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INSECTICIDAL POTENTIAL OF INDIGENOUS *BACILLUS THURINGIENSIS* STRAINS AGAINST PUMPKIN FRUIT FLY

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ABSTRACT

The sustainable development of *Bacillus thuringiensis* (*Bt*)-based biopesticides against *Zeugodacus tau*, a destructive vegetable pest susceptible to Cry proteins, necessitates extensive laboratory and field-scale bioassays to replace hazardous chemical pesticides. In this study, 44 indigenous *Bt* strains harbouring various *cry* genes were evaluated against early third-instar larvae of *Zeugodacus tau* (Walker) (Diptera: Tephritidae: Dacinae) following enhanced Cry protein expression in T3 broth. Among the tested strains, nine demonstrated >70% larval mortality, with *Bt* JSd1 (92%), SaS6 (85%), JDc1 (83%), TaSa4 (75%), RaSa2 (74%), NaSc3 (72%), Ksa2 (72%), FhSb3 (70%), and MuSc2 (70%) showing the highest efficacy. Probit analysis was used to determine the lethal concentration (LC_{50}, LC_{99}) and lethal time (LT_{50}, LT_{99}) of each strain. The lowest LC_{50} was observed for *Bt* JSd1 (0.472 mg/ml), while the highest was for FhSb3 (0.753 mg/ml). LC_{99} values ranged from 3.992 mg/ml (*Bt* JSd1) to 6.926 mg/ml (MuSc2). The shortest LT_{50} was recorded for *Bt* JSd1 (55.17 hrs), while the shortest LT_{99} was noted for *Bt* RaSa2 (241.73 hrs). Biological quality parameters, including minimum number of pupae, pupal weight, adult emergence, sex ratio (M:F), percent adult emergence, wing length, flight ability (% fliers), percentage of half-emerged and un-emerged individuals, and prolonged larval and pupal durations were all significantly affected in *Z. tau* infested bitter gourd and cucumber treated with the highly potent *Bt* JSd1 strain. In contrast, the control group exhibited the highest values for all quality parameters. *Bt kurstaki* HD-73 served as the reference strain in this study. The combined dose and time-dependent bioassay results, along with quality parameter assessments, identified *Bt* JSd1 as the most promising candidate for environmentally safe and effective control of *Z. tau*. Upon successful field validation, *Bt* JSd1 holds strong potential for large-scale production and sustainable application in integrated pest management strategies.

***Keywords*:** Mortality, Quality parameters, Cry protein, *Zeugodacus tau,* Tephritidae

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INTRODUCTION

The subtropical climate of Bangladesh is highly conducive to the production of a diverse range of vegetables and crops. However, fruit flies one of the most destructive agricultural pests infest a wide variety of fresh fruits and vegetables in tropical and subtropical regions. Among them, members of the family *Tephritidae* are considered the most damaging insect pests of horticultural crops worldwide (Vargas *et al.* 2015). These flies lay their eggs within the flesh of fruits and vegetables, and the emerging larvae feed on the decaying tissue, rendering the produce inedible and causing post-harvest losses of 10–30%. Infested fruits often drop prematurely, leading to severe yield reductions in cucurbitaceous fruits, vegetables, and berries, with damage ranging from 30% to 100% depending on fruit species and seasonal conditions (Dhillon 2005, Sapkota 2010).

Many countries enforce strict quarantine regulations on produce imported from regions affected by *Tephritid* species and require pre-import treatment to prevent infestation (Vargas 2008). In Bangladesh, recent surveys have identified two new species, *Bactrocera carambolae* and *Zeugodacus madhupuri*, bringing the total number of *Tephritidae* species to 37 comprising 29 from the tribe Dacini and eight from other tribes (Leblanc *et al.* 2013, 2014, 2019; Khan *et al.* 2015, 2017). Since 2013, extensive field surveys have been conducted, initially focusing on rural and agricultural zones (Leblanc *et al.* 2013, 2014), and later expanding to protected forest areas in 2016 (Leblanc *et al.* 2019). These efforts have increased the known Dacini species count in Bangladesh from seven to 34 (Leblanc *et al.* 2013, 2014, 2019, 2021), with two additional species recently reported (Khan *et al.* 2024) and 12 new country records added, including a checklist of non-Dacini species (Hossain *et al.* 2024).

Zeugodacus (Zeugodacus) tau, previously known as *Bactrocera (Zeugodacus) tau* until the elevation of *Zeugodacus* to genus status (Virgilio *et al.* 2015), is a widely distributed and economically important pest species in Southeast Asia. It primarily affects cucurbit crops but can be misidentified due to its membership in a complex of morphologically similar species. *Z. tau* is a major quarantine pest whose females insert eggs beneath the fruit epidermis using a specialized ovipositor, while

the larvae feed internally, causing substantial damage. In China, *Z. tau* infestations have severely impacted fruit and vegetable production, with estimated losses to the pumpkin industry reaching ¥23.16 billion. Initially classified under the genus *Dacus* by Walker in 1849 upon its discovery in Fujian, China, *Z. tau* is now recognized for its phytophagous nature and latent larval feeding behavior (Guo *et al.* 2023). Its broad host range, high fecundity, and environmental adaptability make it a key quarantine pest in many countries (Hasyim *et al.* 2016), and one of the primary targets of this study.

