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Toxicological effect of underutilized plant, *Cleistanthus collinus* leaf extracts against two major stored grain pests, the rice weevil, *Sitophilus oryzae* and red flour beetle, *Tribolium castaneum*

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ABSTRACT

Toxicity and repellency activity of *Cleistanthus collinus* (Roxb.) (CC) leaf extracts were evaluated against rice weevil, *Sitophilus oryzae* (L.) and red flour beetle, *Tribolium castaneum* (Herbst) under laboratory condition. Five concentration(s) (1%, 1.5%, 2%, 2.5% and 4%) with two controls (acetone and water) treatments along with deltamethrin were used for direct and contact residual toxicity. The insect that survived after CC exposure were transferred to an untreated feeding substrate and the population buildup of subsequent two generation were recorded after 30 (F_1) and 60 days (F_2). In the contact residual toxicity, highest CC concentration (4%) produced 75% mortality in *S. oryzae* and 62.5% mortality in *T. castaneum* during 7 days of exposure, whereas in direct toxicity the mortality were 81% and 58% respectively, for *S. oryzae* and *T. castaneum*. The long term effect of CC was apparent in both the insect species, where F_2 populations were significantly decreased in the CC treatments. CC treatment at 4% produced similar adult mortality in comparison to deltamethrin at 1%. In addition, repellent activity of CC extracts was observed against both *S. oryzae* and *T. castaneum*. This is the first step towards assessing the scientific basis for the understanding the effectiveness of CC extracts against stored grain pests and it could be a viable eco-friendly option for stored grain insect pest management.

1. Introduction

Rice holds the record of being staple food for more than 1.6 billion people around the world, particularly in Asia, Latin America, and some parts of Africa. It is invariably infested by insect pests at all stages starting from their growth until they are being processed and consumed. Tropical and sub-tropical regions of the world facing difficulty to increase their food grain production; however they are more quantity of produce lost between harvest and consumption by storage insect pest (Kumar and Kalita, 2017). Storage is the one of the important post-harvest practices that when properly executed, helps in reducing food shortage problems. Among the different losses in post-harvest operation, storage holds a major share (7.5%). According to food and agricultural organization (FAO), approximately 70% of the farm produce is stockpiled by farmers for various purposes. In India, farmers generally use locally designed storage structures for bulk storage of their commodities which in most cases are not insect or moisture proof (Grover and Singh, 2013).

Storage insect pests inflict both qualitative and quantitative loss to cereals, making them unfit for sowing (loss viability) or for food or feed. Further, it also creates serious problems for food industries and export commodities via., food contamination (Rajendran, 2002). United Nation (UN) reports suggest that, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), are the two foremost storage insect pest of rice throughout the world (Sallam, 2014). These are most devastating stored insect pests that causes about 30–40% weight losses thereby have greater economic importance. Moreover, pest infestation may have upto 90% damage potential within 5–6 months after infection.

It is extremely difficult to eradicate any species of insect pest as they are coevolved with plants, so it can only be managed by bringing down the population to below economic injury level (EIL). In storage also different management options like physical, bio-control agents, sex pheromones, botanicals, fumigants and synthetic insecticides *etc* (Golob, 1997; Shaaya et al., 1997; Rosell et al., 2008; Islam et al., 2010; Isman, 2017) have been evaluated and recommended to farmers.

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Constant use of these synthetic chemicals in storage has led to the development of resistant insect strains. Earlier works (Guedes et al., 1996, DARB, 2003; Benhalima et al., 2004) suggested that constant insecticide pressure over the period of time applying the selection pressure to insect, which in turn inducing them to the develop the insecticide resistant strains. Thereby, the recommended concentration or effective concentration of insecticides increased that owing to various health and environmental hazards (Bell and Wilson, 1995; Pimentel et al., 2009). Because of this awareness, worldwide researchers mostly concentrate on the development of sustainable and alternative mode of insect pest management in storage condition, with emphasizes on reassessment of plant derived usage or green chemistry (Rajendran and Sriranjani, 2008; Erler et al., 2010; Saroukolai et al., 2010; Yang et al., 2010; Ayvaz et al., 2010; Isman, 2017)

Phyto-products or botanicals are promising alternatives to synthetic insecticides for stored grain protection due to their environment-friendly nature (Copping and Menn, 2000). The plant products are indigenous and abundantly available at low cost and have wide spectrum of activity such as insecticide, anti-feedents, repellents, larvicidal, ovicidal and insect growth regulators (Isman, 2006; Guleria and Tiku, 2009). To deter the insect in storage, neem leaves were mixed with stored grains in earlier days and this practice is still followed in many developing countries. Plant products are generally harmless to the environment due to their rapid recycling; thereby preventing the development of resistance in insects as well as accumulating in the non-targeted organism and environment (Shaaya et al., 1997; Saroukolai et al., 2010). Thus, botanicals emerge as one of the leading approach to protect the grains in storage. Utility of plant products against insect pest of storage have been studied and important active components exhibiting potentiality as a grain protection agent had been reported earlier (Isman and Akhtar, 2007; Rajendran and Sriranjani, 2008; Guleria and Tiku, 2009). However, only a countable number of botanicals like neem have been extensively used as a commercial product in insect pest management programmes (Regnault-Roger et al., 2012; Pavela, 2016).

