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Report on Micro moth Phthorimaea operculella (Lepidoptera: Gelechiidae) infestation in Capsicum chinense Bhut Jolokia fruit

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Abstract: *The study was aimed at reporting the pest infesting the Capsicum chinense Bhut Jolokia fruits collected from Nagaland, Northeast India. The larvae present in the stored dry fruits were reared into adult in the laboratory and identified. The amount of capsaicinoids ingested by the larvae and accumulated in the adult after the pupation was estimated by UV Spectrophotometer and HPLC analysis. The pest was identified as Phthorimaea operculella (Lepidoptera: Gelechiidae) for the first time in Naga chilli. About 54.07% of fruit weight loss and 100% of fruit damage was caused by larva that reduces the fruit quality and quantity during storage. The amount of capsaicinoids ingested was 6.99 µg/mg and 2.65µg/mg in the larvae and adult respectively. The HPLC analysis confirmed the presence of capsaicin and dihydrocapsaicin peak in the acetonitrile extract of larvae as 71.87µg/ml and 78.37µg/ml respectively. The estimation confirmed the ingestion of capsaicin by the larvae there by reduce the quality of fruit. In practice, capsaicin was used as insect repellent but the reported species tolerate and survive the highly pungent environment. Hence the Lepidoptera infestation in Capsicum chinense has to be considered as it poses high rate of quarantine risk during storage and export.***

Keywords: *Capsicum chinense, Phthorimaea operculella, Micro moth, Gelechiidae, quarantine risk*

I. Introduction

Capsicum belongs to the Solanaceae family. There are 25–30 species of Capsicum, of which C. annuum L., C. frutescens Mill., C. baccatum L., C. chinense and C. pubescens Ruiz and Pavon have been commercially cultivated [1]. Capsicum chinense Bhut Jolokia (also called Naga Jolokia, Naga Morich, Bih Jolokia etc.) is in fact the world's hottest known chilli pepper with a pungent level of 879,953 to 1,001,304 SHU [2]. India is the largest producer, consumer and exporter of chillies in the world. It contributes about 36% to global chilli production and exports about 20% of its total production [3]. In 2008–2009, the value of chilli commodities including chilli powder, fruits and seeds exported was Rs. 25,838.27 Lacs [4]. According to Steve, Personal Communication, March 2010 a rough estimate, 1000 tonnes of Naga chilli is produced every year in north-eastern India, which under favourable weather conditions could increase up to five times [5]. The Naga chilli has immense scope in domestic as well as international market due to its remarkably high capsaicin content. Insects and mites are major pests of chilli which include thrips, aphids, whiteflies, fruit borers, cutworms, plant bug, mites and other minor pests [6]. Dried chilies when stored are often attacked by drugstore beetle. There is overall reduction in quantity and quality due to infestation of insect pests and vectors mainly on chilli (C. annuum), potato, tomato and aubergine in India [7,8]. Stegobium paniceum (Linn), cigarette beettle Lasioderma serricorne and Artbrodeis species feed on chillies, though the loss caused by them is negligible [9]. Insect pests not only feed on leaves and foliage, they also infest the fruits. Therefore, chilli peppers must be free of internal and external damage caused by pests. Pest damage affecting the flesh makes the chilli unfit for eating. Capsicum sp. are not only cultivated as vegetable and condiment crop, but also have recognizable pharmacological properties due to the presence of alkaloids collectively known as capsaicinoids [10]. The economic, nutritious and pharmacological importance of this genus is responsible for its high demand. It has a high value chemical priced at $325 per gram for 97% pure capsaicin [11] and its analogues in the fruit. Thus quality chilli fruit with high yield of capsaicin content was major target of chilli cultivators. The present study aims in reporting the pest infestation in the stored fruits of C. chinense.

II. Materials and Methods

2.1. Larva Collections

The C. chinense fresh and dry fruits collected from Nagaland and Manipur were screened for larvae infestation. The dried chilli fruits were stored in the container for a month. The larvae were collected from the stored fruits and observed under light microscope. The morphological details were documented.