Bacillus thuringiensis (*Bt*), a spore-forming soil bacterium, produces proteinaceous insecticidal crystals known as δ-endotoxins during sporulation. These toxins specifically target insects in the orders Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera, Homoptera, Orthoptera, and Mallophaga, as well as other invertebrate larvae (De Meyer *et al.* 2015). *Bt*-based biopesticides play a critical role in Integrated Pest Management (IPM) by providing a sustainable, environmentally friendly alternative to chemical pesticides and ensuring the presence of natural enemies of pests. The key insecticidal components Cry and Cyt proteins are encoded by *cry* and *cyt* genes (Soberon 2018). Over decades, *Bt* biopesticides have proven effective in agriculture, forestry, and vector control, and are a core element of IPM strategies aimed at reducing chemical resistance and maintaining pest control efficacy (Salehi *et al.* 2005).

Microbial insecticides, including *Bt*, have been in use in the United States since the 1960s and have been isolated from various environments such as soil, insect cadavers, agricultural habitats, and storage systems (Salehi *et al.* 2005, Kumar *et al.* 2008). Their targeted toxicity has been well established against *Bactrocera cucurbitae*, the melon fruit fly (Ansari *et al.* 2012, Shishir *et al.* 2012, 2014, 2015, Bari *et al.* 2021). However, the indiscriminate use of persistent chemical pesticides has led to environmental degradation, food chain disruption, pest resistance, and health hazards. Consequently, there is growing public demand for eco-friendly alternatives and organic pest control solutions, such as *Bt*-based biopesticides, especially in a country like Bangladesh where sustainable agriculture is critical. These biopesticides offer a long-term, cost-effective, and environmentally safer method of pest management, ensuring better food safety and ecosystem preservation.

Therefore, this study was undertaken to evaluate the efficacy of indigenous *B. thuringiensis* strains against the pumpkin fruit fly, *Z. tau* (Walker), and to develop a viable field-level application strategy. The ultimate goal is to promote the large-scale production of potent *Bt* biopesticides for sustainable delivery to farmers and to replace hazardous chemical pesticides with environmentally friendly alternatives for the management of major Tephritid fruit flies.

MATERIALS AND METHODS

***Bacillus thuringiensis* (*Bt*) strains and culture conditions:** A total of 44 *Bacillus thuringiensis* (*Bt*) strains were used in this study, including *B. thuringiensis* subsp. *kurstaki* (*Btk*) and *indiana* strains, namely: AgS2, ChSd2, CiSa5, DSe1, DSf4, DSh4, FhSb2, FhSb3, JDb1, JDc1, JSd1, KSa2, KSb1, KSe2, MeSa1, MeSb1, MuSa1, MuSc2, MuSc4, MuSe4, NaSc3, NaSd2, NoS2, NoS3, NsSe1, NsSe2, RaSa1, RaSa2, RaSb1, RaSc1, RaSd1, RhSa2, RhSb2, SaS4, SaS6, SaS7/19s, Soi1/li, SpSc1, SSb2, SSe2, TaSa4, TaSb3, TaSc1, and USc3. These strains were obtained from Lab 215 of the Department of Microbiology, University of Dhaka, along with reference strains *Bt kurstaki* HD-73, *Bt sotto* T84A1, and *Bt japonensis* Buibui from the *Bt* stock collection at Okayama University, Japan. These isolates were previously collected from different locations throughout Bangladesh (Shishir *et al.* 2014) and preserved at -80˚C. All *Bt* strains were cultured on Luria–Bertani (LB) agar for maintenance, subculturing, and spore enumeration. Incubation was carried out at a constant temperature of 30˚C. Liquid cultures were incubated with shaking at 180 rpm using an orbital shaker.

Inoculum preparation: *Bt* strains were streaked onto LB agar plates from slants and incubated overnight at 30°C. A single isolated colony was aseptically picked from the LB agar plate using a sterile loop and inoculated into a 100 ml Erlenmeyer flask containing 20 ml of LB broth. The culture was incubated overnight at 30°C with shaking at 180 rpm. After incubation, the cell density of the culture was measured at OD_{600} nm using uninoculated LB broth as a blank. The overnight culture was then used as the inoculum for all subsequent sporulation experiments, which were carried out in T3 medium (or modified Yousten agar medium). Unless stated otherwise, the inoculum was added to each experimental medium to achieve a starting OD_{600} nm of 0.1.

Estimation of spore count: The spore counting was performed by taking 1.0 ml of spore-crystal suspension and treating with heat at 80°C for 10 min. The heat-treated suspension was then serially diluted and plated on the LB agar medium by spread plate technique. The plates were then incubated at 37°C for 24 hrs. The spores survived the heat but all other vegetative cells were killed in this process. So, the colonies that were germinated from the spores after incubation were counted and multiplied by the dilution factor to estimate the actual number.

Protein concentration measurement: The protein concentration was measured using the previously known Bradford technique (Bradford 1976). The Bradford assay is based on a change in light absorption characteristics of the dye Coomassie Brilliant Blue G-250 caused by protein binding. When dissolved in an acidic solution (85% phosphoric acid), the dye absorbs the lightest at 465 nm. When protein is added, the dye's maximum absorption shifts to 595 nm, and the light absorbance at this wavelength increases linearly with protein concentration. This rise in absorbance can be detected using a spectrophotometer (Genesys 5, Thermospectronic).

