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HOST RANGE, DAMAGE EXTENT AND LEAF CONSUMPTIONS BY THE BAGWORM, *THYRIDOPTERYX EPHEMERAEFORMIS* HAW. (LEPIDOPTERA: PSYCHIDAE) IN BANGLADESH

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Abstract

Bagworm, *Thyridopteryx ephemeraeformis* Haw. (Lepidoptera: Psychidae) is a polyphagous pest in Northern America and southern Australia, causing severe damage to several host plants. A study was conducted to record the host plants, damage extent, and morphometrics of bagworms in Patuakhali Science and Technology University (PSTU) from 2015 to 2018. Results revealed a limited number of host plants for bagworm namely guava (*Psidium guajava*), arborvitae/juniper (*Thuja standishii*), rangan or jungles, geranium (*Ixora grandiflora/Ixora coccinea*), mussaenda (*Mussaenda philippica*), cropperleaf (*Acalypha wilkesiana* 'Ceylon), henna (*Lawsonia inermis*), mango (*Mangifera indica*), pomegranate (*Punica granatum***)** and betel nut (*Areca catechu*). The length of tiny larvae, fully-grown larvae, pupa, adult winged male moths, and wingless adult maggot-like females was 1.02 mm, 24.8 mm, 14 mm, 15 mm, and 48 mm, respectively. The average length of the bag was 24.7 mm with a range of 21-28 mm, and the average breadth in the middle of the bag was 6.9 mm with a range of 6-8 mm. The highest number of larval cases per branch was found in guava (56), and the lowest was in copperleaf (8). The highest percent of leaf damage (86.23%) per plant was also observed in the guava plant, and the lowest percent of leaf damage (37.46%) was in copperleaf. The highest number of infested leaves and bags per leaf was observed in the lower stratum of the guava branch, while the lowest infestation was in the top stratum. The highest percentage of leaf area (30%) damage was recorded 72 hours after release (HAR), and the lowest percentage of leaf area (10%) damage was at 24 HAR in guava. The maximum amount of leaf was consumed by bagworm larva at 72 HAR and the minimum amount was consumed at 24 HAR in guava. The maximum leaf area (13.46 cm^2) consumption was recorded at 72 HAR, while the minimum amount (3.42 cm^2) was consumed at 24 HAR. No significant damage by *T. ephemeraeformis* was observed in the case of mango, pomegranate, and betel nut plants.

Key words: Bagworm, Damage extent, Host plant, Leaf Consumption, *Thyridopteryx ephemeraeformis*

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Introduction

The Bagworm, *Thyridopteryx ephemeraeformis* (Haworth) (Lepidoptera: Psychidae), is native to Pennsylvania, USA a serious pest of many trees and shrubs of a deciduous and evergreen group. The alternate names of this bagworm are eastern bagworm, evergreen bagworm, common basket worm, common bagworm, or North American bagworm due to feeding on plant species of different groups. Psychidae family has approximately 1,000 species (Rhainds *et al.*, 2009), in which all species' larvae are concealed in a bag, and most species have wingless adult females. The larval stage of *T. ephemeraeformis* is reported to feed on over 125 different plant species consisting of 50 families (Hoover, 2000; Moore and Hanks, 2004; Rhainds *et al.*, 2009). Its populations may build up very rapidly and become serious pests due to its potential to cause damage. It spreads slowly because the female has no wings; however, it can move to other host plants by crawling and spreading through infested nursery stock (Mazzey and Masiuk, 2013). Evergreen plants such as arborvitae (*Thuja* spp.), fir (*Abies* spp.), hemlock (*Tsuga* spp.), juniper (*Juniperus* spp.), southern red cedar (*Juniperus silicicola*), pine (*Pinus* spp.) and spruce (*Picea* spp.); Hosts such as honey locust (*Gleditsia triacanthos),* black locust (*Robinia pseudoacacia*), sweetgum (*Liquidambar styraciflua)* and sycamore (*Platanus occidentalis*) and like ornamental conifers, box elder, cedar, cypress, elm (*Ulmus* spp.) are stated as Deciduous plants, fruit and nut trees, live oak (*Quercus virginiana*), maple (*Acer* spp.), Indian hawthorn (*Raphiolepis indica*), ligustrum (*Ligustrum japonica*), viburnum (*Viburnum* spp.), persimmon, salt, sumac, wild cherry, willow (*Salix* spp.) serve as host plants for bagworm species (Mazzey and Masiuk, 2013). At the initial stage, feeding by larvae on evergreen trees causes brown and unhealthy branch tip damage (Baxendale and Kalisch, 2009). Severe infestation can destroy the aesthetics and health of host plants (Ellis *et al.*, 2005; Ellis *et al.*, 2005; Rhainds and Sadof, 2008).

During summer, larvae of *T. ephemeraeformis* can cause severe defoliation that leads to the death of evergreen plant species. Larval development of *T. ephemeraeformis* occurs in localized infestation as they move only a few distances from their original host plant and results in maximum population density in some plants, while adjacent plants may have very few bagworms. This type of dispersal also led bagworms in spreading out in the host plant and showed a high population of the bagworm year after year (Rhainds and Sadof, 2008). After selecting a suitable host, the larva starts feeding and incorporating materials foe preparing bag such as pieces of twigs, leaves, and silk into its bag (Peterson, 1969). The spindle-shaped silk bag camouflaged with bits of foliage, bark, and other debris materials is formed by this pest (Shetlar, 2010). In whole larval instars, the larva increases its bag size because of growing and can live long periods without taking food

during the later stages of its development (Rhainds *et al.*, 2009). Completed bags range from 37.5 to 62.5 mm long (Shetlar, 2011). The mature caterpillar is about 25 mm long and takes up to 4 months to develop, depending on temperature (Rhainds *et al*., 2009). Larva attaches its bag firmly with a thick silken strand to its host plant or disperse to another structure. Before moulting and pupation, the bagworm seals the frontal part of the bag (Leonhardt *et al.*, 1983). The common bagworm caterpillar develops through seven instars before transforming into a pupa (Rhainds and Sadof, 2008). Immature caterpillars feed on the upper epidermis leaving small brown spots upon foliage. Mature caterpillars consume whole leaves of susceptible deciduous plant species, leaving only the larger veins (Baxendale and Kalisch, 2009). Recently the bagworm, *T. ephemeraeformis* has become a serious problem at PSTU campus because of damaging guava, thuja, mussenda, ixora plants, nuts, and palms. The Sharupkathi variety guava plants are seriously infested, and no fruiting occurrs in attacked plants. Many thuja plants have already died due to the attack of bagworms. So far, this insect, its host range, and its damage extent has not been reported yet in Bangladesh. Hence, the aims of the study were to know the host range, damage extent, and morphometrics of bagworms.

Materials and Methods

Location and duration of the study: Studies were conducted at the PSTU campus from January 2015 to October 2018.

Data collection procedure: The bagworms and damaged parts of the infested host plants were observed and collected at weekly interval. Before collecting samples, the infested plant was divided into three strata, lower, middle, and top. Ten infested leaves per branch of each stratum were collected, and the number of bags per branch was recorded. The number of holes per leaf from 10 infested leaves of guava, rangan, mussenda, copperleaf, and henna was also recorded. Bagworms were identified by cone-shaped bags, which were made of silk and bits of leaves and twigs (Plate 1). The exterior part of early larval stages is shiny black, and the undersides of their body are dull amber. The mature bagworms are a dull, dirty, and grey, with darker markings towards the head (Plate 2). The adult male transforms into a moth that can fly, but females do not transform into moths but remain inside the bag, which looks maggot having no functional eyes, legs, mouthparts, or antennae. Immature stages of bagworms, known as caterpillars, having chewing mouthparts (Plate 3).

Morphometric study: The length and breadth of tiny larvae, fully-grown larvae, pupa, adult winged male moths and wingless adult maggots like female bagworms (Plate 4) and bags were measured by using a scale (Plate 5).

Leaf area consumption: The collected specimens were kept in a polybag and then processed, mounted, and labeled for studying leaf area consumption. The damaged area of the guava leaf by the larva was assessed under laboratory condition. Thirty leaves consisting of 10 per replication were used in leaf consumption. Leaf consumption by bagworm larvae was measured by leaf area meter at 24, 48, and 72 hours after the release of the larva. Leaf consumption based on weight was measured at 24, 48, and 72 hours after the release of larva. During foliage feeding, the larvae emerged from the top of the bag and hung onto the host plant with a silken thread and their legs. The lower end of the bag remains open to pass out fecal materials or frass from the body.

Plate 1. Rearing of bagworm on the henna plant in glass jar and petri dish. A. Leaf case moth. B. stick case and leaf case moths, C. Leaf case moths.

Statistical analysis: WASP 1.0 (Web-based Agricultural Statistical Package) software and Excel program were used to analyze data.

Plate 2. Different stages of bagworms with their case. A. Male. B. Male. C. Female. D. Mature Larva. E. Pupa. F. Larva inside the soft bag. G. Pupa inside rough bag

Plate 3. Adult male (left) and adult female (right). (Source: Shetlar, 2010).

Plate 4. Bagworm eggs. (Source: Shetlar, 2010).

.

Plate 5. Measurement of bag made by bagworm

Results and Discussion

Host range: Host plants infested by bagworm, *T. ephemeraeformis* were identified as guava (*Psidium guajava*), arborvitae/juniper (*Thuja standishii*), rangan or jungle geranium (*Ixora grandiflora/I. coccinea*), mussaenda (*Mussaenda philippica*), cropperleaf (*Acalypha wilkesiana* 'Ceylon), henna (*Lawsonia inermis*), mango (*Mangifera indica*), pomegranate (*Punica granatum***)** and betel nut (*Areca catechu*) (Table 1) in PSTU campus. Numerous plants such as evergreen trees, broadleaf, and shrubs like arborvitae and other ornamental conifers, cedar, cypress, box elder, elm fruit, nut trees, maple, locust, juniper, live oak, persimmon, salt cedar, sumac, sycamore, wild

cherry, willow, and many other ornaments served as bagworm host plants (Baxendale and Kalisch, 2009; Mazzey and Masiuk, 2013).

Table 1. List of host plants infested by bagworm, *T. ephemeraeformis*.

Sl. No.	Common name	Scientific name	Family
1	Guava	*Psidium guajava*	Myrtaceae
2	Arborvitae/Juniper/Thuja	*Thuja standishii*	Cupressaceae
3	Rangan or jungle geranium	*Ixora grandiflora/I. coccinea*	Rubiaceae
4	Mussaenda	*Mussaenda philippica*	Rubiaceae
5	Copperleaf	*Acalypha wilkesiana*	Euphorbiaceae
6	Henna	*Lawsonia inermis*	Lythraceae
7	Mango	*Mangifera indica*	Anacardiaceae
8	Pomegranate	*Punica granatum*	Lythraceae
9	Betel nut	*Areca catechu*	Arecaceae

Morphological characteristics and morphometrics of *T. ephemeraeformis*

The tiny larva was 1.02 mm long. The length and breadth of full-grown larvae were 24.8 mm and 4.8 mm, respectively, which appeared medium to dark brown. The length and breadth of the pupa were 14 mm and 5 mm, respectively, which appeared dark brown to black. The adult male moth was a hairy and charcoal-black body with feathery antennae. The length and breadth of the male moth were 15 mm and 5 mm, respectively. The wings of the male moth were membranous or clear having a length of 12.5 mm and a breadth of 6.5 mm while the length and breadth of female moth were 48 mm and 8 mm, respectively (Table 2). The results are supported by Peterson (1969); Rhainds and Sadof (2008).