2.2. Rearing of adult

Ten larvae isolated from the infected fruits were placed in separate rearing containers containing healthy uninfected 25 dry fruits. The fruits were weighed before the infestation. After a month the infested fruits (used for rearing) were weighed and the percentage of damage was calculated. The larvae matured and pupated within cocoons. After emergence, the moths were collected, frozen, wings were spread, and specimens were labeled and identified by M. Gabriel Paulraj, Entomology Research Institute, Loyola College, Chennai, South India.

2.3. Estimation of capsaicin ingested by the larva by UV spectrophotometer

Five larvae were weighed and ground individually with acetonitrile for the extraction of capsaicinoids present in the gut of the larvae. The homogenate was centrifuged at 10000rpm for 10min. The supernatant was scanned for the presence of capsaicin peak using UV visible spectrophotometer (Hitachi). The amount of capsaicinoids present in the larva was expressed in µg/mg of larvae calibrated using standard Capsaicin curve. The simple linear regression curve was developed using standard capsaicin purchased from Sigma Chemical. A stock solution of one milligram capsaicin per milliliter of ethanol was dissolved and different concentrations were prepared ranging from 10µg to 100 µg. The optical density was recorded at 280nm. The linear regression equation ($Y = mX + C$) was generated using the online Statistics and forecasting software (www. wessa.net).

2.4. Quantification of capsaicin ingested by the larva by HPLC

The acetonitrile extract of the larvae was filtered through 0.45 µm (Millipore filter) using a 1 ml disposable syringe (Millipore, Bedford, MA) into a sample vial, capped and stored at 5ºC in refrigerator until analysis. A Shimadzu (LC-10, Shimadzu, Japan) HPLC system equipped with LC- 10AS multi solvent delivery system, a SPD-10A UV-Vis detector at wavelength fixed at 280 nm and parameters controlled with system controller unit (SCL-10A) was used. The analysis was carried out following the conditions: column temperature 30ºC, mobile phase flow rate; 1 ml.min-1 and data acquisition with Class LC-10 software. All analyses were performed isocratically using degassed HPLC grade 50% acetonitrile (Merck, Germany) and 50% milli 'Q' water as a mobile phase. The reverse-phase chromatographic column (Discovery C18 (250 x 4.6 mm, 5 mm), Supelco, Bellefonte,PA, USA) was used for the detection of capsaicin and dihydrocapsaicin. The simple linear regression curve was plotted using standard capsaicin purchased from Sigma Chemical. A stock solution of one milligram capsaicin per milliliter of methanol was prepared from which different concentrations viz. 1000µg, 600 µg, 200 µg, 50 µg and 1 µg was made.

2.5. Scoville Heat Unit Conversions

According to the commonly accepted Scoville organoleptic test, the spicy strength of the investigated samples was calculated by converting the capsaicin content expressed in grams of capsaicin per gram of pepper. This conversion to Scoville heat units was done by multiplying the capsaicin content in pepper dry weight by the coefficient corresponding to the heat value for pure capsaicin, which is 1.6×10^7 [12].

III. Results

3.1. Morphology

The adult was identified by the scientist from Entomology Research Institute, Loyola College as micro moth Phthorimaea operculella **(Fig.1F).** The identified pest belongs to the Class Insecta, Order: Lepidoptera, Family: Gelechiidae. Genus: Phthorimaea. Synonyms: Gnorimoschema operculella Zell., Phthorimaea terella Wlk., and Gelechia solanella Boisd. The identified moth was small and light gray with roof-like wings. Forewings are widely lanceolate; wingspan 12-14 mm. Dark points form along the median crease. Hind wings were almost as wide as forewings with folded external edge. Antennae were gray with well-designated joints. Last abdominal segment is almost equal to 1/3 the length of the abdomen in the male. In female, the anal segment is of usual length. The apex of male abdomen is strongly pubescent, covered in dense, hairy bunches. Egg was oval, 0.4-0.6 mm in length, to 0.4 mm in width, at first pearl-white, later yellow and dark. The older larva flesh was colored yellowish red, with a pale longitudinal stripe along the median of its back, 10-12 mm in length, 1.5 mm in width. Pupa was brown, 5.5-6.5 mm in length. The abdominal apex had a small cremaster and chaetae. It also developed in cocoon of grayish with pale red color (Fig: 1E) that reaches 10 mm in length. Larval development continues for 11-14 days while caterpillars pass through 4 instars. Pupation occurs inside hidden cocoons located in various shelters. Pupal stage lasts 6-8 days. In storehouses, the pest reproduction proceeds continuously.

