Laboratory Study of Repellent Property of Bhut Jolokia Chilli against *Sitophilus oryzae* (Coleoptera: Curculionidae) in Stored Wheat[1]

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Abstract This study investigated the repellent property of 'Bhut Jolokia' chilli pepper, a *Capsicum chinense* Jacquin cultivar, against the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), in stored wheat, *Triticum aestivum* L., in laboratory assays using a modified cup bioassay apparatus. Contact and fumigation repellency assays were conducted with the Bhut Jolokia chilli dry powder at concentrations of 3 and 5% (w/w) of the total weight of grain and the Bhut Jolokia chilli ethanol extract at volumes of 3 and 5 ml. In the contact repellency test, the number (mean ± SE) of test insects repelled (7.90 ± 0.23) increased with increasing concentration (5%) and time interval (24 h), whereas in the fumigation repellency test, the mean number of repelled insects increased (8.2 ± 0.2) with increasing volume (5 ml) and decreased (3.80 ± 0.13) with increasing time interval (60 min). Gas chromatography–mass spectrometry (GC-MS) analysis identified that the active compounds present in the Bhut Jolokia chilli ethanol extract were the capsaicinoids capsaicin (21.50%) and dihydrocapsaicin (4.44%) and the noncapsaicinoids n-hexadecanoic acid (29.34%), n-pentadecylacetamide (12.92%), and others. Our findings provide a preliminary assessment of the repellent property of Bhut Jolokia chilli against *S. oryzae* in stored wheat under laboratory conditions and serve as an impetus for continued research.

Key Words Bhut Jolokia chilli, contact repellency, fumigation repellency, *Sitophilus oryzae*, stored grain

Wheat (*Triticum* spp.) is the most important crop worldwide. With the increase in wheat production, many insect pests threaten wheat storage quantitatively and qualitatively. *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae) are the primary pests of stored wheat (Burges 2008). Grain damage by *S. oryzae* larvae and adults decreases the grain's nutritional quality and germination capacity and lowers the market price (Moino et al. 1998). Pesticides with fumigation properties are commonly used to manage stored grain pests. However, there are concerns about the risk of chemical residues and the development of insecticide resistance insect populations; therefore, alternatives to these

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conventional insecticides for controlling stored-product insects are of great interest (Phillips and Throne 2010).

Plants offer a rich source of secondary metabolites with the potential as ecofriendly insecticides, attractants, repellents, and growth inhibitors against a broad spectrum of insect pests (Rajashekar et al. 2014). Numerous studies have been conducted using plant extracts and whole plant materials for the management of insects (Ali et al. 2019). In addition, plant products such as essential oils and their constituents were studied for their fumigant effect because it is believed that natural compounds with plant origins can be superior to conventional fumigants, owing to their low mammalian toxicity, quick degradation, and local availability (Rajendran and Sriranjini 2008). Chilli peppers (*Capsicum* spp.) are popular spices that are prevalent in many parts of the world and are known for their pungency, a property that is mainly associated with capsaicinoid compounds (Sarwa et al. 2013). Among the 20 distinct capsaicinoids identified from different *Capsicum* spp. (Crapnell and Banks 2021), capsaicin and dihydrocapsaicin together account for approximately 90% of the pungency (Lu et al. 2017, Purkayastha et al. 2012). Some of these red pepper powders have been tested in various bioassays to determine their effectiveness against stored grain pests (Ashouri and Shayesteh 2010, Mahdi and Rahman 2008). The cultivar 'Bhut Jolokia' is one of the world's hottest chillis (*Capsicum chinense* Jacquin); it is indigenous to the northeastern part of India and grows primarily in the state of Assam. It contains higher capsaicin content and a remarkable pungency level (466,992 Scoville heat units) (Amruthraj et al. 2013, Liu and Nair 2010, Meghvansi et al. 2010, Popelka et al. 2017). To date, no reports are available on the preliminary analysis of the repellent property of Bhut Jolokia chillis against stored grain insect pests. Therefore, we undertook this study to determine the repellent activity of Bhut Jolokia chilli against *S. oryzae*.

Materials and Methods

Insects. The *S. oryzae* specimens used in this study were obtained from the postharvest laboratory II stock culture in the Department of Agricultural Engineering, Assam Agricultural University, Jorhat, India. The rearing of the selected individuals ($n = 100$) was on the sterilized wheat grains in 5-L plastic jars with 250 g of grain per jar. Adults were removed after 24 h of ovipositional activity. Eventually, emerging adults were transferred to jars containing fresh wheat grains to maintain a pure culture. The insect culture was maintained at 27 ± 1°C and 70 ± 5% RH. Adult weevils that were 1 week old were used for the repellency tests.