Preparation of spore-crystal mixture: Spore and crystal mixtures were prepared for bioassay from *cry1* gene-positive strains (Shishir *et al.* 2014) using 100 ml of T3 liquid medium (Obeidat *et al.* 2004). Cultures were incubated at 30°C for three to four days with constant shaking at 250 rpm. Although LB broth has traditionally been used for preparing bacterial cultures, T3 medium was used in this study due to its enhanced sporulation efficiency. Following incubation, cultures were centrifuged at 5000 rpm for 15 minutes to separate the biomass from the culture supernatant. The pellet was washed with 50 ml of sterile, deionized water and centrifuged again for 5 minutes at 5000 rpm. The resulting spore-crystal pellet was retained and used for bioassay. This pellet suspension was further incubated for an additional two days at 30°C with shaking at 250 rpm to enhance sporulation and crystal protein yield. The protein concentration in the supernatant was quantified using the Bradford method (Bradford 1976).

Preparation of the dose suspensions: Spore-crystal suspensions of *Bt* strains that exhibited more than 50% average mortality were serially diluted in sterile distilled water at 2^{-1}, 2^{-2}, and 2^{-3} fold dilutions. Each dilution was thoroughly mixed with the

larval diet. The number of dead larvae at different concentrations across treatments (spore-crystal mixtures of *Bt* strains) was recorded for subsequent analysis to determine LC_{50} and LC_{99} values. All bioassays were performed in triplicate.

Egg collection from fruit flies for bioassay: To obtain a sufficient number of eggs, mature, ripe, and fresh pieces of sweet gourd were partially husked and placed inside the *Z. tau* adult rearing cages to encourage oviposition. After 30 minutes, the infested sweet gourd samples were collected and placed into plastic bowls lined with dry sawdust to allow for larval development. Fresh sweet gourds were periodically provided as food to support larval growth until early 3rd instar stages for bioassay.

Insect rearing: Larvae were maintained on a laboratory-developed artificial diet. Adult fruit flies were reared in stainless-steel-framed cages (120×120×90 cm^3 and 183×76×38 cm^3), covered with stainless-steel mesh. Each cage contained approximately 5,000–7,000 or 7,000–10,000 adult flies, respectively. Newly hatched larvae were provided with preferred hosts for development. Adults were fed two types of artificial diets: a liquid diet (baking yeast:sugar:water in a 1:3:4 ratio) and a dry diet (yeast extract: casein: sugar in a 1:1:2 ratio). Water-soaked cotton in a conical flask was placed in the cage to maintain humidity. The rearing room conditions were maintained at 28±2°C temperature and 70–80% relative humidity (RH). Rearing activities were conducted during 2018 to 2019 at the Insect Biotechnology Division (IBD), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh.

Bioassay evaluation: The toxicity of *Bt* strains was tested in vivo against early 3rd instar larvae of pumpkin fruit fly, *Z. tau*, through bioassay. Spore-crystal mixtures were prepared using cry1A gene-positive strains (Shishir *et al.* 2014, Obeidat *et al.* 2004). A total of 1.0 ml of spore-crystal suspension was mixed with 10 g of mashed sweet gourd, on which 20 early 3rd instar larvae were placed in each Petri dish. The dishes were incubated at 28±2°C and 70±10% RH with a 16:8 h (L:D) photoperiod. Mortality was recorded daily for up to 7 days. A parallel control was maintained using sterile distilled water. Mortality data were corrected using Abbott's formula (Abbott 1925). *Bt* kurstaki HD-73 was used as a reference strain. All assays were conducted in triplicate at the Insect Biotechnology Division (IBD). Institute of Food

and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh.

Preferred host collection: Uniform-sized, mature, ripened, and blemish-free preferred hosts, including bitter gourd and cucumber, were collected fresh from local farmers during harvest. The fruits were cleaned with a soft cloth and packed in polyethylene bags for transport to the laboratory. These were used to study biological quality parameters after infestation by *Z. tau* and treatment with selected potential *Bt* strains.

Hosts placement on adult rearing cages for infestation: Twelve uniform samples each of bitter gourd and cucumber were placed inside adult *Z. tau* rearing cages to allow oviposition. After 30 minutes, all infested hosts were collected and maintained under laboratory conditions for further study.

***Bt* treatment and biological quality parameter assessment:** After 24 hours, nine infested host samples were treated with three selected *Bt* strains JSd1, SaS6, and JDc1 by injecting 5 ml of suspension per host, evenly distributed across four sides under a biosafety cabinet. Each treated sample, along with three untreated controls, was placed into separate small bowls, which were then placed in 12 larger plastic containers lined with sawdust. Observations included larval and pupal periods, pupation rate, adult emergence, and associated developmental parameters. After 6–8 days, sawdust was sieved to collect pupae, which were then transferred to labeled Petri dishes for further evaluation. Data recorded included pupal yield, pupal weight, adult emergence rate, sex ratio (M:F), percentage of fliers, wing length, and percentages of partially and un-emerged adults. This procedure was applied similarly to both treated preferred hosts, bitter gourd and cucumber. Experiments were conducted during 2018 to 2019 at the Insect Biotechnology Division (IBD), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh.

Data analysis: The bioassay was conducted in two phases: initial screening for effective strains, followed by LC value determination for those causing >50% mortality. Initially, equal volumes of undiluted spore-crystal suspension were added

to the diet for each strain, and larval mortality was recorded. Mortality percentages were corrected using Abbott's formula (Abbott 1925). Experiments were done in triplicate, and the average mortality for each strain was calculated. Strains showing >40% mortality were retested, and those causing >50% mortality were selected for LC determination. Spore-crystal suspensions of selected strains were serially diluted (2^{-1}, 2^{-2}, and 2^{-3}) and mixed with larval diet. Larval mortality was recorded at each concentration. LC_{50} and LC_{99} values were estimated using Probit Analysis based on the Lognormal Distribution (Finney 1971). All biological quality parameters-pupal recovery, larval and pupal durations, pupal weight, adult emergence, sex ratio, percentage of fliers, wing length, and emergence anomalies were documented for each infested and treated host. All experiments were performed in triplicate. Data were analyzed using one-way ANOVA to determine statistically significant differences (LSD test), with $p \leq 0.05$ considered significant. Graphical analysis was performed using Python (https://www.w3schools.com/python/trypython.asp?filename=demo_string_quotes), an open-source software platform.