Morphometrics of bag formed by bagworm larvae

The length and breadth in the middle of the bag formed by the full-grown larva of the bagworm are presented in Table 3. The average length of the bag was 24.7 mm, with a range of 21-28 mm and standard error of 0.667. The average breadth in the middle of the bag was 6.9 mm, with a range of 6-8 mm and a standard error of 0.179.

Table 2. Morphological characteristics, length and breadth of *T. ephemeraeformis.*

Stages of bagworm	Morphological characteristics	Size of bagworm	
		Length (mm)	Breadth (mm)
Egg	Smooth, cylindrical in shape, covered by tuft-like waxy layer.	-	-
Tiny larva	Tiny long caterpillars attached themselves on silken threads to new leaf and make a very small conical shaped bag which they bear upright as they move	1.02	-
Full-grown larva within a bag	The posterior part of the caterpillar was medium to dark brown, with dorsal part of the first 3 segments are white to yellow with a dark brown pattern. Caterpillar developed through 7 instars before it transformed into a pupa	24.8	4.8
Pupa	Dark brown to black pupa remained inside the bag. The duration of pupal period was 7-10 days.	14	5
Adult winged male moth	Hairy and charcoal black body with feathery antennae	15	5
Male wing	Wings were transparent or membranous	12.5	6.5
Adult wingless maggot-like female	Females were not transformed into moths, but remained concealed into the bag, which appeared as maggot having no functional legs, eyes, antennae or mouthparts.	48	8

Table 3. Length and breadth of bag formed by the full-grown larva of bagworm

No. of observation	Bag size formed by the larva	
	Length (mm)	Breadth (mm) at middle
1	28	7
2	27	8
3	21	7
4	26	7
5	25	6
6	26	7
7	23	6
8	24	7
9	23	7
10	24	7
Mean	24.7	6.9
Range	21-28	6-8
SE	0.667	0.179

Plate 6 Contd.

Plate 6. Bagworms and their damage on leaves of guava (A&B), thuja (C&D), rangan (E&F), mussaenda (G&H), copperleaf (I&J), henna (K&L), and mango (M&N) at PSTU campus. A. Leaf case moth, B. Leaf case moth, C. Cone case moth, D. Leaf case moth, E. Leaf case moth, F. Leaf case moth, G. Stick case moth, H. Stick case moth, I. Cone case moth, J. Cone case moth, K. Leaf case moth, L. Leaf case moth, M. Leaf case moth, N. Leaf case moth

Damage symptoms and extent of the damage: The immature caterpillars fed on the upper epidermal tissues leaving small brown spots on the foliages. Mature caterpillars consumed whole leaves of host plants leaving only the larger veins. Sometimes major leaf veins were also removed by mature caterpillars. Host plants were completely defoliated when female bagworms were abundant in infested plants (Plate 6). Highly infested thuja plants were died at the PSTU campus (Plate 7). The mature caterpillars usually attached their bags to a branch by folding extra silk that didn't spoil rpidly. This silk made band may have girdled the branch of the host plant as it grows, resulted in dead branches several years Shetlar (2010) supported this results, who explained that female bagworms could not fly and local populations can build rapidly on an established preferred host. Numerous caterpillars may eat the buds of attacked conifers, causing branch dieback of the host plants. Moderate defoliation might be unsightly but excessive defoliation of conifers may cause whole plant death during the next season.

Plate 7. Dead thuja plant as a result of bagworm infestation at PSTU campus.

Number of larval cases per branch: The highest number of larval cases per branch was observed in guava (55.8), followed by thuja (38.4) and mussaenda (26.9) while the lowest number of larval cases per branch was in copperleaf (8.3) followed by henna (14.9) and rangan (18.2) (Fig. 1).

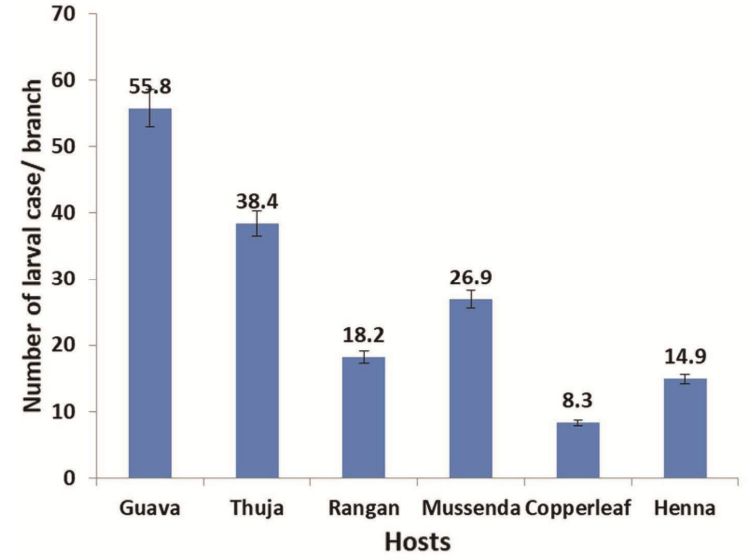

Fig. 1. Number of larval cases per branch of selected host plants.

Percentage of leaf damage: The highest percent of leaf damage was observed in guava (86.23%), followed by thuja (75.24%) and mussaenda (72.15%), and the lowest percentage of foliage damage was in copperleaf (37.46%) followed by henna (42.45%) and rangan (46.32%) (Fig. 2).

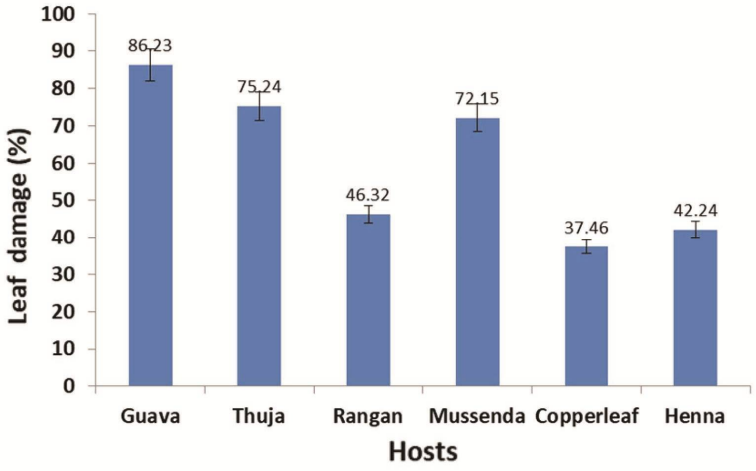

Fig. 2. Percentage of leaf damage caused by bagworm larva in selected host plants.

Number of holes per leaf: The highest number of holes per leaf was recorded in the guava plant (7.75), followed by mussaenda (7.5), while the lowest number of holes per leaf was in the copperleaf (4.5) plant, followed by rangan (Fig. 3).

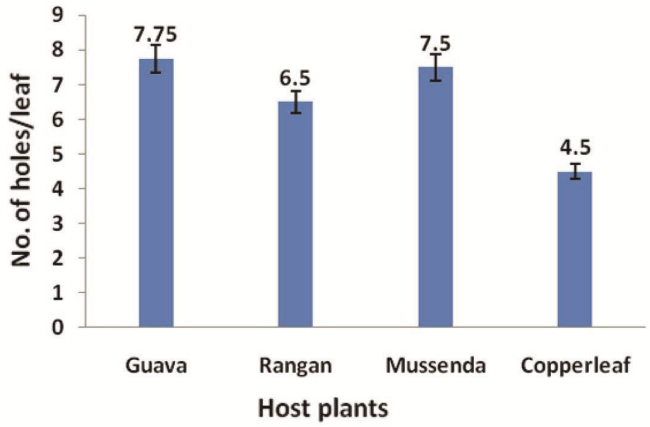

Fig. 3. Number of holes per leaf caused by bagworm larva in four selected host plants.

Number of infested leaves on guava: The highest number of infested leaves was observed in the lower stratum (17.3), followed by the middle strata (6.3). The lowest infestation was observed in the leaves of the top (2.5) stratum (Fig. 4).

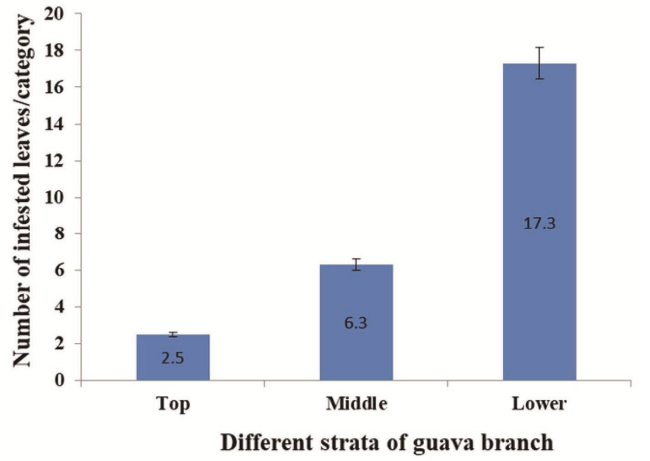

Fig. 4. Number of infested leaves at different strata of guava branch.

Number of bags per leaf on guava: The highest number of bags per leaf (2) was observed in the middle, and lower strata leave compared to the top (0.7) stratum (Fig. 5).

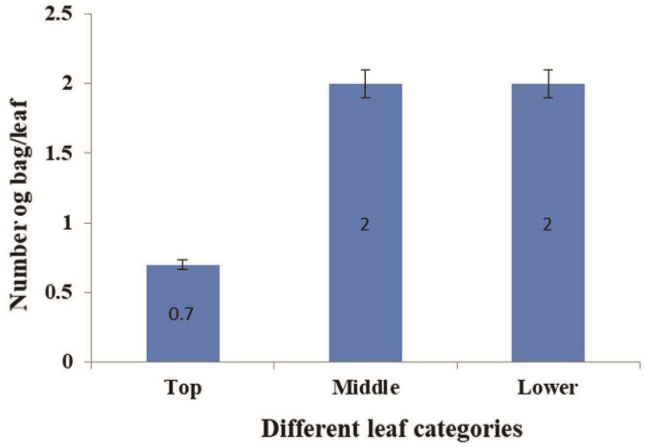

Fig. 5. Number of bags per leaf formed by bagworm larvae on guava.

Percentage of leaf area damage in guava under laboratory conditions: The highest percentage of leaf area (30%) damage was recorded 72 hours after release, followed by 48 hours (20%), and the lowest leaf area (10%) damage was at 24 hours after release (HAR) (Fig. 6).

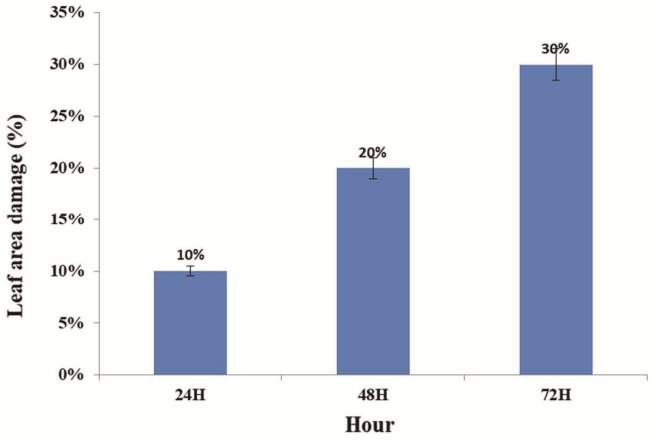

Fig. 6. Percentage of leaf area damaged by bagworm larva in guava under laboratory conditions.

