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Short communication

Potential of using phorbol esters as an insecticide against *Spodoptera frugiperda*

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ABSTRACT

Jatropha curcas oil is a promising candidate for biodiesel production. The oil is also rich in bioactive diterpenoids (phorbol esters, PEs). In the present study, the extracted PEs (PEs enriched fraction, PEEF) from Jatropha oil was evaluated for insecticidal activity against *Spodoptera frugiperda* (third instar larvae), which is a common pest in corn field across the tropical and subtropical areas. The PEEF exhibited contact toxicity with an LC_{50} of 0.83 mg ml^{-1} (w/v). The corn leaves treated with PEEF also declined the food consumption (33%), relative growth (42%) and food conversion efficiency (38%) at a concentration of 0.25 mg ml^{-1} (w/v). Higher reduction (39 and 45%) in the relative consumption rate was observed at 0.625 and 0.125 mg PEs ml^{-1} (w/v) of PEEF. Considering the rapid growth in Jatropha biodiesel industry, large amount of PEs can be harnessed, which can be further utilized as a biocontrol agent.

1. Introduction

Terpenoids are the largest class of plant metabolites comprising more than 40,000 structures (Bohlmann and Keeling, 2008). In plants, they may have different physiological, metabolic and structural roles or may have more discrete, specialized interactions with other organisms. The specialized interactions may include interaction with other organisms in context of reproduction, defence or symbiosis (Gershenzon and Dudareva, 2007). The role of specialized terpenoids may include, among others, as repellents, anti-feedentants, attractants, toxins or antibiotics. Due to their array of biological activities in nature, they have been widely exploited by humans as industrially relevant compounds in crude or purified forms for a long time, for example as a flavours, fragrances, pharmaceuticals, food supplements (vitamins), sweetners or biocontrol agents (pesticides) (Bohlmann and Keeling, 2008). The chemical diversity of terpenoids often originates from complex biosynthetic pathways and they may serve as a large volume feedstock resource for the production of industrial biomaterials. This resource can be utilized both in their naturally occurring forms or metabolically engineered forms in agriculture, forestry and horticulture (Bohlmann and Keeling, 2008). Harnessing the bioactive and economically valuable terpenoids requires interdisciplinary research which involves chemistry, biology and pharmacology, among others, giving ample opportunity and new means for the exploitation of terpenoids in agricultural and pharmaceutical applications.

In the present study, the diterpene from the *Jatropha curcas* plant is studied. Jatropha (Euphorbiaceae) seeds are an attractive feedstock resource of seed oil for biodiesel production. The rapidly increasing commercial cultivation gives ample opportunity to harness highly bioactive compounds as coproducts (Kumar and Sharma, 2008). Among the secondary metabolites, the Jatropha plant contains variety of terpenoids. So far, more than 65 types of diterpenes have been identified (Devappa et al., 2011). Among them, phorbol esters (PEs, a group of tigliane diterpenes) are the most investigated and these compounds possess high potency and bioactivity. There are 6 types of PEs present in the Jatropha oil (Haas et al., 2002). Previous reports suggest that the aqueous/organic solvent extracts from Jatropha oil/seed are effective as insecticidal and antimicrobial agent in vitro; and in majority of the studies, the activities are attributed to the presence of PEs (Devappa et al., 2010b). In the present study, preliminary investigation has been carried out to evaluate the potential of phorbol ester enriched fraction (PEEF), isolated from Jatropha oil, as an insecticide or insect deterrent in the insect, *Spodoptera frugiperda*. The *S. frugiperda* (Lepidoptera: Noctuidae) is a polyphagous species that commonly attacks economically important crops in several countries. This insect damages following crops: corn, sorghum, rice, wheat, alfalfa, beans, peanuts, tomato, cotton, potatoes, cabbage, spinach, pumpkin and cabbage (Cruz et al., 1999; Praça et al., 2006).

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2. Material and methods

2.1. Materials

J. curcas seeds (toxic Indian variety) were collected from wild trees (mature, approx. age 15 years) existing in places around Jaipur (geographical coordinates: 26°55′0″N, 75°49′0″E), Rajasthan, India. Phorbol 12-myristate-13-acetate (PMA), other chemicals was obtained from Sigma (St. Louis, USA) and all other chemicals/solvents used were of analytical grade.

2.2. Extraction of phorbol ester enriched fraction

J. curcas seeds were mechanically pressed using a screw press to obtain oil. The oil was centrifuged at 3150 × *g* for 20 min to remove residues. The extraction of phorbol esters enriched fraction (PEEF) was carried as reported by Devappa et al. (2010a). The oil was mixed with methanol (1:2, w/v) and the mixture was mixed at 55 °C for 15 min using a magnetic stirrer (300 rpm). Further, the mixture was centrifuged (3150 × *g* for 5 min) to get upper methanolic and lower oily layers. The methanol layer was rotaevaporated (Buchi, Germany) to get oily PEEF. The oily PEEF was stored at 4 °C for 4 months until further analysis.

2.3. Preparation purified phorbol ester rich extract (PEs-rich extract)

In brief, the PEEF were subjected to flash chromatography on 50 g of silica gel (40–63 μm, Merck), which had been preconditioned with dichloromethane (DCM). The column was eluted successively with DCM (500 ml), 1% DCM in MeOH (500 ml) then 5% DCM in MeOH (250 ml, collected in 50 ml fractions). Fractions containing PEs eluted with the 5% DCM in MeOH mixture and were concentrated in vacuo to yield a yellow PEs-rich extract. The PEs-rich extract was stored at 4 °C for 4 months until further analysis.