RESULTS AND DISCUSSION

Among the forty-four native *Bacillus thuringiensis* (*Bt*) strains harboring different *cry* genes that were tested in vivo against *Zeugodacus tau*, sixteen strains caused more than 50% larval mortality. These strains were: *Bt* DSe1 (50%), DSf4 (65%), FhSb3 (70%), JDc1 (83%), JSd1 (92%), KSa2 (72%), MuSc2 (70%), MuSc4 (52%), NaSc3 (72%), RaSa2 (74%), SaS4 (52%), SaS6 (85%), SaS7/19s (65%), Soi1/li (55%), SSe2 (54%), and TaSa4 (75%). Of these sixteen strains, nine exhibited over 70% larval mortality against early 3rd instar larvae of *Z. tau*, while only three, *Bt* JSd1, SaS6, and JDc1were found to cause more than 80% mortality. In contrast, the reference strain *B. thuringiensis kurstaki* (*Btk*) HD-73 and the untreated control exhibited 94% and 10% larval mortality, respectively. Some larval deaths also occurred in the control group, which were accounted for using Abbott's formula to correct test mortalities.

The toxicity of the selected potential *Bt* strains against early 3rd instar larvae of *Z. tau* followed the order: JSd1>SaS6>JDc1>TaSa4>RaSa2>NaSc3>KSa2>Fh Sb3>MuSc2, all of which caused more than 70% larval mortality. The efficacy of

these strains was compared to that of the reference strain, *Btk* HD-73, and the results were graphically presented in Figs. 1, 2A and 2B.

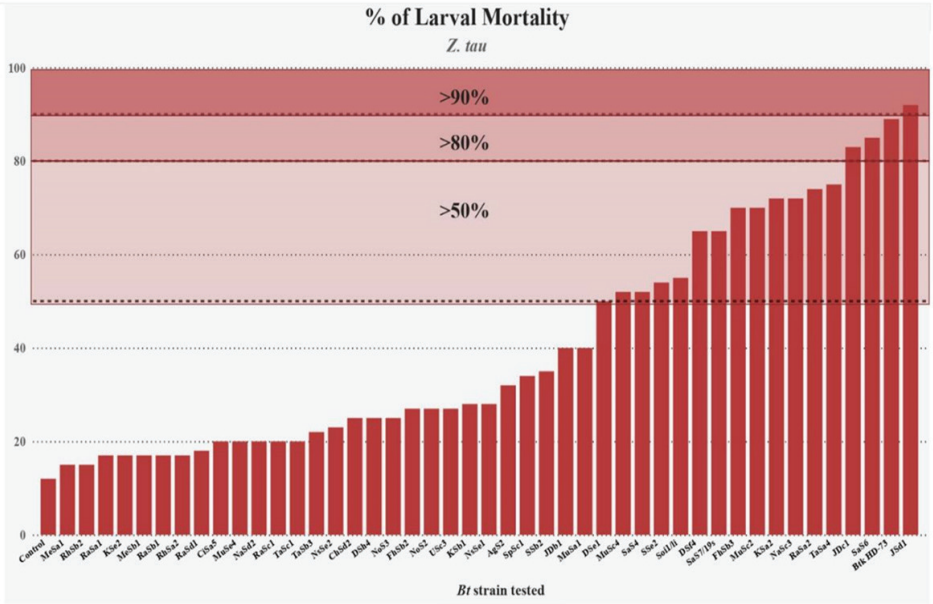
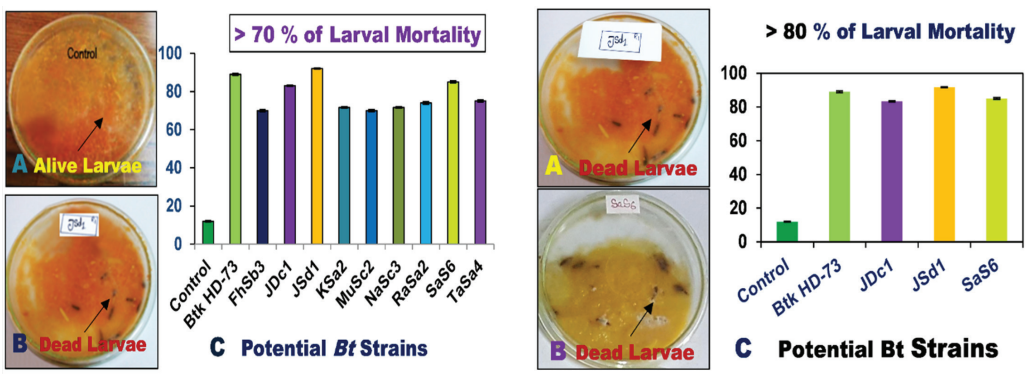


Fig. 1. Larval mortality caused by the spore-crystal protein mixture of total tested Bt strains against 3rd instar larvae of *Z. tau*



A B

Fig. 2 A & B. Bioassay performed with spore-crystal protein mixture of potential *Bt* strains tested against *Z. tau* (early 3rd instar larvae) caused >70% and >80% mortality.