Genus, *Cleistanthus* belonging to the family Phyllanthaceae consists of 140 species are widespread herb in many parts of rural India. Among these, *Cleistanthus collinus* (Roxb.) is well known by different local names in various parts of India. The entire plant is poisonous and different kind(s) of toxic compounds were reported from extracts of different plant parts (Subrahmanyam et al., 2003). The main chemical constitution of the plants belong to arylnaphthalene lignan lactone glycosides which include cleistanthin A, cleistanthin B, collinusin and diphyllin (Subrahmanyam et al., 2003; Kettimuthu et al., 2011).

From the earlier works it is evident that this plant plays multiple role(s) in-terms of their therapeutic usage such as insecticidal, larvicidal, antifungal, antiseptic, antimicrobial, diuretic and anticancer properties (Tamilselvan et al., 2014). The versatile actions of such plants are mostly possible because of the existence of diverse group of phytochemicals in the plant. In this context, the present study was focused to provide the first information on the toxicity and repellency of *C. collinus*, against two important storage insect pests, *S. oryzae* and *T. caestaneum*. Detailed evaluations of survival capacity of parent generation (F_0) were performed by contact residual and direct toxicity trail (immediate effect) and their effects (long-term trans-generational effect) were assessed on their progenies (F_1 and F_2).

2. Materials and methods

2.1. Insect rearing

Populations of *S. oryzae* and *T. castaneum* were collected from National Rice Research Institute (NRRI) (20°45′ N latitude, 85°93′ E longitude and 36 m altitude), Cuttack, India storage godowns, to start the initial laboratory culture on local rice variety (Naveen). Rice grains and flour were used for rearing of *S. oryzae* and *T. castaneum*, respectively and were kept separately in glass jar (15.5 × 10.5 cm) inside the Biological Oxygen Demand (BOD) incubator at 27 ± 2 °C temperature and 70 ± 5% relative humidity (RH). The jars were covered with perforated lids for aeration. Maximum of 3 days were allowed for mating and oviposition after that parent insects were removed. Later, grains and flour containing the eggs were transferred to fresh rearing jars covered with muslin cloth, fastened with rubber bands to prevent the contamination of grains and escape of beetles. Before starting the experiments the progenies were reared for about 5–6 generations. For the continuous supply of insects, sub-culturing was done at regular intervals. In the present study newly emerged (1–3 days old) *S. oryzae* and *T. castaneum* adults were used for bioassay.

2.2. Preparation of Cleistanthus collinus leaf extracts

Leaves of *C. collinus,* were collected from NRRI fields and were thoroughly washed in tap water followed by rinsed with distilled water twice. The cleaned leaves were dried at room temperature (28 ± 2 °C) in the absence of sunlight for 3 days to achieve constant weight and grounded into powder using a mixer grinder. Soxhlet extraction apparatus was used to extract the *C. collinus* (CC) leaf compounds by placing 200 g leaf powder in 1 L acetone (AR grade) (Valladares et al., 1997; Ali et al., 2012). CC extract was filtered under suction through Buchner funnel into another conical flask and it was concentrated at 40 °C with 220 rpm using a rotary evaporator (Heidolph, Germany) to obtain crude extract. After preparation the extracts were kept under 4 °C. These pure extracts were dissolved in required quantity of pure acetone to get desired concentrations (1%, 1.5%, 2%, 2.5% and 4%).

2.3. Contact toxicity bioassay

Impregnated paper assay (Kljajic and Peric, 2006), that is consistent with FAO method 15 (Busvine, 1980) was used to test the efficacy of five CC concentrations against *S. oryzae* and *T. castaneum*. Whatman No. 1 filter papers were cut into two equal parts and immersed in different CC concentrations for 30 s. In order to test the impact of solvent, acetone alone included as separate treatment and water was used as control. The treated filter papers were air dried for 2–3 min and then placed individually in different pedri-dish. Consecutively, adults of *S. oryzae* and *T. castaneum* were released into the pedri-dish. Each treatment comprised of four replications and each replication had 25 adults (male and female) per treatment. Observations on insect survival were recorded 3, 5 and 7 days after the insect release by touching it with hair brush (Camel No - 23202) and considered dead when no visible stimulus is observed. All the dead insects were removed from the petri-dish after observation. Similarly 0.05%, 0.1%, 0.3%, 0.5% and 1% of deltamethrin (2.5 WP) efficacy were also tested for both *S. oryzae* and *T. castaneum* with above said methodology and insect survival were recorded 1, 3 and 7 days after the insect release.

2.4. Direct toxicity bioassay

The insecticidal activity of different concentration (1%, 1.5%, 2%, 2.5% and 4%) of CC extracts and control (water and acetone) against adults of *S. oryzae* and *T. castaneum* were evaluated by direct toxicity bioassay. All tests were conducted in petri-dishes with a surface area of 63 cm^2. One day before the tests, 25 g of CEM I 52.5 N material were added into petri-dishes to create the concrete surface. Twenty five numbers of *S. oryzae* and *T. castaneum* adults were placed in the pedri-dish and 1 ml of CC extracts were sprayed as a fine mist using a hand atomizer. After spraying, 5 g of milled rice kernel and flour respectively, for *S. oryzae* and *T. castaneum* as they were the most preferred diets were placed on the pedri-dishes. Each concentration was replicated four times. Observation on insect mortality was recorded at 1, 3 and 5 days after treatment (DAT). All the dead insects were removed from the petri-dish after observation. Similar procedure was also taken up for