applied to 150 mung beans (10 g) placed in 6 × 6 × 6 cm separate plastic jars. The grains were shaken well to ensure uniform distribution