Determinations of lethal concentrations (LC): The bioassay was performed in triplicate using native *Bt* strains that caused more than 70% larval mortality to determine the LC_{50} and LC_{99} values against the test insects.

Table 1. $LC_{50,}$ LC_{99} values, 95% confidence limits, χ^2 values and p values of potential *Bt* strains against *Z. tau* (early 3rd instar larvae)

Bt Strains (Potential)	LC_{50} values (mg/ml)	LC_{99} values (mg/ml)	Regression equations	95% confidence limits		χ^2 values (df)	p-value
				Lower	upper		
Against the 3rd instar larvae of *Zeugodacus tau*							
FhSb3	0.753	6.868	Y=2.5663 + 2.7756 X	0.621	0.911	1.368 (2)	1.11
JDc1	0.608	4.220	Y=2.4292 + 3.2792 X	0.524	0.704	3.465 (2)	2.81
JSd1	0.472	3.992	Y=2.9105 + 3.0974 X	0.356	0.627	7.826 (2)	4.36
KSa2	0.720	6.280	Y=2.5500 + 2.8576 X	0.600	0.863	1.119 (2)	7.25
MuSc2	0.770	6.926	Y=2.3340 + 3.0056 X	0.643	0.923	1.599 (2)	4.18
NaSc3	0.726	6.567	Y=2.6222 + 2.7612 X	0.601	0.877	2.415 (2)	1.88
RaSa2	0.698	6.095	Y=2.7185 + 2.7033 X	0.578	0.842	2.594 (2)	7.07
SaS6	0.557	4.798	Y=2.8392 + 2.8961 X	0.475	0.652	5.362 (2)	4.68
TaSa4	0.652	5.598	Y=2.8528 + 2.6351 X	0.543	0.784	3.497 (2)	4.05
Btk HD-73	0.450	4.478	Y=3.0733 + 2.9463 X	0.333	0.609	8.204 (2)	1.75

For each strain, the initial spore-crystal suspension was serially diluted to 2^{-1}, 2^{-2}, and 2^{-3}. The spore concentrations for each dilution were estimated on a logarithmic scale, and the corresponding larval mortality rates were recorded (Table 1). The LC_{50} and LC_{99} values for *Z. tau* ranged from 0.472 to 0.753 mg/ml and 3.992 to 6.926 mg/ml, respectively. The lowest LC_{50} (0.472 mg/ml) and LC_{99} (3.992 mg/ml) values were recorded for the indigenous *Bt* strain JSd1, indicating its highest potency in causing 50% and 99% larval mortality. In contrast, the highest LC_{50} (0.753 mg/ml) was observed for FhSb3, while the highest LC_{99} (6.926 mg/ml) was recorded for

MuSc2, suggesting that these strains required higher spore concentrations to achieve comparable mortality. The LC_{99} value of *Bt* JSd1 (3.992 mg/ml) further confirmed its potential as an effective biocontrol agent, whereas MuSc2 was the least effective based on its high LC_{99} value. Regression equations, 95% confidence limits (lower and upper bounds), and chi-square (χ^2) values were calculated based on the LC_{50} data. For the reference strain *Btk* HD-73, the LC_{50} and LC_{99} values against *Z. tau* were 0.450 mg/ml and 4.478 mg/ml, respectively (Table 1).

Determination of lethal time (LT): The LT_{50} and LT_{99} values for the potential *Bt* strains ranged from 55.17 to 148.17 hours and 241.73 to 855.31 hours, respectively, against *Z. tau*. The strain *Bt* JSd1 exhibited the lowest LT_{50} value (55.17 hours), indicating the highest efficacy in killing 50% of the larvae within approximately four days (Table 2).

Table 2. LT_{50} and LT_{99} values estimated for the Potential *Bt* strains against *Z. tau* (early 3rd instar larvae).

Bt Strains (Potential)	*Zeugodacus tau*	
	LT_{50} (hrs)	LT_{99} (hrs)
FhSb3	137.05	329.09
JDc1	136.43	345.11
JSd1	55.17	330.19
KSa2	137.19	855.31
MuSc2	148.13	853.09
NaSc3	136.11	470.03
RaSa2	148.17	241.73
SaS6	94.08	345.59
TaSa4	135.97	467.17
*Bt*k HD-73	56.19	335.41

In contrast, RaSa2 showed the highest LT_{50} value (148.17 hours), suggesting a slower rate of larval mortality. Regarding LT_{99} values, RaSa2 demonstrated a relatively fast action (241.73 hours), while KSa2 had the highest LT_{99} value (855.31 hours), indicating the longest time required to achieve 99% larval mortality. For the reference strain *Btk* HD-73, LT_{50} and LT_{99} values were recorded as 56.19 hours

and 335.41 hours, respectively (Table 2). Thus, based on dose–response and time–response bioassays, the most effective strains *Bt* JSd1 and *Bt* SaS6 demonstrated both high toxicity and rapid action against 3rd instar larvae of *Z. tau* in laboratory conditions. These findings suggest their strong potential for large-scale production and sustainable use in integrated pest management programs for farmers.