Leaf consumption in guava plant: Based on weight, the maximum quantity (0.43 g) of the leaf was consumed by bagworm larva at 72 HAR followed by 48 HAR (0.27 g), and the minimum quantity (0.13) was consumed at 24 HAR (Fig. 7). Similar trend was also observed in case of area of leaf consumption. The maximum quantity (13.46 cm^2) of leaf area consumption was recorded at 72 HAR followed by 48 HAR (7.73 cm^2), while the minimum quantity (3.42 cm^2) was consumed at 24 HAR (Fig. 8) (Plate 8).

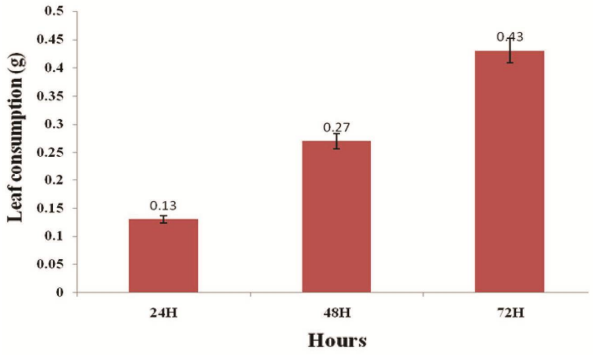

Fig. 7. Guava leaf consumption in weight by bagworm larva under laboratory conditions.

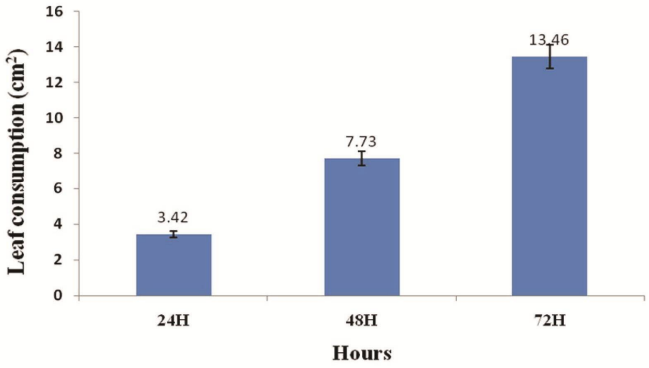

Fig. 8. Area of guava leaf consumption by bagworm larva under laboratory conditions.

The present study's findings are in conformity with Rhainds *et al.* (2009) who stated that bagworms can eat on numerous various kinds of plants, and *Thyridopteryx ephemeraeformis* can feed on over 50 families of deciduous and evergreen trees and shrubs (Rhainds *et al.*, 2009). As the caterpillars increase in size and shape, their feeding

Plate 8. Area of guava leaf consumed by different aged larvae of bagworms.

symptoms appeared as more visible and prominent due to their requirement for a higher quantity of food sources (Baxendale and Kalisch, 2009). Due to enough food, they may resident on the similar host plant as their mother causing significant damage (Rhainds and Sadof, 2008). Woody plant can tolerate less than 10% damage on it by consumers (Lemke *et al*., 2005), and as few as 4 bagworm larvae can cause a four-foot thuja to be unmarketable for sale during the summer months (Sadof and Raupp 1987). The presence of bagworms often remains unnoticed until they become mature and extensive damage is appeared (Hoover, 2000).

Conclusion

Nine plants were recorded as hosts of *T. ephemeraeformis* for the first time in Bangladesh. The fully-grown larva was longer than the pupa, while the wingless maggot-like female was larger than the male moth. The leaves of guava, thuja, rangan, mussaenda, henna, and copperleaf were found severely infested by bagworm larvae at the PSTU campus. The highest infestation in respect of infested leaves, number of bags per leaf, percentage of leaf area damage, and leaf consumption was observed in guava, followed by thuja, which was the lowest in copperleaf.

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AN ETHNOBOTANICAL SURVEY OF MEDICINAL PLANTS FOCUSING ON CARDIOVASCULAR DISEASES USED BY THE LOCAL PEOPLE IN AND AROUND DINAJPUR DISTRICT, BANGLADESH

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Abstract

This study aimed to document the medicinal plants through semi-structured interviews, key informant discussions, and informal conversations with local people in and around the Dinajpur district, Bangladesh. A total of 109 medicinal plant species distributed in 60 families with 210 formularies to treat 55 ailments were recorded. The most frequently utilized plant populations were herbs, followed by trees, shrubs, and climbers. Oral consumption was the main mode of treatment in the study area and was followed by external application. The highest factor of informant consensus (Fic) values was found in heart disease, followed by diabetes, gastrointestinal disorders, skin disease, respiratory disorders, sexual disease, and cuts and wounds. In the present survey, eight species have attained a fidelity level of 100 percent (Fl). Among the plants, 25 species have been used to treat cardiovascular diseases. The most cited medicinal plants for cardiac management are *Terminalia arjuna* (Roxb. *ex* DC.) Wight & Arn., *Baccaurea ramiflora* Lour., *Dillenia indica* L., *Allium sativum* L., *Tamarindus indica* L., *Rauvolfia serpentina* (L.) Benth. *ex* Kurz., *Terminalia chebula* Retz., *Phyllanthus emblica* L., *Averrhoa carambola* L. and *Spondias pinnata* (L. f.) Kurz. The ethnobotanical uses of the documented plants provide basic data, and further investigation focusing on pharmacological research is essential to confirm the results. Numerous threats to medicinal plants were identified during the ethnobotanical survey in the study area. Some recommendations are provided to mitigate the threats and the conservation of medicinal plants.

Key words: Ethnobotanical survey, Medicinal plants, Dinajpur District, Cardiovascular disease, Threats, Conservation.

Introduction

Ethnobotanical studies are significant for discovering modern drugs from native medicinal plant resources. There are appropriate sources of information about useful medicinal plant species, which can be targeted for management and domestication

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(Njoroge *et al.,* 2004; Mahmood *et al.,* 2013). Dinajpur is the largest district among all sixteen northern districts of the Rangpur division of Bangladesh. It is famous for producing rice, lychee, and wheat and is highly rich in natural resources like coal. Recently, according to the Geological Survey of Bangladesh (GSB), the presence of iron reserves in the form of magnetite was found at Isabpur village in Hakimpur Upazila of the district (GSB, 2019). Bangladesh is divided into distinct, culturally diverse areas. Dinajpur is such one area where ethnic communities such as Santals, Oraon, Mahali, Malpahari, and Kol live (Bangladesh Population Census, 2001). Cardiovascular diseases (CVDs) are problems with the heart or blood vessels (Uddin *et al*., 2019; Olorunnisola *et al*., 2011). CVDs are a variety of diseases, including peripheral vascular diseases, coronary heart disease (CHD), heart failure, heart attack (myocardial infarction), stroke, cardiomyopathies, dyslipidemias, and hypertension (Reiner *et al*., 2019). According to the World Health Organization (WHO, 2017), an estimated 17.9 million people died from CVDs in 2016, representing 31% of all global deaths. Of these deaths, 85% were due to heart attacks and strokes. Bangladeshis had the highest prevalence of CVD risk factors among five South Asian countries, with a prevalence of self-reported history of hypertension (14.3%), abdominal obesity (43.3%), and current and former smoking (59.9%) (Uddin *et al*., 2019; Joshi *et al*., 2007).

From the beginning of society, humans relied on plants to create a new field for discovering of plant-derived drugs. These drugs effectively cure certain diseases and have drawn attention to herbal medicines in a new way. Medicinal herbs continue to be an alternative treatment approach for several diseases, including CVD (Shaito *et al.*, 2020). It is estimated that about 30% of pharmaceuticals are prepared from plant derivatives (Leta *et al.*, 2002; Gillman *et al*., 1995). Several research studies have been conducted to discover the plants and natural food sources, the supplements of which have antithrombotic (anticoagulant and antiplatelet) effects, and there is an indication that consuming such foods leads to the prevention of coronary events and stroke (Ratnasooriya *et al.*, 2008; Joshipura *et al.*,1999; Liu *et al.*, 2000).

In Bangladesh, a number of plants are known to possess cardioprotective properties, resulting in their use by traditional healers for the treatment of chest complaints, high cholesterol, high blood pressure, and general heart problems, which are the most common symptoms of cardiovascular diseases. Although the beneficial effects of thrombolytic therapy are now well established (Collen, 1996), the biochemical mechanisms of thrombolytic therapy have been explained. The search for alternative therapies continues because of availability, affordability, diversity, and easy access to natural resources. Due

to their biological activities, plants may serve as alternative sources for developing new anticoagulant agents (Uddin *et al.*, 2019).

Ethnobotanical knowledge of medicinal plants needs proper documentation and evaluation before declining from the natural source of the study area. To protect such knowledge, documentation of ethnobotanical plants has already been started. In Bangladesh, a number of research has been done in this field, focusing mainly on a particular community or particular diseases or particular areas, such as Mia and Haque (1988); Hassan and Khan (1986, 1996); Alam (1992); Alam *et al*., (1996); Uddin *et al*., (2001, 2006, 2012, 2017); Khan *et al*., (2002); Ghani (2003); Uddin and Hassan (2004); Yusuf and Uddin (2006); Yosuf (2006); Uddin and Roy (2007); Roy *et al*., (2008); Emily *et al*., (2010); Uddin (2013); Haque *et al*.,(2014); Uddin *et al.* (2015a,b). But there is no record of ethnobotanical plant species useful for cardiovascular diseases in and around the Dinajpur district. In order to document and corroborate ethnobotanical plant species for cardiovascular diseases in and around Dinajpur district, an attempt was made to achieve the following objectives: to record, assimilate, and document all scattered distribution of traditional healthcare knowledge of medicinal plants, along with discovering any threats to medicinal plants in the study area, and to focus the traditional knowledge of medicinal plants for cardiovascular diseases.

Materials and Methods

Study area: The total area of Dinajpur district is 3437.98 km^2, located between 25°10' and 26°04' north latitudes and 88°23' and 89°18' east longitudes. It is bounded by Thakurgaon and Panchagarh districts in the north, Gaibandha and Joypurhat districts in the south, Nilphamari and Rangpur districts in the east, and the state of West Bengal, India in the west. There are 13 Upazillas in the study area, where Birganj is the biggest and Hakimpur is the smallest. The Singra Shal forest in Birganj Upazila has a vast collection of plant resources. It is also a protected area in Bangladesh as a National Park.

Data collection: The study area was visited five times in different seasons from July 2018 to April 2019. Each field trip lasted five to eight days. Data on medicinal plants was recorded in three ways, i.e., semi-structured interviews, key informant interviews, and informal conversations with local people, including herbal practitioners. A total of 300 informants were interviewed using a questionnaire. Among them, 57% were male and the rest, 43%, were female (Fig. 1). age ranged from 21 to 70 years old (Fig. 2).

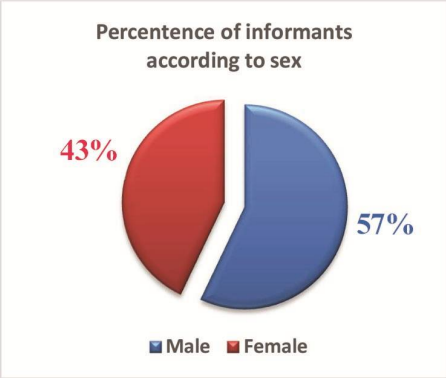

Fig. 1. Percentage of informants according to sex.