Grain and chilli materials. Wheat grains (variety HD2967, suitable for the upper zone of the Brahmaputra valley, Assam, India) used in the study were obtained from the stock maintained at the Assam Agricultural University farmhouse, Jorhat district. All the plant debris, inert matter, weed seeds, and other crop seeds were manually removed and then the grains were sun dried to bring the moisture level to 12%.

The Bhut Jolokia chilli used in the study was collected from a local farm in Assam during November 2020. The collected chilli variety was dried in a hot air oven at 60–70°C for 48 h and then ground using a lab hammer mill to a fine powder and stored in an airtight container until the onset of the tests.

An ethanol extract of the chilli was used for an assessment of the fumigant activity against *S. oryzae* adults. The extraction method was that of Ashwini et al.

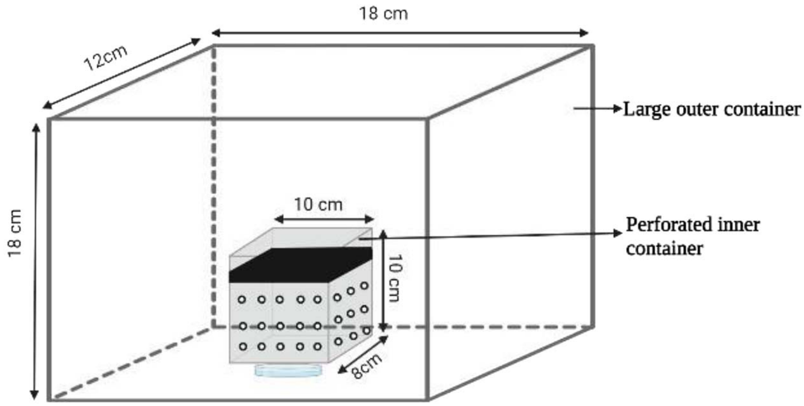

Fig. 1. Bioassay arena for evaluation of repellency of Bhut Jolokia chilli extracts against *S. oryzae* adults in wheat grain.

(2015) and involved grinding 20 g of Bhut Jolokia chillies by using a laboratory hammer mill and placing the powder in a Soxhlet extraction apparatus (Sigma-Aldrich, St. Louis, MO). Ethanol (Sigma-Aldrich) was added and allowed to percolate through the sample until no red color appeared in the percolated ethanol. The extracts were then cooled and brought to a total of 200 ml by using ethanol. The extracts were kept separately in amber bottles in the refrigerator (10 $\pm$ 2°C) until used in bioassays.

Bioassay arena. The bioassay arena (Fig. 1) used in these tests consisted of a transparent perforated inner container that can hold up to 500 g of wheat grain. It was constructed from a polyacrylic sheet (4 mm thick) with 2-mm perforations on all four sides and the bottom and shaped into a cube measuring 10 cm long, 8 cm wide, and 10 cm high with a movable polystyrene lid (1 cm thick) to close the top. This container was placed in a larger container (18 cm long $\times$ 12 cm wide $\times$ 18 cm high) constructed from the same material, but lacking perforations, and covered with muslin cloth as a top. The wheat and weevils are placed in the inner container, whereas the outer container serves to collect the weevils that exit the inner container through its perforations. The rate at which weevils exit the treated wheat grains in the inner container can be determined by counting the weevils that are collected from the outer container; thereby, providing a measure of the contact repellency of a test material.

This apparatus can also be used to determine the repellency of volatile compounds. For such testing, the apparatus should remain closed and the grain or insects should not come in direct contact with the volatile material. To ensure this, the bottom of the inner container was covered with a top or bottom of a 9-cm-diameter Petri dish, which acted as a barrier to prevent direct contact between the test compound placed beneath the inner container and the grain and insects in the inner container. This design of the inner container allowed the processed volatiles to permeate throughout the outer and inner containers to assess repellency.

Contact repellency assay. The Bhut Jolokia chilli powder was tested at 0, 3, and 5% (w/w) of the total mass of 100 g of wheat in the inner container. The powder was wrapped in a muslin cloth and kept in contact with wheat grain. The control (0%) treatment was handled in the same manner, but without the chilli powder. Ten 24-h-old adult *S. oryzae*, irrespective of their sex, were released into the center of the wheat grain in the inner container by using a long-stemmed funnel. At this time in the test, the perforations in the walls of the inner container were covered with parafilm for 10 min to allow the introduced insects to acclimate in the grain and the container. Once the parafilm was removed, the number of weevils that had exited the inner container to become trapped in the outer container at 1, 12, and 24 h were recorded as per Kumar et al. (2004) and Shayesteh and Ashouri (2010). The degree of repellency was related to the speed of response by *S. oryzae* adults exiting the container with the treated wheat. The test was performed at ambient room temperature, and the treatments were replicated 10 times. To avoid active ingredient concentration accumulation in the arena over time, each replicate was performed in different arenas.