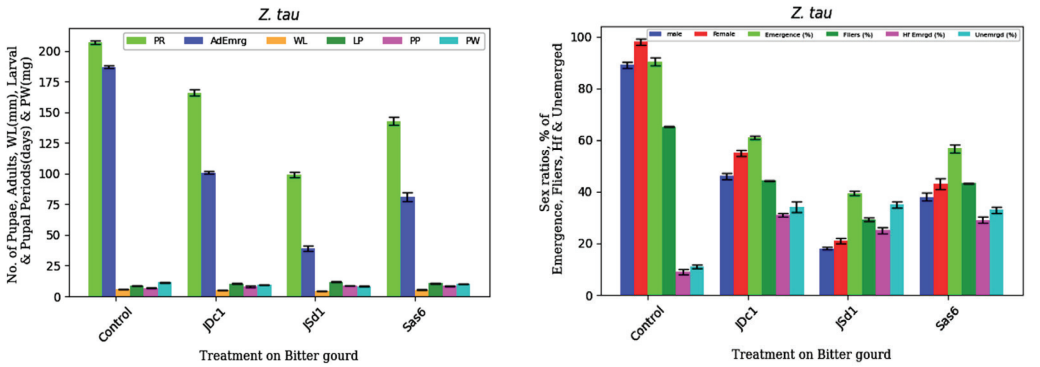
Influence of potential *Bt* biopesticide application on the biological quality parameters of *Z. tau* on infested and treated bitter gourd and cucumber: Only three potential *Bt* strains; JSd1, SaS6, and JDc1which exhibited more than 80% larval mortality, were selected for evaluating the biological quality parameters of *Z. tau* infesting treated bitter gourd and cucumber, the preferred host.

Pupation number (Pupal yield): The number of pupae recorded from treated bitter gourd were 99 ± 2.081 (JSd1), 143 ± 3.511 (SaS6), and 166 ± 2.645 (JDc1), and from treated cucumber were 160 ± 2.081 (JSd1), 196 ± 6.658 (SaS6), and 218 ± 3.382 (JDc1). The lowest pupation numbers were observed in the JSd1-treated group for both bitter gourd (99 ± 2.081) and cucumber (160 ± 2.081), while the highest pupation numbers were found in the control groups 207 ± 1.527 (bitter gourd) and 319 ± 1.527 (cucumber).

Pupal weight: Mean pupal weights for *Z. tau* developing in treated bitter gourd were 8.25 ± 0.37 mg (JSd1), 9.89 ± 0.08 mg (SaS6), and 9.19 ± 0.134 mg (JDc1); and for cucumber, 9.53 ± 0.217 mg (JSd1), 11.03 ± 0.164 mg (SaS6), and 10.09 ± 0.121 mg (JDc1). The lowest pupal weights were recorded in the JSd1 treatment group, while the highest weights were observed in the control batches: 11.25 ± 0.155 mg (bitter gourd) and 12.19 ± 0.083 mg (cucumber).

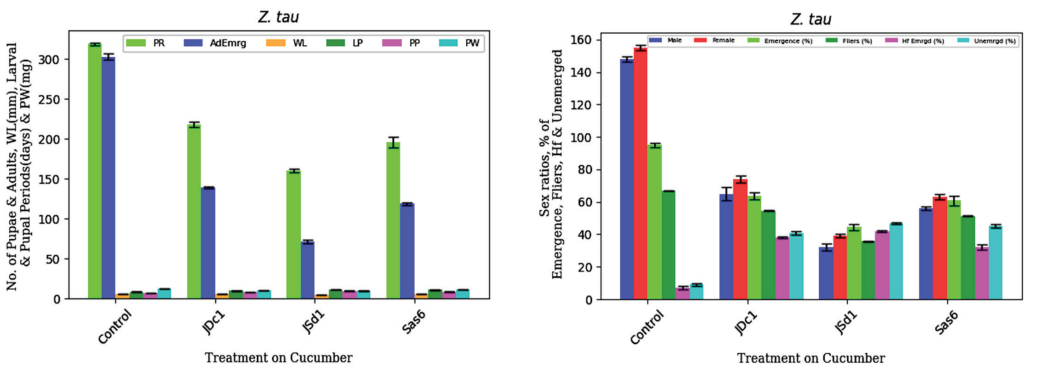
Adult emergence (Adult yield): The number of adult flies emerging from treated bitter gourd were 39 ± 2.083 (JSd1), 81 ± 3.464 (SaS6), and 101 ± 1.155 (JDc1); and from cucumber were 71 ± 2.083 (JSd1), 119 ± 1.132 (SaS6), and 139 ± 1.154 (JDc1). The lowest adult emergence occurred in the JSd1 treatment group for both bitter gourd (39 ± 2.083) and cucumber (71 ± 2.083), while the highest emergence was recorded in the control groups 187 ± 1.154 (bitter gourd) and 303 ± 4.163 (cucumber).

Sex ratios and % of adult emergence: Male and female emergence ratios (M:F) were recorded as follows for *Z. tau* reared on bitter gourd and cucumber treated with the potential *Bt* strains JSd1, SaS6, and JDc1. In bitter gourd: JSd1: 18 ± 0.577: 21 ± 1.154, SaS6: 38 ± 1.527: 43 ± 2.081 and JDc1: 46 ± 1.154: 55 ± 1.157, Cucumber: JSd1: 32 ± 2.081: 39 ± 1.154, SaS6: 56 ± 1.154: 63 ± 1.527 and JDc1: 65 ± 4.041: 74 ± 2.081.