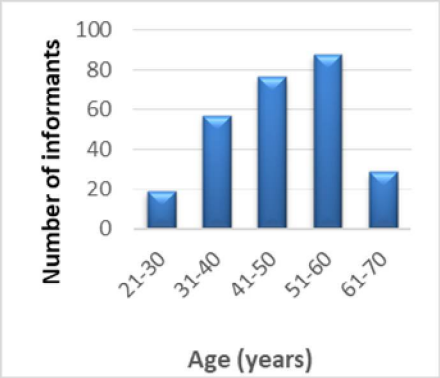

Fig. 2. Number of informants in each age group.

Key questions about medicinal plants were on the local name of a medicinal plant, particularly, types of disease treated, mode of treatment and method of preparation for remedy, plant parts used, fresh or dry plant parts used, mode of administration, and requirement for doses.

Collection and identification of the plant materials: According to the list, medicinal plants were collected from fields, gardens, forests, and the habits of these plants were documented. The collected plant specimens were pressed, dried, poisoned, mounted, and processed using standard herbarium techniques (Hyland, 1972; Alexiades, 1996).

Data analysis: The factor of informant consensus (Fic) was calculated using the following equation: Fic = $N_{ur} – N_{taxa}/N_{ur}$-1**,** where N_{ur} is the number of use reports in each category and N_{taxa} is the number of species in each category (Heinrich *et al*., 1998). The fidelity level was calculated following the equation: Fl (%) = (Np/N) 100, where Np is the number of informants who claim to have used a plant species to treat a specific disease and N is the number of informants who use the plants as medicine to treat any given disease (Friedman *et al.*, 1986). Cf values of medicinal plants were estimated by Friedman *et al. in* 1986. Using a Microsoft Office Excel sheet, the data were summarized. Descriptive statistical methods were applied for analyzing and summarizing the ethnobotanical data, such as frequency and percentage.

Results and Discussion

The study has resulted in the recording of 109 medicinal plant species belonging to 60 families. The local people use these species to treat 55 ailments through 210 formularies

in and around the Dinajpur district. For each species, scientific name, local name, family, habit, part used, ailment, and treatment mode are provided (Table 1).

Table 1. Ethnobotanical data on medicinal plants and uses in the study area (S=Shrub, C=Climber, H=Herb, T=Tree).

Scientific name	Local name	Family	Habit	Parts use	Ailments	Treatment mode
Abroma augustum (L.) L. f.	Ulotkombal	Sterculiaceae	S	Leaf	Jaundice	Leaf juice is taken
					Dysentery	Juice is taken
Justicia adhatoda L.	Basak	Acanthaceae	S	Leaf	Cold treatment	Leaf is chewed
					Bronchitis	Leaf juice is taken with ginger and honey
Aegle marmelos (L.) Corr.	Bel	Rutaceae	T	Fruit	Dysentery	Juice is taken twice per day
					Stomach problem	Juice is taken
				Leaf	Diarrhoea	Leaf juice is taken
Albizia procera (Roxb.) Benth.	Koroi	Mimosaceae	T	Leaf	Skin disease	Leaf paste is applied to the affected area
Allium cepa L.	Piaz	Liliaceae	H	Bulb	Flu	Juice is taken
Allium sativum L.	Rosun	Liliaceae	H	Clove	Blood pressure	Clove is eaten raw
					Heart disease	2-3 cloves are eaten
Alocasia cucullata (Lour.) G. Don	Biskachu	Araceae	H	Rhizome	Body ache	Cooked rhizome is taken
					Rheumatic pain	Cooked rhizome is taken
Aloe vera (L.) Burm. f.	Aloevera	Aloaceae	H	Leaf	Stomachache	Leaf juice is taken
					Weight loss	Juice is taken
					Hair treatment	Latex paste is applied to hair
Alstonia scholaris (L.) R. Br.	Chatim	Apocynaceae	T	Latex	Ringworm	Latex is applied to the affected area
Amaranthus tricolor L.	Lalsak	Amaranthaceae	H	Leaf	Blood purifier	Cooked leaf is taken
					Blood pressure	Cooked leaf is taken
Amorphophallus paeoniifolius (Dennst.) Nicolson	Olkachu	Araceae	H	Rhizome	Rheumatic pain	Cooked rhizome is taken
Anacardium occidentale L.	Bhela	Anacardiaceae	T	Fruit	Antiseptic	Fruit juice is applied to the affected area
Andrographis paniculata (Burm.f.) Nees	Kalomegh	Acanthaceae	H	Leaf	Cold treatment	Leaf is chewed

Table 1 contd.

Scientific name	Local name	Family	Habit	Parts use	Ailments	Treatment mode
					Constipation	Leaf juice is taken
					Ulcer	Leaf juice is taken.
Annona squamosa L.	Sharifa	Annonaceae	T	Fruit	Anthelmintics	Juice is taken
Arachis hypogaea L.	Badam	Fabaceae	H	Fruit	Heart disease	Fruit is taken
Areca catechu L.	Supari	Arecaceae	T	Fruit	Stomachache	Crushed fruit is taken
Aristolochia indica L.	Iswarmul	Aristolochiaceae	C	Root	Dysentery	Root juice is taken
Artocarpus heterophyllus Lamk.	Kathal	Moraceae	T	Fruit	Nutritive	Fruit is eaten
				Leaf	Skin disease	Paste is applied
Asparagus racemosus Willd.	Satamuli	Asparagaceae	C	Root	Gastric	powdered root is taken
Averrhoa carambola L.	Kamranga	Oxalidaceae	T	Fruit	Cold treatment	Fruit is taken
					Heart disease	Fruit juice is taken
Azadirachta indica A. Juss.	Neem	Meliaceae	T	Leaf	Diabetes	Juice is taken
					Skin disease	Leaf boiled water is applied affected to the area
					Jaundice	Juice is taken
Baccaurea ramiflora Lour.	Lotkon	Euphorbiaceae	T	Fruit	Heart disease	Fruit juice is taken
					Anti-oxidant	Fruit juice is taken
Bambusa tulda Roxb.	Talla bash	Poaceae	T	Stem	Impotence	Cooked stem is taken
Basella alba L.	Puisak	Basellaceae	C	Leaf	Wound	Leaf paste is applied to the affected area
Bombax ceiba L.	Shimul	Bombacaceae	T	Root	Impotence	Root juice is taken
Borassus flabellifer L.	Tal	Arecaceae	T	Young apex	Cough	Juice is taken
Bryophyllum pinnatum (Lamk.) Oken	Pathorkuchi	Crassulaceae	H	Leaf	Diabetes	Juice is taken
					Cold treatment	Juice is taken
					Cuts & wounds	Paste is applied
					Blood dysentery	Juice is taken
Cajanus cajan (L.) Millsp.	Orhor	Fabaceae	S	Leaf	Jaundice	Leaf juice is taken
Calotropis procera (Ait.) R. Br.	Akanda	Asclepiadaceae	S	Leaf	Ringworm	Leaf paste is applied over affected area
Careya arborea Roxb.	Kumbhi	Lecythidaceae	T	Bark	Weakness	Bark juice is taken

Table 1 contd.

Scientific name	Local name	Family	Habit	Parts use	Ailments	Treatment mode
Carica papaya L.	Pepe	Caricaceae	S	Fruit	Gastric pain	Boiled fruit is taken
					Constipation	Cooked fruit is taken
					Constipation	Raw fruit is taken
				Latex	Ringworm	Latex is applied to the affected area
Cassia fistula L.	Sonalu	Caesalpiniaceae	T	Leaf	Ringworm	Leaf paste is applied to the affected area
				Fruit	Laxative	Fruit is taken
Catharanthus roseus (L.) G. Don	Noyontara	Apocynaceae	H	Leaf	Diabetes	Leaf juice is taken twice per day
				Leaf	Diabetes	Leaf is chewed in empty stomach
				Flower	Diabetes	Flower chewed early in the morning
Centella asiatica (L.)	Thankuni	Apiaceae	H	Leaf	Brain tonic	Leaf chewed
Urban				Leaf	Skin disease	Leaf paste is applied to the applied area
				Leaf	Constipation	Leaf paste is taken
Cinnamomum tamala Nees & Eberm.	Tejpata	Lauraceae	T	Leaf	Asthma	Bud is eaten
					Digestion	Bud is eaten raw
					Cold treatment	Bud is boiled with tea & then taken
Citrus aurantifolia (Christm. & Panzer)	Kagojilebu	Rutaceae	S	Fruit	Vomiting	Juice is taken.
Swingle					Vomiting	Juice is taken
					Toothache	Juice is taken
Citrus limon (L.) Burm. f.	Lebu	Rutaceae	S	Fruit	Jaundice	Juice is taken
Clerodendrum viscosum Pers.	Vat	Verbenaceae	S	Leaf	Dysentery	Leaf paste is taken
					Fever	Leaf juice is taken
					Dysentery	Leaf paste is taken
				Root	Daud	Root blended with Zinger then the paste is applied to the affected area
				Stem	Jaundice	Juice is taken
Coccinia grandis (L.) Voigt	Telakucha	Cucurbitaceae	C	Leaf	Diabetes	Leaf paste is taken
					Constipation	Leaf juice taken with black cumin
					Diabetes	4 to 5 leaves are chewed in empty stomach in the morning

Table 1 contd.

Scientific name	Local name	Family	Habit	Parts use	Ailments	Treatment mode
					Diabetes	Paste taken with boiled rice
					Dysentery	1 glass of leaf juice is taken
					Jaundice	1 cup of juice is taken
					Constipation	Leaf juice taken with black cumin
Cocos nucifera L.	Narikel	Arecaceae	T	Fruit	Jaundice	Water is taken
Colocasia esculenta (L.) Schott	Kochu	Araceae	H	Leaf	Brain tonic	Cooked and taken
				Stem	Brain tonic	Cooked stem is taken
Coriandrum sativum L.	Dhonia	Apiaceae	H	Seed	Reducing cholesterol	Soaked in water then water is taken
Crinum asiaticum L.	Birpiaj	Liliaceae	H	Root	Ring worm	Root paste is applied to the affected area
Cucurbita siceraria Molina	Lau	Cucurbitaceae	C	Fruit	Toothache	Cooked fruit is taken
Curcuma longa L.	Halood	Zingiberaceae	H	Rhizome	Blood purifier	Juice is taken
					Skin disease	Paste is applied to the affected area
Cuscuta reflexa Roxb.	Swarnalota	Cuscutaceae	C	Stem	Jaundice	Juice is taken
					Deworming	Juice is taken
Cyperus rotundus L.	Gandhavadlu	Cyperaceae	H	Leaf	Diarrhoea	Cooked leaf is taken
Dalbergia sissoo Roxb.	Shishu	Fabaceae	T	Leaf	Dysentery	Leaf juice is taken
Datura metel L.	Dhatura	Solanaceae	S	Leaf	Paralysis	Dried crushed leaf is applied to the affected area
					Skin disease	Leaf paste is applied to the affected area
					Skin disease	Cooked leaf is taken
Daucus carota L.	Carot	Apiaceae	H	Root	Heart disease	Root juice is taken
Dillenia indica L.	Chalta	Dilleniaceae	T	Leaf	Diarrhoea	Leaf paste is applied to the affected area
					Headache	Paste is applied to the affected area
				Leaf	Tumor	Paste is applied to the affected area
				Fruit	Heart disease	Fruit juice is taken
Diospyros malabarica (Desr.) Kostel.	Gab	Ebenaceae	T	Leaf	Headache	Leaf paste is applied to the affected area

Table 1 contd.