3.2. Estimation of capsaicin ingested by the larva

The larvae appeared red to orange in color due to the ingestion of capsicum fruit as a diet. Fruit weight loss of 54.07% and 100% of damage to the fruits was recorded (**Fig 1D)**. The UV spectrum of acetonitrile extract of the larvae and adult showed a peak at 280nm corresponding to the standard capsaicin peak. The amount of capsaicin present was calculated using the standard curve and found to be 34.98µg/ml for larvae and

5.3µg/ml for adult. The HPLC chromatogram of the acetonitrile extract of the larvae showed capsaicin and dihydrocapsaicin peak, corresponding to the standard capsaicin retention time. The amount of capsaicin and dihydrocapsaicin in the larvae was 71.87µg/ml and 78.37µg/ml respectively. The estimation confirmed the ingestion of capsaicin by the larvae there by reduce the quality of fruit.

3.3. Pungent level (SHU)

The pungent level in acetonitrile extract of C. chinense fruits showed 1,276,944 SHU. The pungent levels in infested larvae and adult were 559,080 SHU and 34, 500 SHU respectively
(Fig.2)

IV. Discussion

Chilli is an important cash crop in India and is grown for its pungent fruits, used throughout the world as a spice and also in the making of beverages and medicines. In our study the infested larvae from the stored dried fruits of C. chinense was reared into adult in the lab and identified as P. operculella based on the morphology. It is one of the most serious pests on plants of the Solanaceae family, either in the field or in stores [13]. In Ethiopia the damage due to P. operculella could reach up to 91% in 90 days in potatoes [14]. In our study, we observed these larvae more often penetrate green fruits. They create twisting tunnels in fruits and form web-like interlacing inside the fruit between the seed and the pericarp. The fruit borer larvae feed on flesh of the fruit and not seeds. Soon after hatching, larvae enter young fruits making a very small entrance hole (0.5 mm) which is difficult to detect. Before pupation, they leave the fruit, leaving large exit holes which facilitate secondary fungal or bacterial infections. The presence of a single larva inside a fruit is sufficient to render it unmarketable (and up to 18 larvae have been observed in a single fruit). Infestation also leads to premature fruit fall. In attacked crops, damage usually becomes more evident near harvest. Capsaicinoids are considered as the major bioactive components in hot peppers [15-18]. Capsaicin and dihydrocapsaicin, the two major capsaicinoids, were responsible for up to 90% of the total pungency of pepper fruits [19]. The UV-VIS spectrophotometric method is one of the most inexpensive and accessible for capsaicin quantification. High-pressure liquid chromatography (HPLC) is considered the most reliable and rapid method [20] available for the identification and quantification of capsaicinoids. In our study, the identified larvae tolerate the high pungency up to 559,080 SHU after ingesting capsaicinoids as a diet. In our study the amount of capsaicinoids in the larvae estimated by UV was 34.98µg/ml and that by HPLC analysis showed 71.87µg/ml respectively. However, analysis is restricted to capsaicin solutions with microgram-level concentrations. We confirmed that fruit damage in chilli caused by these larvae reduce the fruit quality and quantity during storage. Capsaicin is generally used as a pest repellent. It deters such insects such as cotton pests, maize weevil (Sitophilus zeamais) or red flour beetle (Tribolium castaneum) [21]. It is also effective as a repellent against Arion lusitanicus, an important pest of rapeseed [22]. In insects, capsaicin's toxicity appears to be through metabolic disruption, membrane damage, and nervous system dysfunction. Capsaicin has also been shown to repel insects as well as kill them [23]. Ingestion of large amounts of capsacin has been reported to cause histopathological and biochemical changes, including erosion of gastric mucosa and hepatic necrosis [24]. Recent research showed that capsaicin inhibits acetyl cholinesterase action in vitro [25]. But in our study the ingestion of capsaicin by P. operculum showed to be high in larvae and adult. Thus we confirmed this pest tolerates the effect of capsaicin and consume it for its growth. There is a need to investigate the further hypothesis on metabolism break down of high pungent toxic capsaicin in P. operculella, which might provide for control of these species. Quinalphos and diflubenzuron reduced damage caused by P. operculella, and the yield was highest in plots treated with quinalphos in India [26]. The efficacy of nine insecticides against P. operculella on potatoes was tested in Maharashtra, India. Treatment with phenthoate, chlorpyrifos, fenitrothion, phoxim, permethrin, cypermethrin, deltamethrin and fenvalerate at fortnightly intervals reduced pest populations. One of these insecticides should be applied on the appearance of pests with two or three repeat applications [27]. Quarantine actions include the inspection of cargo and vehicles from countries in which the pest is distributed; disinfecting vehicles and industrial cargo, monitoring with pheromone traps 3-5 km around primary import stations and strict quarantine of solanaceous cultures arriving from the infected regions. Control measures include before drying and fumigation with bromic methyl according to instructions regular elimination of nightshade weeds, insecticide treatments in fields in zones of wide and limited distribution of the pest according to regular recommendations and the use of bio-preparations. To avoid insect pests, inspect plants daily for signs of infestation, and get rid of any diseased plants immediately. The quarantine check of P. operculella commercially important spice was added on information. Hence the outcome of this report will be considered highly important in storage management and exporting of C. chinense fruits.