2.4. Phorbol ester analysis

The PEs were determined at least in duplicate according to Makkar et al. (2007a), based on the method of Makkar et al. (1997). Briefly, 0.5 g of PEEF or PEs-rich extract was dissolved in 2% tetra hydrofuran containing methanol. A suitable aliquot was loaded into a high-performance liquid chromatography (HPLC) fixed with a reverse-phase C_{18} LiChrospher 100, 5 mm (250 × 4 mm id, from Merck (Darmstadt, Germany) column. The column was protected with a head column containing the same material. The separation was performed at RT (23 °C) and the flow rate was 1.3 ml min^{-1} using a gradient elution Makkar et al. (2007a). The four phorbol ester peaks (containing 6 PEs) appeared between 25.5 and 30.5 min were detected at 280 nm. Phorbol-12-myristate 13-acetate was used as an external standard which appeared between 31 and 32 min. The area of the four phorbol ester peaks was summed and the concentration was expressed equivalent to PMA. The PEs detection limit in the HPLC was 3–4 μg.

2.5. Contact toxicity

Stock solutions of PEEF and PEs-rich extract was dissolved in acetone and applied topically (10 μl total volume) to the dorsal region of the thorax of third instar larvae of *S. Frugiperda* using a Hamilton microsyringe. The test included different concentrations (0.0313, 0.0625, 0.125, 0.25, 0.5, 1 and 20 mg ml^{-1}) of PEEF and PEs-rich extract; and a control (only acetone). Treated groups consisted of 10 larvae at each dose, replicated two times. The mortality of larvae was recorded after 24 h (25 ± 2 °C). Larvae that were unable to make coordinated movements within 10 s of prodding were assigned as dead. Results were corrected for mortality in the untreated larvae group and analyzed using the probit method.

2.6. Ingestion toxicity assay

The bioassay was carried out on *S. frugiperda* using leaf disc by no choice method to analyze the effects of PEEF on insect development by survival and for antifeedant activity of the insect. One *S. frugiperda* larvae (third instar) was placed in the centre of Petri dish and fed on corn leaves (1.5 cm in diameter) previously dipped into one of the treatment solutions of either the pure compounds in acetone (0.0625, 0.125 and 0.25 mg ml^{-1}) or solvent (acetone). Ten larvae were used for each dose with two replications. The ingestion toxicity was recorded after 24 h for 10 days (25 ± 2 °C). Data collected were: % mortality, % food consumption (visual), larvae weight (mg) and corn leaf disc weight (mg). The nutritional indexes calculated were: Relative growth rate (RGR) = (fw − iw)/(gw × *T*) [where fw, final weight; iw, initial weight; gw, geometric weight calculated as $(iw \times fw)^{1/2}$ and *T*, time (ten days)]; relative consumption rate (RCR) = *I*/(gw × *T*) [where *I*, ingested food calculated as (initial weight of food × % food consumption); gw, geometric weight and *T*, time (ten days)]; and food conversion efficiency (FCE) = (fw − iw)/*I* × 100 [where, fw, final weight; iw, initial weight; *I*, ingested food calculated as (initial weight of food × % food consumption)].

3. Results and discussion

The pests infesting economically important crops inflict marked losses in the agrarian production. The insects have always been recognised as one of the most serious agricultural problems. When compared to synthetic insecticides, usage of biological control agents offer the advantage of being compatible with the environment, often with high specificity, and represent a long-term solution for controlling insects that are particularly resistant to organic chemical based controlling agents. Therefore, many efforts have been made for controlling insects using natural biocontrol agents, such as plant phytochemicals. In the present study, the insect (*S. frugiperda*) chosen is a pest commonly present in corn fields in the tropical/subtropical countries such as Mexico and Brazil.

The concentration of PEs in PEEF and PEs-rich extract was 62.82 mg g^{-1} and 1.8 g g^{-1}, respectively. In the present study, the test concentration of PEs present in PEEF and PEs-rich extract was expressed equivalent of PMA. The contact toxicity of PEs present in PEEF and PEs-rich extract on *S. frugiperda* is shown in Fig. 1. The PEEF exhibited dose dependent increase in mortality of larvae, exhibiting insecticidal activity. The LC_{50} of PEEF was 0.83 mg of PEs ml^{-1} (w/v). However, no effect was observed for the PEs-rich extract. This may be due to degradation of PEs during storage (4 months at 4 °C) prior to conducting the experiment. The PEs content in the PEs-rich extract (in the dried form) were reduced by 48% in 98 days when stored at 4 °C (Roach et al., 2012). In another study, purified TPA decomposed slowly within 3 months when kept under dark at 4 °C and the degradation was extensive in 3 months at 25 °C in the diffused daylight. During decomposition 7-hydroperoxide was formed as a major product (Schmidt and Hecker, 1975). Similar decomposed products may have been formed during the PEs-rich extract storage in our study and thereby losing its bioactivity. In addition, TPA stored as a powder for 3 months at 25 °C (under diffused light) formed hydroperoxides, and at 4 °C (under dark) the hydroperoxides were formed but to a very limited extent. Whereas, the PEs present in the PEEF were found to be stable even after 2 years at 4 °C (Devappa et al., 2009).