Scientific name	Local name	Family	Habit	Parts use	Ailments	Treatment mode
Bacopa monnieri (L.) Pennell	Brammi	Scrophulariaceae	H	Leaf	Hair treatment	Juice applied to hair for hair growth
Eclipta prostata (L.) Mant.	Keshoraj	Asteraceae	H	Leaf	Hair treatment	Leaf paste is applied to hair
Enhydra fluctuans Lour.	Helencha	Asteraceae	H	Leaf	Jaundice	Juice is taken
					Ulcer	Leaf juice is taken
					Anti-oxidant	Cooked leaf is taken
					Eye treatment	Cooked leaf is taken
Ficus racemosa L.	Jogdumur	Moraceae	T	Leaf	Diabetes	Cooked leaf is taken
Glinus oppositifolius (L.) A. DC.	Gemashak	Molluginaceae	H	Leaf	Blood pressure	Dried leaf is taken
Gloriosa superba L.	Ulotchandal	Lilliaceae	C	Root	Stomachache	Juice is taken
				Leaf	Head lice	Paste is applied
Glycosmis pentaphylla (Retz.) A. DC.	Motkilla	Rutaceae	S	Leaf	Diarrhea	Juice is taken
				Stem	Toothache	Stem used as brushing teeth
				Root	Skin disease	Paste is applied to the affected area
					Eczema	Paste is applied to the affected area
Hibiscus rosa-sinensis L.	Joba	Malvaceae	S	Leaf	Dysentery	Leaf juice is taken internally twice a day
					Liver disease	Leaf soaked in water at night then taken in the next morning
					Hair tonic	Leaf paste is boiled with oil and then applied over hair
				Flower	Hair fall	Flower paste is applied over head
					Weakness	Flower buds are taken
Hyptis suaveolens (L.) Poit.	Tokma	Lamiaceae	H	Seed	Blood pressure	Seed is taken
					Constipation	Seed is taken
Ipomoea aquatica Forssk.	Kalmisak	Convolvulaceae	H	Leaf	Eye treatment	Leaf is cooked
Lawsonia inermis L.	Mehedi	Lythraceae	S	Leaf	Hair treatment	Leaf paste is applied to the affected area
Leucas aspera (Willd.) Link	Dondokolosh	Lamiaceae	H	Leaf	Cold treatment	Juice is taken
Litsea glutinosa (Lour.) Robinson	Menda	Lauraceae	T	Leaf	Diarrhoea	Leaf juice is taken
				Bark	Dysentery	Bark soaked in water is taken

Table 1 contd.

Scientific name	Local name	Family	Habit	Parts use	Ailments	Treatment mode
Lycopersicon esculentum Mill.	Tomato	Solanaceae	H	Fruit	Heart disease	Juice is taken
Mangifera indica L.	Aam	Anacardiaceae	T	Fruit	Stomach problem	Fruit juice is taken
				Seed	Reducing cholesterol	Crushed seed is taken
				Leaf	Teeth ache	Chewed leaves
					Dysentery	Grinded and taken in empty stomach once per day
					Heart disease	Young leaves are eaten in empty stomach
					Diarrhoea	Grinded and taken in empty stomach once per day
				Stem	Weakness	Stem soaked water is taken
Mentha arvensis L.	Pudina	Lamiaceae	H	Leaf	cold treatment	Leaf juice is taken
Mikania cordata (Burm. f.) Robinson	Assamilata	Asteraceae	C	Leaf	Cuts & wounds	Leaf paste is applied to the affected area
					Diarrhoea	Leaf juice is taken
Mimosa pudica L.	Lajjaboti	Mimosaceae	H	Whole plant	Blood purifier	Decoction of the whole plant is taken
				Root	Fistula	Juice is taken
				Leaf	Toothache	juice is taken
Moringa oleifera Lamk.	Sajna	Moringaceae	T	Bark	Gastric	Crushed bark is taken
					Body ache	Juice is taken
					Ulcer	Bark paste is taken
Murraya paniculata (L.) Jack	Kamini	Rutaceae	T	Leaf	Toothache	Leaf paste is applied
Musa acuminata Colla	Kola	Musaceae	H	Latex	Skin disease	Latex is applied to the affected area
				Fruit	Digestion	Fruit is eaten
				Flower	Heart disease	Paste is taken
Neolamarckia cadamba (Roxb.) Bosser	Kadam	Rubiaceae	T	Leaf	Rheumatic pain	Heated leaf is applied to the affected area
Nicotiana plumbaginifolia Viv.	Tamak	Solanaceae	H	Leaf	Cuts & wounds	Leaf paste is applied
Nigella sativa L.	Kalojira	Ranunculaceae	H	Seed	Liver control	Crushed seed is taken internally
Ocimum gratissimum L.	Raamtulsi	Lamiaceae	S	Leaf	Asthma	Leaf juice is taken

Table 1 contd.

Scientific name	Local name	Family	Habit	Parts use	Ailments	Treatment mode
Ocimum sanctum L.	Tulshi	Lamiaceae	H	Leaf	Cold treatment	Leaf is chewed
					Cold treatment	Leaf is boiled with tea and then taken
					Constipation	Dried crushed leaf is taken with raw honey
					Ringworm	Leaf paste is applied to the affected area
					Tuberculosis	Leaf juice is taken
Oryza sativa L.	Dhan	Poaceae	H	Seed	Diarrhoea	Powder is taken after meal twice per day
Paederia foetida L.	Gandhaveduli	Rubiaceae	C	Leaf	Diarrhoea	Leaf juice is taken
Persicaria hydropiper (L.) Spach	Biskatali	Polygonaceae	H	Leaf	Skin disease	Leaf paste is applied to the affected area
Phyllanthus emblica L.	Amloki	Euphorbiaceae	T	Fruit	Toothache	Fruit juice is taken
					Hair problem	Paste is applied on hair
					Hair tonic	Fruit juice is boiled with oil and then applied on hair
					Heart disease	Fruit juice is taken
Senna alata (L.) Roxb.	Dadmardan	Caesalpiniaceae	H	Leaf	Ring worm	Paste is applied to the affected area
					Skin disease	Paste is taken
Piper betle L.	Pan	Piperaceae	C	Leaf	Bone ache	Leaf paste applied to the affected area
Psidium guajava L.	Peyara	Myrtaceae	T	Leaf	Dysentery	Young leaves are eaten
				Fruit	Nutritive	Fruit is taken
Punica granatum L.	Dalim	Punicaceae	S	Fruit	Heart disease	Juice is taken
Rauvolfia serpentina (L.) Benth. *ex* Kurz	Sarpagandha	Apocynaceae	S	Root	Heart disease	Powder is taken after meal twice per day
					Blood purifier	Paste is taken
Ricinus communis L.	Verenda	Euphorbiaceae	S	Seed	Constipation	Seed oil is used
Saccharum officinarum L.	Akh	Poaceae	H	Stem	Jaundice	Juice is taken
Saraca asoca (Roxb.) de Wild.	Ashok	Caesalpiniaceae	T	Bark	Anti-leukemia	Bark soaked in water then taken in empty stomach
Scoparia dulcis L.	Chinipata	Scrophulariaceae	H	Leaf	Diabetes	Juice is taken
					Dysentery	Juice is taken
					Diarrhoea	Leaf juice is taken

Table 1 contd.

Scientific name	Local name	Family	Habit	Parts use	Ailments	Treatment mode
Sesamum indicum L.	Til	Pedaliaceae	H	Seed	Skin disease	Oil is applied
Smilax macrophylla Roxb.	Kumarilata	Smilacaceae	C	Leaf	Stomach ache	Leaf juice is taken
Solanum melongena L.	Begun	Solanaceae	S	Fruit	Reducing cholesterol	Boiled fruit eaten internally.
Solanum tuberosum L.	Alu	Solanaceae	H	Tuber	Cuts & wounds	Paste is applied
Spondias pinnata (L. f.) Kurz	Amra	Anacardiaceae	T	Fruit	Blood pressure	Fruit juice is taken
Syzygium cumini (L.) Skeels	Kalojam	Myrtaceae	T	Seed	Diabetes	Seed powder is taken
					Dysentery	Seed powder is taken
				Bark	Toothache	Decoction of bark is taken
				Seed	Diabetes	Seed paste is taken
Syzygium samarangense (Blume) Merr. & Perry	Jamrul	Myrtaceae	T	Leaf	Stomachache	Juice is taken
Tagetes erecta L.	Gada	Asteraceae	H	Leaf	Cut	Juice is applied to the affected area.
				Flower	Dysentery	Flower is taken
Tamarindus indica L.	Tetul	Caesalpiniaceae	T	Fruit	Blood pressure	Fruit juice is taken
Terminalia arjuna (Roxb. *ex* DC.) Wight &Arn.	Arjun	Combretaceae	T	Bark	Heart disease	Bark soaked water is taken in empty stomach
					Heart disease	Powdered bark is taken in empty stomach early in the morning
					Heart disease	Bark mixed with Amloki,Horitoki and Bohera and then taken in empty stomach
					Gastric pain	Bark soaked water is taken in empty stomach
					Chest pain	Bark juice is taken
Terminalia bellirica (Gaertn.) Roxb.	Bohera	Combretaceae	T	Seed	Skin disease	Seed oil is used
				Fruit	Diabetes	Juice is taken
Terminalia chebula Retz.	Haritaki	Combretaceae	T	Fruit	Blood purifier	Fruit soaked water is taken
					Gastro intestinal disorders	Fruit soaked water is taken

Table 1 contd.

Scientific name	Local name	Family	Habit	Parts use	Ailments	Treatment mode
					Diabetes	Fruit juice is taken
					Dysentery	Unripe fruit is taken
Tinospora crispa (L.) Hook. f. & Thoms.	Gulancha	Menispermaceae	C	Stem	anthelmintics	Juice is taken
Streblus asper Lour.	Sheora	Moraceae	T	Leaf	Diabetes	Leaf juice is taken
Vitex negundo L.	Nishinda	Verbenaceae	S	Leaf	Insomnia	Leaf is kept under pillow
Zingiber officinale Rosc.	Ada	Zingiberaceae	H	Rhizome	Cold treatment	Boiled with tea then taken
					Digestion	Taken with salt before meal
					Gastric	Juice taken with salt.
Ziziphus mauritiana Lamk.	Boroi	Rhamnaceae	T	Leaf	Cuts & wounds	Boiled water is applied to the affected area.

It was observed that medicinal plants recorded in the study area belonged to 60 families. Among them, the maximum number of species belonged to 40 families and other species to the families Fabaceae, Rutaceae, Araceae, Asteraceae, Apiaceae, Solanaceae, Cucurbitaceae, Combretaceae, Myterceae, and Euphorbiaceae (Fig. 3).

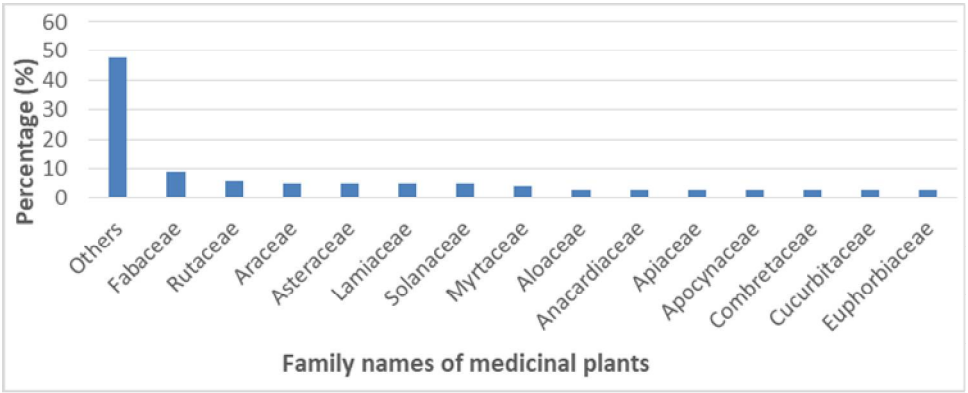

Fig. 3. Medicinal plants used for medicinal purposes.