Fumigation repellency assay. The Bhut Jolokia chilli ethanol extract was placed in 9-cm-diameter Petri dishes in volumes of either 3 or 5 ml, and the dish was placed below the bottom of the inner container, but within the outer container. Wheat grains (100 g) were placed in the inner container. Ten 1-d-old *S. oryzae* adults, irrespective of their sex, were introduced into the inner container. The number of weevils that had exited the inner container to be trapped in the outer container was counted at 20, 40, and 60 min after introduction (Perera and Karunaratne 2015). The assay was conducted at ambient room conditions with 10 replicates (treatments). An untreated control was performed without the addition of the ethanol extract to the arenas. To avoid contamination by active ingredient concentrations, each replicate was performed in different arenas.

Active compound analysis. For active compound analysis, the ethanol extract of the Bhut Jolokia chilli was subjected to GC-MS (QP2020 single quadrupole gas chromatograph mass spectrometer, Shimadzu Corp., Kyoto, Japan) equipped with a mass detector, Turbo Mass Gold containing an Elite-1 dimethyl silicone, 30 m $\times$ 0.25 mm ID $\times$ 1 mM df. The specifications followed during GC-MS analysis include an auto injector–based injection with a column oven temperature of approximately 70°C and an injection temperature of 240°C. The injection mode was splitless; the flow control mode pressure was 61.3 kPa; the total flow was 14.00 ml min^{-1}; and the carrier gas used was helium, with a flow rate of 1.00 ml min^{-1} and a linear velocity of 36.7 cm s^{-1}. The detector used in the detection was a mass spectrometer in full scan mode, with an interface temperature of approximately 280°C. The major constituents were identified with the aid of a computer-driven algorithm and by matching the mass spectrum of the analysis with that of a National Institute of Standards and Technology library (version 2.0, 2005). The software used for GC-MS was Tubro Mass 5.1.

Statistical analysis. The *t* test (Ross and Willson 2017) compared the number of test insects repelled between the treatments and the control ($P = 0.05$). The normality of the data was examined by Shapiro–Wilk's test, with a null hypothesis wherever the data followed a normal distribution (Shapiro and Wilk 1965). The homogeneity of the variance assumption was examined using Levene's test. All the data in the dataset followed a normal distribution and had equal variances.

Results and Discussion

Our contact repellency assay with Bhut Jolokia chilli powder revealed that the two concentrations tested, 3 and 5% (w/w), compelled significantly more adult

Table 1. Contact repellency of Bhut Jolokia chilli dry powder against *S. oryzae* adults in wheat grains.

Concentration of Powder (%)*	No. of Repelled Weevils at Intervals after Exposure (mean ± SE)**		
	1 h	12 h	24 h
3	3.80 ± 0.13	4.70 ± 0.15	5.40 ± 0.22
5	5.90 ± 0.10	6.80 ± 0.20	7.90 ± 0.23
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
t value	−12.60	−8.35	−7.77
df	18	18	18
Significance level	$P < 0.05$	$P < 0.05$	$P < 0.05$

* Percentage concentration based on the amount (w/w) of chilli powder in 100 g of wheat grain.
** Each value is the mean of 10 replicates. Statistical significance determined by t test ($P = 0.05$).

weevils to exit the inner container with the powder and the wheat grain than the control treatment with no powder (Table 1). The number (mean ± SE) of repelled insects increased with increased concentration of the powder and increased duration of exposure with the highest number of repelled insects recorded with 5% powder after 24 h (7.90 ± 0.23) followed by 5% at 12 h (6.80 ± 0.20). The lowest number recorded was with 3% powder at 1 h after treatment. These findings corroborate those of Shayesteh and Ashouri (2010), who determined that the repellency of chilli pepper (*Capsicum annuum* L.) powder against *R. dominica*, *Sitophilus granarius* (L.), and *T. castaneum* increased with concentration and exposure time.

In the fumigation repellency assay, the number of repelled insects (e.g., the number exiting the inner container following exposure) increased with the increased volume of the Bhut Jolokia chilli ethanol extract. The number repelled, however, decreased with the duration of exposure (Table 2). The highest number (mean ± SE)

Table 2. Fumigant repellency Bhut Jolokia chilli ethanol extract against *S. oryzae* adults in wheat grain.