A B

Fig. 3 A & B. Biological quality parameters observed on infested bitter gourd treated with potential *Bt* strains tested against *Z. tau.*



A B

Fig. 4 A & B. Biological quality parameters observed on infested cucumber treated with potential *Bt* strains tested against *Z. tau.*

The percent of adult emergence was in bitter gourd: JSd1: 39.39 ± 0.874%, SaS6: 56.64 ± 1.533% and JDc1: 60.84 ± 0.635%, Cucumber: JSd1: 44.37 ± 1.771%, SaS6: 60.71 ± 2.791% and JDc1: 63.76 ± 2.246%. The lowest sex ratios and adult emergence percentages were observed in the JSd1-treated groups for both hosts, while the highest sex ratios (89 ± 1.155: 98 ± 1.154 for bitter gourd; 148 ± 1.527: 155 ± 1.732 for cucumber) and adult emergence (90.33 ± 1.477% for bitter gourd; 94.98 ± 1.346% for cucumber) were recorded in the control groups.

Wing length and flight ability: Bitter gourd: JSd1: 4.09 ± 0.046, SaS6: 5.23 ± 0.222, JDc1: 5.06 ± 0.051 mm, Cucumber: JSd1: 4.57 ± 0.165, SaS6: 5.16 ± 0.04, JDc1: 5.33 ± 0.06 mm

Percent of fliers: Bitter gourd: JSd1: 29.32 ± 0.584%, SaS6: 43.27 ± 0.216%, JDc1: 44.34 ± 0.145%, Cucumber: JSd1: 35.79 ± 0.158%, SaS6: 51.34 ± 0.196%, JDc1: 54.52 ± 0.267%

The lowest wing lengths and flight percentages were observed in JSd1 treatments, whereas the control batches showed the highest values: Wing length: 6.00 ± 0.005 mm (bitter gourd), 6.03 ± 0.011 mm (cucumber), Flight ability: 65.19 ± 0.12% (bitter gourd), 66.88 ± 0.054% (cucumber)

Retardation in growth: Half-emerged percentages**:** Bitter gourd: JSd1: 25 ± 1.155%, SaS6: 29 ± 1.154%, JDc1: 31 ± 0.577%, Cucumber: JSd1: 42 ± 0.577%, SaS6: 32 ± 1.528%, JDc1: 38±0.578%

Un-emerged percentages: Bitter gourd: JSd1: 35 ± 1.154%, SaS6: 33 ± 1.157%, JDc1: 34 ± 2.081% and Cucumber: JSd1: 47 ± 0.579%, SaS6: 45 ± 1.154%, JDc1: 41 ± 1.154%

Larval period (days): Bitter gourd: JSd1: 11.66 ± 0.333, SaS6: 10.66 ± 0.333, JDc1: 10.33 ± 0.333, Cucumber: JSd1: 11.33 ± 0.333, SaS6: 10.66 ± 0.333, JDc1: 9.66 ± 0.333

Pupal period (days): Bitter gourd: JSd1: 8.66 ± 0.333, SaS6: 8.33 ± 0.333, JDc1: 8 ± 0.577 and Cucumber: JSd1: 9 ± 0.573, SaS6: 8.33 ± 0.333, JDc1: 7.66 ± 0.333 Maximum developmental delays were observed in JSd1-treated groups, while the shortest

durations were seen in the control groups: Half-emerged: 9 ± 1.154% (bitter gourd), 7 ± 1.155% (cucumber), Un-emerged: 11 ± 0.577% (bitter gourd), 9 ± 0.578% (cucumber) Larval period: 8.66 ± 0.333 days (bitter gourd), 8.33 ± 0.333 days (cucumber), Pupal period: 6.66 ± 0.333 days (both hosts).

These results demonstrate that the application of potential *Bt* strains, particularly JSd1 and SaS6, significantly affects the biological development and vitality of *Z. tau* in both bitter gourd and cucumber under laboratory conditions. This supports their potential for large-scale biopesticide development and field use (Figs. 3A & 3B and Figs. 4A & B).

Phenotypic characterization of potential *Bt* strains: Of the total examined strains, nine strains exhibited the most lethal against *Z. tau* early third instars larvae. Only nine of 44 indigenous *Bt* strains tested in this study had their phenotypic features observed with the phase-contrast microscope after sporulation (Table 3).

Table 3. Phenotypic Characteristics of the potential *Bt* strains tested against *Z. tau* (early 3rd instar larvae)

Bt Strains (Potential)	Size	Shape	Color	Margin	Consistency	Elevation	Gram Staining
FhSb3	Medium to large	Round	Off white	Wooly	Opaque	Raised	+
JDc1	Medium to large	Round	Off white	Wooly	Opaque	Raised	+
JSd1	Medium to large	Round	Off white	Wooly	Opaque	Raised	+
KSa2	Medium to large	Round	Off white	Wooly	Opaque	Raised	+
MuSc2	Medium to large	Round	Off white	Wooly	Opaque	Raised	+
Nasc3	Medium	Round	Off white	Entire	Opaque	Raised	+
RaSa2	Large	Round	Off white	Entire	Opaque	Raised	+
SaS6	Large	Round	Off white	Entire	Opaque	Raised	+
TaSa4	Medium	Round	Off white	entire	Opaque	flat	+

Spore counting and protein concentration determination: Only nine of the forty-four evaluated effective indigenous *Bt* strains were utilized in this experiment against selected *Z. tau* (early 3rd instar larvae) to measure the number of spores/ml suspension and the protein concentration in mg/ml, and the results are reported in Figs. 5 & 6.