Leaves were the most commonly utilized plant part with 43% application in traditional medicinal recipes, followed by fruit (18%), root (7%), seed (7%), stem (5%), bark (4%), rhizome (4%), latex (3%) , flower (3%) and others (flower bud, bulb, clove, tuber, whole plant, young apex) 1% (Fig. 4).

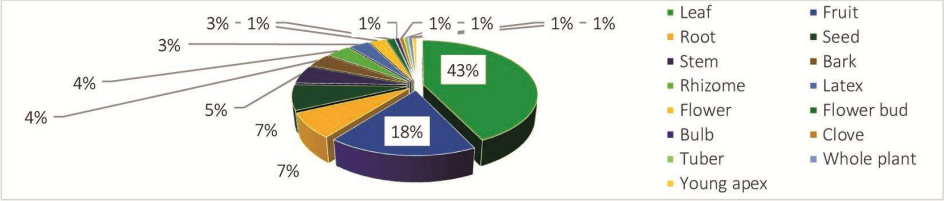

Fig. 4. Plant parts used for medicinal purposes

Plant species were classified into four groups based on their habitat: herbs (37%), trees (36%), shrubs (16%), and climbers (11%) (Fig. 5). It was observed that local healers use herbs more than trees, shrubs, and climbers to cure different kinds of diseases; it may be due to their easy accessibility, collection, fewer side effects, and abundance in the area. Local inhabitants of the study area use different methods, i.e., juice, paste, crushed, decoction, cooked, etc., to prepare a recipe for the treatment of various ailments. Out of 210 formularies, 71% were internal applications, and the rest (29% were external) (Fig. 6).

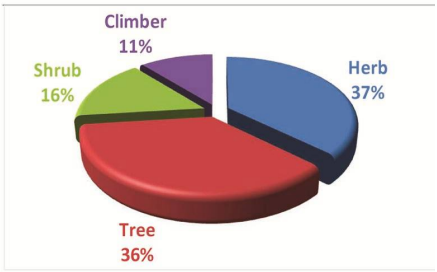

Fig. 5. Vegetation analysis of medicinal plants based on habit.

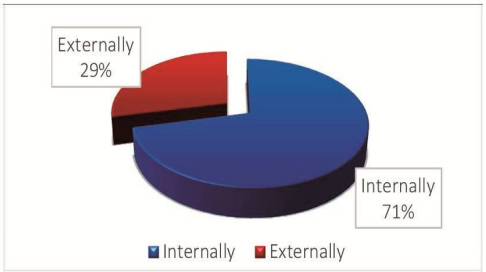

Fig. 6. Application mode of medicinal plants.

The informant consensus factor (Fic): To calculate Fic, the reported ailments were first classified into 8 different disease categories based on their usage reports. Among the

major disease categories, heart disease (more than 0.93) attained the highest Fic value (Table 2).

Table 2. Values of the factor of informant consensus in the uses of medicinal plants among the informants.

Disease category	Ailments	Most cited plants	N_{ur}	N_{taxa}	Fic value
1	Heart disease	*Terminalia arjuna* (Roxb. *ex* DC.) Wight & Arn., *Baccaurea ramiflora* Lour., *Dillenia indica* L., *Allium sativum* L., *Tamarindus indica* L., *Rauvolfia serpentina* (L.) Benth. *ex* Kurz., *Terminalia chebula* Retz., *Phyllanthus emblica* L.,*Averrhoa carambola* L.,*Spondias pinnata* (L. f.) Kurz	424	25	0.943
2	Diabetes	*Coccinia grandis*(L.) Voigt	117	12	0.905
3	Gastro-intestinal disorders	*Agle marmelos* (L.) Correa	237	46	0.809
4	Skin disease	*Azadirachta indica* A. Juss.	189	27	0.862
5	Respiratory disorder	*Justicia adhatoda* L.	136	22	0.844
6	Impotence	*Bombax ceiba* L.	30	3	0.931
7	Cuts & wounds	*Bryophyllum pinnatum*(Lamk.) Oken	19	7	0.667
8	Others	*Centella asiatica* (L.) Urban	172	32	0.819

N_{ur}= The number of use reports in each category; Ntaxa =The number of species in each category; Fic= Factor of informant consensus.

The fidelity level (Fl) of the 21 most important plant species ranged from 35% to 100%. *Terminalia arjuna* (Roxb. *ex* DC.) Wight & Arn., *Baccaurea ramiflora* Lour., *Dillenia indica* L., *Lycopersicon esculentum* Mill., *Tamarindus indica* L., *Lawsonia inermis* L., *Azadirachta indica* A. Juss., *Nigella sativa* L. indicated 100% Fl against heart disease, blood pressure, hair treatment, skin disease, and liver control respectively (Table 3).

Citation frequency values varied from species to species, as indicated in Table 4. *Terminalia arjuna* (Roxb. *ex* DC.) Wight & Arn. scored the highest Cf% value, meaning that such species are very popular in the study and used to treat heart disease. *Allium sativum* L., *Dillenia indica* L., *Baccaurea ramiflora* Lour., *Tamarindus indica* L, *Spondias pinnata* (L. f.) Kurz, *Rauvolfia serpentina* (L.) Benth. *ex* Kurz, *Terminalia chebula* Retz., *Phyllanthus emblica* L., and *Averrhoa carambola* L. were the most cited species in the study area.

The therapeutic potential of herbs in the healthcare system is well known worldwide, whether for a diseased state or for proper health maintenance (Malik, 2007). Herbs for cardiovascular diseases such as congestive heart failure, systolic hypertension, angina

pectoris, atherosclerosis, cerebral insufficiency, and arrhythmia have been prevalent since ancient times (Ray and Saini 2021; Mashour *et al*., 1988).

Table 3. Fidelity level (Fl) values of the frequently reported plants and their major uses.

Species	Ailments	Np	N	Fl(%)
Terminalia arjuna (Roxb. *ex* DC.) Wight &Arn.	Heart disease	97	97	100
Baccaurea ramiflora Lour.	Heart disease	21	21	100
Dillenia indica L.	Heart disease	23	23	100
Lycopersicon esculentum Mill.	Heart disease	13	13	100
Tamarindus indica L.	Blood pressure	21	21	100
Lawsonia inermis L.	Hair treatment	17	17	100
Azadirachta indica A. Juss.	Skin disease	22	22	100
Nigella sativa L.	Liver control	18	18	100
Averrhoa carambola L.	Heart disease	16	17	94.118
Catharanthus roseus L.	Diabetes	23	27	85.185
Coccinia grandis (L.) Voigt	Diabetes	16	20	80
Ocimum sanctum L.	Cold treatment	22	31	70.968
Syzygium cumini (L.) Skeel.	Diabetes	11	18	61.111
Centella asiatica (L.) Urban	Brain tonic	14	24	58.333
Allium sativum L.	Blood pressure	27	49	55.102
Rauvolfia serpentina (L.) Benth. *ex* Kurz	Blood pressure	19	36	52.778
Phyllanthus emblica L.	Heart disease	16	31	51.613

Np= The number of informants who claim to have used a plant species to treat a specific disease; N= The number of informants who use the plants as medicine to treat any given disease.

Table 4. Citation frequency of most cited medicinal plants.

Scientific name	Local name	Citation	Citation frequency (Cf %)
Terminalia arjuna (Roxb. *ex* DC.) Wight & Arn.	Arjun	45	15
Allium sativum L.	Rosun	27	9
Dillenia indica L.	Chalta	23	7.667
Baccaurea ramiflora Lour.	Lotkon	21	7
Tamarindus indica L	Tetul	21	7
Spondias pinnata (L. f.) Kurz	Amra	19	6.333
Rauvolfia serpentina (L.) Benth. *ex* Kurz	Sarpagandha	17	5.667
Terminalia chebula Retz.	Haritaki	16	5.333
Phyllanthus emblica L.	Amloki	16	5.333
Averrhoa carambola L.	Kamranga	16	5.333

Many ethnobotanical surveys have been carried out in the Dinajpur district of Bangladesh (Rahmatullah *et al*., 2010, 2009; Rahman 2015, 2012; Jamal *et al*., 2012; Uddin *et al*., 2006). None of these focused on ethnobotanical research in connection with the cardiovascular plant. In Bangladesh, several plants are reputed to possess cardioprotective properties, resulting in their use by traditional healers to treat chest complaints, high cholesterol, high and low blood pressure, and general heart problems (Uddin *et al.,* 2019). There is compelling scientific evidence demonstrating that consumping dietary anticoagulants or phytochemicals with anticoagulant properties can ultimately reduce or eliminate the risks of thromboembolic diseases (Uddin *et al*., 2019; Kumar *et al*., 2011; Lee *et al*., 2012; Manicam *et al*.,2010).

The present study revealed that 109 medicinal plant species were used for 55 ailments, with 210 formularies by the local people of the study area. Among them, 25 species have been used to treat cardiovascular diseases. These are *Terminalia arjuna* (Roxb. *ex* DC.) Wight & Arn. *Baccaurea ramiflora* Lour., *Rauvolfia serpentina* (L.) Benth. ex Kurz, *Hyptis suaveolens* (L.) Poit, *Phyllanthus embelica* L., *Averrhoa carambola* L., *Amaranthus tricolor* L., *Glinus oppositifolius* (L) Aug.DC., *Enhydra flactuans* Lour. *Spondias pinnata* (L. f.) Kurz, *Amaranthus tricolor* L., *Daucus carota* L., *Lycopersicon esculentum* Mill., *Solanum melongena* L., *Musa acuminata* Colla, *Mimosa pudica* L., *Arachis hypogaea* L., *Mangifera indica* (L), *Curcuma longa* L., *Cuminum cyminum* L. and *Punica granatum* L. The most cited medicinal plant species for cardiac management are *Terminalia arjuna* (Roxb. *ex* DC.) Wight & Arn., *Baccaurea ramiflora* Lour., *Dillenia indica* L., *Allium sativum* L., *Tamarindus indica* L., *Rauvolfia serpentina* (L.) Benth. ex Kurz., *Terminalia chebula* Retz., *Phyllanthus emblica* L., *Averrhoa carambola* L.