Volume of Extract (ml)	No. of Repelled Weevils at Times after Exposure (mean ± SE)*		
	20 min	40 min	60 min
3	5.10 ± 0.23	4.30 ± 0.15	3.80 ± 0.13
5	8.20 ± 0.20	6.50 ± 0.17	4.70 ± 0.15
t value	−10.09	−9.73	−4.44
Df	18	18	18
Significance level	$P < 0.05$	$P < 0.05$	$P < 0.05$

* Each value is the mean of 10 replicates. Statistical significance determined by t test ($P = 0.05$).

Table 3. Chemical components of the Bhut Jolokia chilli extracts as determined by gas chromatography–mass spectometry analysis.

Chemical Component	Retention Index*	Retention Time (min)	Relative Content (%)
Ethyl 14-methyl-hexadecanoate	2013	5.006	9.03
Cyclopentane, 1-butyl-2-propylcyclopentane	1219	5.250	6.88
n-Hexadecanoic acid	1968	5.338	29.34
Z,*Z*-3,13-Octadecadien-1-ol acetate	2069	6.596	12.56
n-Pentadecylacetamide	1714	6.742	12.92
Capsaicin	2541	10.729	21.50
1-(2,4-Dihydroxyphenyl)-2-(4-methoxy-3-nitrophenyl) ethanone	2728	10.930	3.32
Dihydrocapsaicin	2533	10.976	4.44
		Total	99.99

* Retention index as determined on a dimethyl silicone column by using the homologous series of n-alkanes (Babushok et al. 2011).

of repelled insects was observed with a volume of 5 ml of the extract after 20 min of exposure (8.20 $\pm$ 0.20), whereas the lowest repellency was seen with 3 ml of extract after 60 min of exposure (3.80 $\pm$ 0.13). We postulate that as exposure increases, the active ingredient(s) in the ethanol carrier may have evaporated or otherwise dissipated. These findings correspond with those of Voahanginirina (2018), who inferred that capsaicin may be an insect-repellent and unappetizing agent, particularly at the initial time of the treatment.

Analysis of the components of the Bhut Jolokia chilli extract by using GC-MS identified capsaicin (21.50%) and dihydrocapsaicin (4.44%) as the capsaicinoid compounds and noncapsaicinoid constituents as n-hexadecanoic acid (29.34%), n-pentadecylacetamide (12.92%), *Z*,*Z*-3, 13-octadecadien-1-ol acetate (12.56%), ethyl 14-methyl-hexadecanoate (9.03%), cyclopentane, 1-butyl-2-propyl (6.88%), and 1-(2,4-dihydroxyphenyl)-2-(4-methoxy-3-nitrophenyl) (3.32%) (Table 3). These identified compounds are responsible for the pungent effect of the Bhut Jolokia chilli variety on humans and other animals and are likely the reason for the observed *S. oryzae* repellency in our assays.

Capsicum extracts have been reported to be effective repellents against some species of stored product beetles, including *Sitophilus zeamais* Motsch and *T. castaneum* (Ho et al. 1997). Furthermore, *C. annuum* contains capsaicin and other capsaicinoids as active compounds that cause irritation and respiration effects on most animals, including insects (Walukhu and Nyukuri 2020). Yet, products containing capsaicin are primarily used to repel insects rather than kill them (Madhumathy et al. 2007, Tomita and Endo 2007). The exact mechanism by which the Bhut Jolokia chilli causes repellency against *S. oryzae* has not been defined; however, capsaicin is claimed to disrupt insect metabolism and affect the central nervous system, causing membrane damage with physical repellent action (Copping and Duke 2007, Pavela 2016, Tiroesele et al. 2014). Capsaicin is an alkaloid from *Capsicum* spp. that acts as an irritant for many species ranging from insects to mammals, causing a burning sensation through chemoreceptors and nociceptors (Li et al. 2020). Li et al. (2019) also found that capsaicin has broad-spectrum insecticidal activity against many stored product beetles. Alkaloids, in general, are known to be bitter-tasting nitrogenous compounds that negatively affect animal physiology (Adeyemi 2011). Although the scope of our study did not investigate the repellent effect of individual active chemical constituents of Bhut Jolokia chilli (capsaicinoids and noncapsaicinoids) against *S. oryzae*, such studies should be conducted. Nevertheless, we report here the repellent effects of the Bhut Jolokia chilli powder and ethanol extracts against *S. oryzae* adults in laboratory assays. These findings should serve as an impetus for further research with this plant extract as a repellent for the management of *S. oryzae* and other stored product pests.

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