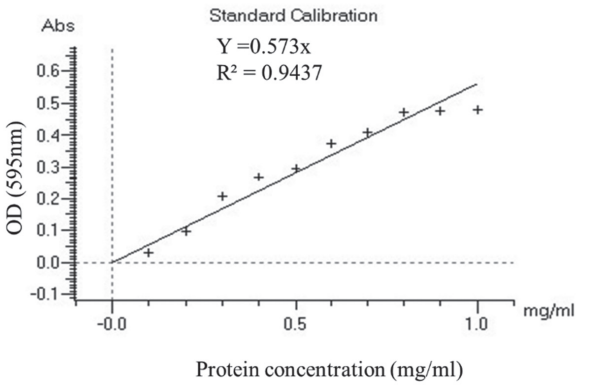


Fig. 5. Standard curve for determining protein concentration by Bradford technique.

A standard (Fig. 5) curve of protein concentration vs absorbance at 595 nm was created by serially diluting a stock of BSA protein and graphing the findings. The equation y = mx depicts the relationship between absorbance (y) at 595 nm and protein concentration (x) in mg/ml. The slope of the standard curve (m) governs the relationship between absorbance and protein concentration.

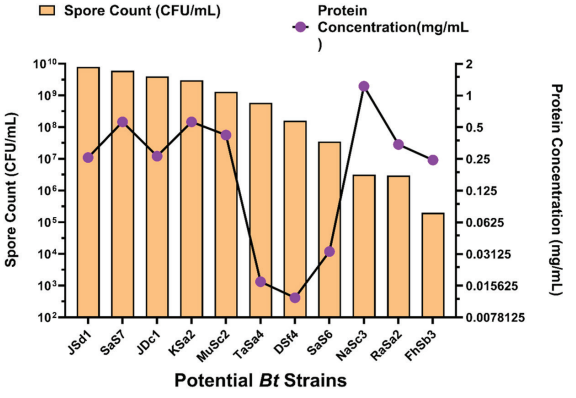


Fig. 6. Spores count and Protein concentration estimation of the potential *Bt* strains tested against *Z. tau* (early 3rd instar larvae).

The use of chemical insecticides in agriculture has led to significant environmental and health issues, including poor soil management, deforestation, and contamination. *Bacillus thuringiensis* (*Bt*)-based biopesticides offer a promising alternative due to their targeted action and minimal environmental impact (Azmi *et al.* 2015). However, the overuse of chemical pesticides has caused environmental damage, posed risks to public health, and contributed to the development of insect resistance. This has underscored the importance of finding sustainable pest management solutions, such as *Bt* biopesticides, which are effective against specific pests without harming non-target organisms (Majeed *et al.* 2017).

Bt is a well-known microbial biocontrol agent due to its production of insecticidal crystal (Cry) and cytolytic (Cyt) proteins, which are toxic to many insect species (Soberon *et al.* 2018). Despite its widespread success, resistance to *Bt* has been reported, reducing its effectiveness over time (Zago *et al.* 2014). This has highlighted the need for novel *Bt* strains with enhanced insecticidal properties. Thus, isolating indigenous *Bt* strains with high toxicity to pests like *Z. tau* is crucial for sustainable pest control (Reyaz *et al.* 2017).

In this study, we isolated indigenous *Bt* strains from soil samples in Bangladesh and evaluated their efficacy against *Zeugodacus tau*, a major pest of cucurbit crops. The results showed that *Bt* JSd1 and *Bt* SaS6 exhibited high insecticidal activity, comparable to the reference strain *Btk* HD-73. *Bt* toxins disrupted larval development, resulting in delayed movement, feeding cessation, and eventual death, highlighting the potential of these strains as biocontrol agents (Figs. 2A & 2B). The LC_{50} and LC_{99} values for *Bt* JSd1 and *Bt* SaS6 were similar to *Btk* HD-73, confirming their potential as effective biopesticides.

The biological parameters of *Z. tau* reared on *Bt*-treated crops were significantly affected. *Bt* JSd1-treated groups showed reduced adult emergence, altered sex ratios, and increased mortality, indicating the strain's ability to impair normal development. These findings suggest that *Bt* JSd1 may be an effective tool for reducing *Z. tau* populations in agricultural settings. Furthermore, the ability of *Bt* JSd1 to impact multiple biological stages of *Z. tau* supports its potential for integrated pest management programs.

Molecular identification through 16S rRNA sequencing confirmed the presence of *Bt* in the isolated strains. Whole-genome sequencing of *Bt* JSd1 revealed the presence of Cry proteins, particularly Cry1Ac, which are responsible for its insecticidal activity (Obeidat *et al.* 2004). This molecular characterization provides insights into the genetic basis of its toxicity and lays the foundation for further research on the strain's potential applications in pest management.

In conclusion, the results of this study underscore the potential of indigenous *Bt* strains, particularly *Bt* JSd1, as eco-friendly alternatives to chemical insecticides for controlling *Z. tau*. By targeting pests without harming the environment or non-target species, *Bt* biopesticides offer a sustainable solution for pest control. The successful isolation and characterization of these strains pave the way for further development and integration of *Bt*-based biocontrol strategies in agricultural practices, contributing to food safety and security in Bangladesh.

CONCLUSION

The current experiment revealed that it will be a valuable resource for further inspiration and research for the isolation of efficient native *Bt* JSd1 strains, as pest controlling agents and replacement of hazardous chemical pesticides by environmentally friendly *Bt* biopesticides, which is today's burning public demand. Resulting, increased volume of advanced work is required, which has the greatest potential to open a new door in future biological control strategies for dealing with the Tephritids fruit fly problem in an environmentally friendly manner, helping to prevent toxic substances from the food chain from bioaccumulation and biomagnification while also improving food safety and security in Bangladesh.

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