Terminalia arjuna (Roxb. *ex* DC.) Wight & Arn. was used to treat heartache in the study area. It is used for the same purpose (Uddin *et al*., 2021, 2012; Uddin and Hassan, 2014) and is also used for stomachaches, coughs, diabetes, menstruation, gastric pain, and dysentery (Uddin *et al*., 2006, 2012, 2017; Islam *et al*., 2014; Uddin *et al*., 2015a, b) reported from the different area from Bangladesh. *Baccaurea ramiflora* Lour. was used for the treatment of heart disease and as an antioxidant. It has been shown to have antioxidant properties (Uddin *et al*., 2021; Goyal *et al.*, 2013; Ullah *et al.*, 2012). The plant was used for diarrhea, flatulence, gastric ulcer, ureterolithiasis, and jaundice (Uddin, 2006). *Dillenia indica* L. was used to treat heart disease, diarrhoea, headaches, and tumors. This plant was also used for jaundice, hair tonic, constipation, dysentery, food poisoning, and cardiac weakness an general weakness (Uddin and Hassan, 2014; Uddin *et al*., 2012; Uddin *et al*., 2015; Uddin, 2006). It has been reported for antioxidant

(Abdille *et al.,* 2005), antihyperlipidemic, and anti-diabetic (Kumar *et al.,* 2011) activities. *Allium sativum* L. has been used to treat heart disease, gastric problems, colds, fevers, chest pain, high blood pressure, and ringworm (Uddin *et al.,* 2015, 2017, 2019). *Tamarindus indica* L. has been used to treat high blood pressure, diarrhea, dysentery, appetizer, constipation, impotence, abscess, and jaundice (Uddin *et al.,* 2012, 2015, 2017; Khan *et al*., 2002). *Rauvolfia serpentina* (L.) Benth. *ex* Kurz. was used to treat high blood pressure, hypertension, mental illness, stomach aches, and gastric ulcers (Islam *et al*., 2014; Roy *et al.,* 2008; Uddin *et al.,* 2004). *Terminalia chebula* Retz. has been shown to have antioxidant, antimicrobial, antidiabetic, hepatoprotective, anti-inflammatory, antimutagenic, antiproliferative, radioprotective, cardioprotective, antiarthritic, anticaries, gastrointestinal motility, and wound healing activity (Bag *et al.,* 2013). *Phyllanthus emblica* L. was used for heart disease (Uddin *et al.,* 2021, 2019;Khatun and Rahman, 2018). Muthu *et al.* (2016) discovered that *Averrhoa carambola* L. has antioxidant properties. *Amaranthus gangeticus* L. was used as a blood purifier (Uddin *et al.,* 2015). *Curcuma longa* L. has been reported as a blood purifier (Uddin *et al.,* 2006). Sujarwo and Keim 2019 reported that *Spondias pinnata* (L. f.) Kurz has a high antioxidant capacity.

Furthermore, *Amaranthus gangeticus* L., *Glinus oppositifolius* (L) Aug. DC., *Musa acuminata* Colla, *Mimosa pudica* L., *Arachis hypogaea* L., *Mangifera indica* (L), *Cuminum cyminum* L., Punica granatum L., *Amaranthus tricolor* L., *Daucus carota* L., *Lycopersicon esculentum* Mill., and *Solanum melongena* L. are also reported as medicinal plants for the cardiovascular diseases in the study area by the local people.

In the course of the study, traditional healers such as Kabiraj (Medicine men) showed their knowledge of the medicinal properties of plant species. The knowledge accumulated by the tribal people, such as the Santal community and the local population, about disease ailments is crucial to discover the latest drugs that can benefit human health. Also, dosages and administration should be standardized with the latest scientific methods. Currently, various developmental activities such as coal mining and stone lifting in Phulbari Upazila are great threats to medicinal plants and their habitats (Uddin *et al.* 2006). The tribals like the Santal community have already converted themselves to other religions, mostly Christianity, because of missionary activities. It gave them opportunities to use modern medicine rather than traditional ones. Sometimes, many medicine men are reluctant to go back to their roots.

From the observations, a variety of dangers to ethnomedicinal plants have been found via field interviews and discussions with local people. The study area's surrounding plantations of exotic timber species, including *Dalbergia sissoo* Roxb. and *Eucalyptus*

camadulensis Dehnh, pose the greatest dangers. Another danger to locally grown medicinal plants is the clearing of forests for constructing exotic monoculture plantations in Phulbari Upazila. Several natural forest sections of the Upazila, Sal Forest and allied species were replaced by *Acacia* spp. and *Eucalyptus* spp. plantations. Due to fragmentation, edge effects, agricultural encroachment, and development activities, the remaining Sal patches are in serious jeopardy.

Moreover, people are not particularly careful in cultivating resources, and care more for ornamental, timber or fruit trees than important medicinal plants. They are starting to take care of the plants in their roof gardens and even balconies. The availability of modern medicine that encourages the local people to use it rather than herbal medicines found in the study area is an additional threat to the medicinal plant. People who are elderly and know herbs are not inclined to share their knowledge with children. In the event of the sudden death of these individuals, the knowledge of herbal remedies in the area will disappear forever.

The present work in the Dinajpur district is very preliminary. The record of these medicinal plant species indicate rich ethnobotanical knowledge among the locals in and around the Dinajpur district. This research could provide an immediate and efficient strategy to investigate the effect of clot lysis on newly developed and known drugs. The results currently reported by medicinal plants are fundamental, and further lengthy studies are essential to verify these results. When conducting the study within the study area, a number of dangers to medicinal plants were discovered and a few suggestions were made to protect beneficial plants within the Dinajpur district. The latest scientific discoveries for further study of bioactive components that could lead to the development of new treatment options for cardiovascular diseases. Along with that, we must cultivate awareness of the importance of medicinal plants among residents, developers, and policymakers.

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PRESENCE OF METHYL PARABEN IN ANTI-DIABETIC HERBAL PREPARATIONS

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Abstract

Plant-based hypoglycemic therapeutics have been increasing in consumption due to the escalation of type 2 diabetes and its related complications. However, the safety of the preparations is little understood. Parabens (alkyl esters of p-hydroxybenzoic acid) are widely used as preservatives in these pharmaceuticals. However, the presence of methylparaben in formulations raises anxiety due to its potential endocrine disruption functions. Endocrine disruption could lead to undesirable health abnormalities and carcinogenic, estrogenic, and adverse reproductive effects. The present investigation directs toward estimating of methylparaben in some anti-diabetic herbal preparations using UV- Vis spectrophotometric method abiding by International Conference on Harmonization (ICH) guidelines for validation. The analytical wavelength of methylparaben in methanol was determined and found at 256.5 nm. The method obeys Beer's law in the analytical range and has a good coefficient of determination (r^2= 0.9881). The limit of detection (LOD) and limit of quantification (LOQ) were 0.19 ppm and 0.57 ppm, respectively. Recoveries were 91.3-98.8% in analyte-free plant matrix and 91-105.8 % in a diluent. The coefficient of variation (CV%) varied between 0.005-0.268% for different standards. Results of forty-eight anti-diabetic herbal preparations showed methylparaben was detected in thirty-four samples in the range of 13.12 – 325.13 mg/day with a mean exposure value of 78.25 mg/day. However, none of the samples raise concerns about safety (the safety ceiling for paraben is 420 mg/day). More investigation is required to determine, whether the herbal drugs are safe to consume in terms of methylparaben.

Key words: Methylparaben, UV-Vis spectrophotometric method, Safety assessment.

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Introduction

Prolonged shelf life is an indication of drug quality. Shelflife is affected by the modification and degradation of a drug due to microbiological, enzymatic, or chemical changes. Preservatives delay or restrict the process (Malik *et al.*, 2010; Mahboubi *et al.*, 2014). However, they can exert an adverse health effect in excess (Andersen *et al.*, 2019; Soni *et al.*, 2002; Nair 2001; Hannuksela *et al.*, 1987; Juhlin 1981; Lahti *et al.*, 1987; Rademaker *et al.*, 1989, Safford *et al.* 1990). The use of methylparaben in pharmaceutical preparations as a preservative is common (Mahboubi *et al.*, 2014). The preservative hails from the alkyl esters of p-hydroxybenzoic acid (parabens), which are a group of a homologous series of chemicals (Fig. 1). Properties of methylparaben like broad activity against bacteria, yeasts, and molds, no apparent odor and taste and chemical stability are thought playing a role behind their application in pharmaceuticals as preservatives (Soni *et al.*, 2002, Anderson *et al.*, 2005; El Hussein *et al.*, 2007). After entering the body parabens quick absorption occurs in the gastrointestinal tract and blood (Darbre *et al.*, 2004; Darbre *et al.*, 2008; Soni *et al.*, 2001).

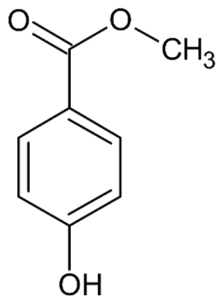

Fig. 1. Chemical structure of methylparaben.

The osteogenic effect of paraben is not mysterious (Routledge *et al.*, 1998). The effect has an association with female breast cancer incidence and the development of male reproductive system malignant melanoma (Tavares *et al*., 2009). Moreover, there are estrogenic effects on children if they consume parabens through drugs (Prudal *et al.*, 2015). The concern is also raised about the safe use of parabens due to their potential endocrine disruption properties in different *in-vitro* and *in-vivo* investigations (Miller *et al.*, 2001; Okubo *et al,*. 2001; Byford *et al.* 2002; Darbre *et al.*, 2008; Boberg *et al.* 2010; Witorsch and Thomsa, 2010). Endocrine disruption coupled with reproductive toxicity related to paraben raises the question about their widespread exposure, thereby

attracting substantial attention by national and international regulatory authorities (Toxicological evaluation of certain food additives with a review of general principles and of specifications, 1974; Scientific Committee on Consumer Safety, 2011). Regulatory authorities ask for data on the quantity of the preservatives in different drugs and foods (CFR, 1999). Therefore, quantification of the analyte in finished drugs must be required.

Rajshahi City is a northern metropolis of Bangladesh. Along with the increasing number of diabetic patients, the consumption of anti-diabetic herbal preparations is increasing. No herbal drug manufacturer provides information on its preservative status. Consequently, the amount of consumption of parabens through herbal formulations has yet to be discovered. If excess parabens are consumed through the preparation, then drug safety could be breached, and an assessment of parabens is becoming mandatory. Therefore, in the current investigation, an assessment of the safety of some anti-diabetic herbal preparations in terms of methylparaben was conducted. Along with chromatographic methods (Sohrabvandi *et al.,* 2015; Baranowska *et al.,* 2004; Can *et al.,* 2011; Yan *et al.,* 2012; Ma *et al*., 2012), UV-Vis spectroscopic methods are common for analyzing preservatives (Mahboubifa *et al.,* 2010). An easy, accurate, and sensitive UV-Vis spectrophotometric analytical method was used to determine paraben concentrations in the herbal preparations. This study helps the local people and the scientific community. The herbal manufacturers can also use the analytical procedure in their lab for routine analysis of parabens.

Materials and Methods

Sample: In total, forty-eight anti-diabetic herbal preparations were procured from different manufacturers, and finished formulations bearing drug administration registration no (DAR No.) were chosen. The location of the manufacturing outlets shows similar characteristics; the collection points were densely populated areas where consumers purchased drugs from their close neighborhood, and the collection points also acted as a junction through which mass movement of people takes place. When returning home from the city, the consumers purchase drugs from the outlets. After collecting samples from shops, they were taken to the research laboratory, Department of Chemistry, University of Rajshahi, where information attached to the drugs (either in the package insert or on the packaging wall) recorded in tabulated form. The drugs were then blindfolded by coding to avoid bias before the assay (Table 1).

Table 1. Sample information.