V. Conclusion

The reported P. operculella larvae caused severe damage to the C. chinense Naga Jolokia in the stored dry fruits. Hence the outcome this report will be considered highly important in storage management and exporting of commercially valuable C. chinense Naga Jolokia fruits the world's hottest known chilli

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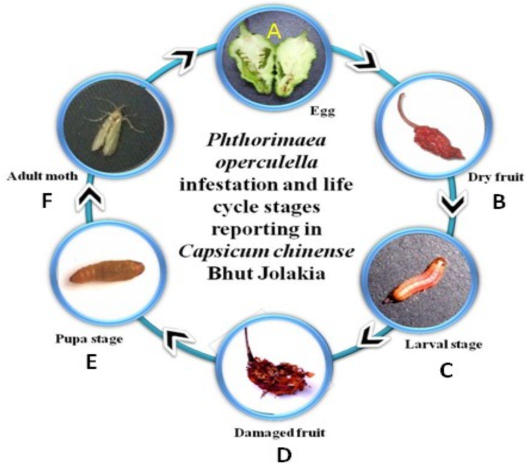

Fig.1. Infestation stages of Phthorimaea operculella: A. Egg, B. uninfected fruit C. larvae after ingestion of chili fruit appear orange red in colour, D. chilli fruit severely damaged by the larval infestation, E. Pupa stage, F. adult moth

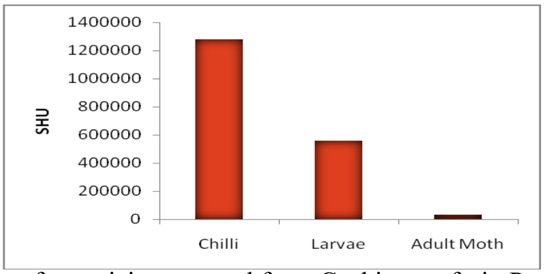

Fig.2. Graph showing SHU value of capsaicin extracted from C. chinense fruit, P. operculella larvae and adult