Sample Code	Batch No.	DAR No.	Manufacturer	Max. dosage/ day	Unit drug wt. (g)
1	1	U-038-A-033	1	2 cap 2 times	0.58
2	3	U-038-A-020	1	2 tab 2 times	0.56
3	11	U-038-A-094	1	3 cap 2 times	0.58
4	54	U-038-A-029	1	2 tab 2 times	0.41
5	81	U-038-A-028	1	2 tab 2 times	0.40
6	1	U-038-A-017	1	2 tab 3 times	0.57
7	4	U-038-A-021	1	2 tab 2 times	0.57
8	8	U-038-A-018	1	2 tab 1 times	0.57
9	1	U-038-A-100	1	4 cap 3 times	0.54
10	1	U-038-A-074	1	1 tab 2 times	0.96
11	6	H-82-A-61	2	2 tab 2 times	0.64
12	5	N/A	2	10 gm 3 times	0.62
13	5	H-82A-054	2	5 tab 3 times	0.59
14	8	H-47A-061	2	2 tab 2 times	0.66
15	6	003-02-94	2	2 tab 3 times	0.65
16	1	003-0002-94	2	2 cap 2 times	0.62
17	9	H-82A-039	2	2 cap 3 times	0.50
18	5	Ayu-210A-007	2	3 cap 2 times	0.46
19	14	H-82A-028	2	3 tea spoon 3 times	1.08
20	5	U19-A-128	3	2 tab 3 times	0.64
21	9	U-19-A-040	3	2 tab 2 times	0.63
22	5	U-19-A-219	3	1 cap 2 times	0.64
23	9	015-0005-94	3	1 cap 2 times	0.60
24	15	Ayu-78A-019	3	1 sachete 3 times	3.83
25	1	015-14-94	3	2 cap 2 times	0.61
26	2020-01/1(1)	Ayu-4A-013	4	3 tab two times	0.28
27	2019-03/1(1)	Ayu-4A-011	4	3 tab two times	0.28
28	2020-02/1(1)	Ayu-4A-384	4	3 tab two times	0.23
29	2020-01/1(1)	Ayu-A-014	4	3 tab two times	0.44
30	2020-10/1(4)	Ayu-A-121	4	3 tab two times	0.64
31	1	Ayu-4A-058	4	4 tea spoon 2 times	0.02
32	1	Ayu-4A-061	4	4 tea spoon 2 times	0.02
33	3	Ayu-4A-063	4	4 tea spoon 2 times	0.02
34	2	Ayu-4A-119	4	4 tea spoon 2 times	0.02
35	2020-02/1(2)	N/A	4	3 tab two times	0.50
36	2020-11/1(2)	Ayu-A-301	4	3 tab two times	0.46
37	2020-02/1(1)	Ayu-A-318	4	3 tab 2 times	0.22
38	2019-06/1(2)	Ayu-A-310	4	3 tab 2 times	0.25
39	2019-06/1(1)	Ayu-A-309	4	3 tab 2 times	0.50
40	12	Ayu-18A-054	5	1 cap 3 times	0.57
41	20914001	U-137A-027	5	2 cap 2 times	0.60
42	LTZD	U-114A-006	5	2 tab 2 times	0.68
43	1	U-124-A-38	5	2 tab 2 times	0.53
44	2	U-17A-027	5	2 cap 2 times	0.52
45	46	U-301A-051	5	2 tab 2 times	0.60
46	B01M21E22	Ayu-26A-019	5	1 tab 2 times	0.57
47	1	5A-144	5	1 tab 2 times	0.57
48	1	U308A13	5	2 cap 3 times	0.60

Tab= tablet, cap= capsule, DAR No.= Drug administration registration number

Apparatus used

- A UV- Vis Spectrophotometer with low stray light (0.5 % max) and ultra-fast scanning (29000 nm/min) (Shimadzu 1900i, Shimadzu Corporation, Kyoto, Japan)
- An electronic balance (Shimadzu ATY 224) with good precision (≤ 0.1mg) and linearity (±0.2mg)

Chemicals used

- Analytical grade methylparaben (Scharlab S.L., Spain, 99-100% claimed purity into its certification)
- Methanol (assay above 99.99%) manufactured by Merck, Germany.

Standard stock solution and calibration standard preparation

Accurately weighed 100 mg of methylparaben and poured it into a 100 ml volumetric flask. Methanol was added, and the volume was made up to the mark. A 1000 ppm standard stock solution is ready. From this standard stock solution, calibration standards (0 ppm, 1 ppm, 2 ppm, 3 ppm, and 4 ppm) were prepared. Analytical wavelength (λ_{max}) was taken and found at 256.5 nm (Fig. 2).

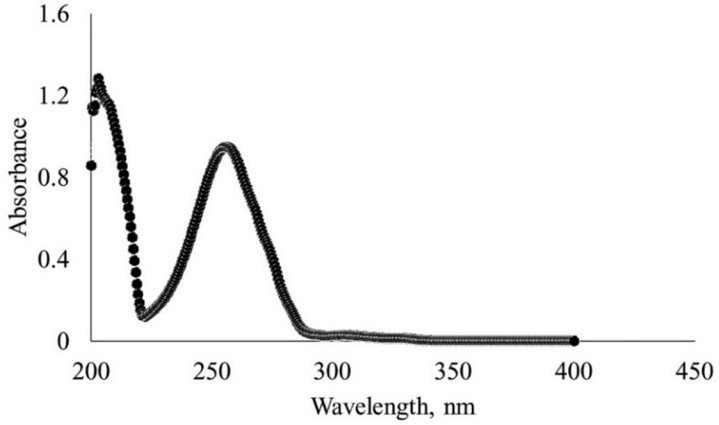

Fig. 2. Analytical wavelength (λ_{max}) determination for methylparaben.

Analysis of samples: Herbal drug samples (one piece for each solid sample and one gram for liquid sample) were macerated for two days with occasional shaking in 50 mL of methanol (Ingle *et al.* 2017, Azwanida 2015, Pandey and Tripathi *et al.* 2014, Doughari

2012). Capsule drugs were holed to escape the inner material into the solvent. The heterogeneous mixture was filtered, and the filtrate was collected. The filtrate was diluted 100 times (0.1 ml filtrate + 9.9 ml methanol). Solvent (methanol) was taken in both cells (sample cell and reference cell) of the spectrophotometer and made auto-zero. A calibration curve was obtained by replacing the solvent in the sample cell with calibration standards one after another in lower to higher concentrations. Then analyte aliquot was added to the sample cell. An instrument response was recorded. The quantity of sodium benzoate in herbal drugs was determined utilizing the following formula:

Daily Exposure (DE) = (C_{HD} x W_{HD} x E)/ 1000 mg (Islam *et al.* 2022, Alhusban and Sawsan *et al.* 2019)

Where,

C_{HD} = Concentration of analyte in mg in drug

W_{HD} = Weight of herbal drug in mg

E=Number of exposures per day.

Method validation: The validation of the utilized method was carried out based on linearity, sensitivity, precision, and accuracy as per ICH guidelines (ICH, 1995A, ICH 1996 B). Calibration and validation sets were shown (Table 2).

Table 2. Calibration and validation sets.

Calibration sets (mg/L)	Validation sets (mg/l)		
	Accuracy Study		Precision study
	Plant matrix	Diluent	
0	3	10	10
1	4	15	15
2	5	20	20
3	6	25	25
4	7	30	30

Linearity study: For the linearity study, calibration standards were added to the sample cell without changing the solvent (methanol) in the reference cell. Concentration data with respective absorbance was obtained and the data sets were transformed into a calibration curve (Fig. 3).

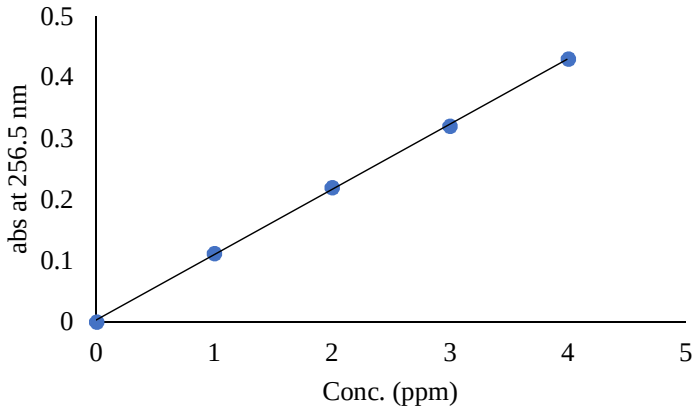

Fig. 3. Calibration plot for methylparaben.

Accuracy and precision study: The accuracy of the analytical method was studied by spiking known analytes in a diluent and analyte-free plant matrix. The sample was split into two portions, namely spiked and unspiked portions. Five different standard solutions containing analytes were added to the diluted sample solution in the spiked portion. The concentration of analytes was measured in both spiked and unspiked portions.

Recovery was calculated as,

$$\%R = \frac{C_S - C_U}{C_A} \times 100$$

Where,

R = Recovery

C_S= Analyte concentration in the spiked portion

C_U= Analyte concentration in the unspiked portion

C_A= Concentration of analyte added.

Additionally, spiking was done in diluent and preservative-free plant extract.

Method sensitivity: Method sensitivity was assessed according to ICH guidelines. The limit of detection (LOD) and limit of quantification (LOQ) were determined by the following formulas,

$$LOD = {(3.3 \times \sigma)}/{S}$$

$$LOQ = {(10 \times \sigma)}/{S}$$

Here, σ is the standard deviation of the response of the blank and S is the slope of the analytical curve.

Result and Discussion

Methylparaben is methanol soluble, used as a blank and solvent during measurement by UV Vis spectroscopy. The analytical wavelength (maximum wavelength, λ_{max}) was 256.5 nm (Table 3).

Table 3. Optical Characteristics of the method.

Parameter	Value
Analytical wavelength for methyl paraben (λ_{max}) nm	256.5
Concentration range obeys Beer's law (ppm)	10-30
Regression Equation, y=mx+c	$y = 0.106 \times x + 0.0028$
Slope	0.106
Intercept	0.0028
Coefficient of Determination, R^2	0.9881
Limit of Detection (LOD) ppm	0.19
Limit of Quantification (LOQ) ppm	0.57

Linearity test: To find the relationship between the predictor (concentration) and response (absorbance) variables, Pearson's Correlation Coefficient (PCC), r was determined. As the Cauchy–Schwarz inequality puts the obtained PCC r-value of 1 at perfect positive linear correlation, the purpose of additively is fulfilled. At a value of zero of the predictor variables (concentration), a value of 0 is obtained as the response value (absorbance) and the homogeneity of the relationship is confirmed. When the requirements of additivity and homogeneity were fulfilled, the model was said to be linear, which satisfies proportionality.

Accuracy and precision study: A recovery study was performed to verify the accuracy of the applied method. The known concentration of analyte was added to a methylparaben - free plant-matrix (diluted) and then directly into the diluent. Recovery was found within the satisfactory level (91.3-98.1%) in mixed plant matrix and (91-105.8%) in direct diluent (Andreasson, 2015) (Table 4). All of this information indicates that the detection of the analyte was unaffected by the interference of excipients present in the plant matrix (Lee *et al.* 2006).

Table 4. Recovery study.

Analyte free plant matrix		Diluent	
Spiked amount ppm	Recovery %	Spiked amount ppm	Recovery %
3	91.3	10	105.8
4	98.1	15	91.0
5	96.9	20	103.3
6	95.2	25	101.8
7	95.2	30	96.5

To assess precision, different standards (10 ppm, 15 ppm, 20 ppm, 25 ppm, and 30 ppm) were prepared and the coefficient of variation was found in the range of 0.005-0.268% (Table 5), indicating good precision.

Table 5. The precision of analytical data.

Standard ppm	Mean conc. found (ppm) n=3	Co-efficient of variation (CV)%
10	10.58	0.005
15	13.64	0.046
20	20.66	0.074
25	25.45	0.191
30	28.96	0.268

Sensitivity: Sensitivity was studied as the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ of the developed method were 0.19 ppm and 0.57 ppm, respectively, for methylparaben (Table 3).

Safety assessment of anti-diabetic herbal drugs: Methylparaben is an alkyl ester of p-hydroxybenzoic acid. The analyte is readily absorbed from the gastrointestinal tract after oral administration. The ester is hydrolyzed to its reactant molecule, para hydroxy benzoic acid. Then it is excreted through urine without accumulating in the body. However, studies showed, allergic reactions upon oral paraben exposure (Soni et al., 2002). Daily exposure to the ester is shown (Fig. 4). The safety limit per day for a 70 Kg human being is 420 mg. With this value, no instances crossed safety (Final Report on the Safety Assessment of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben, 1984).

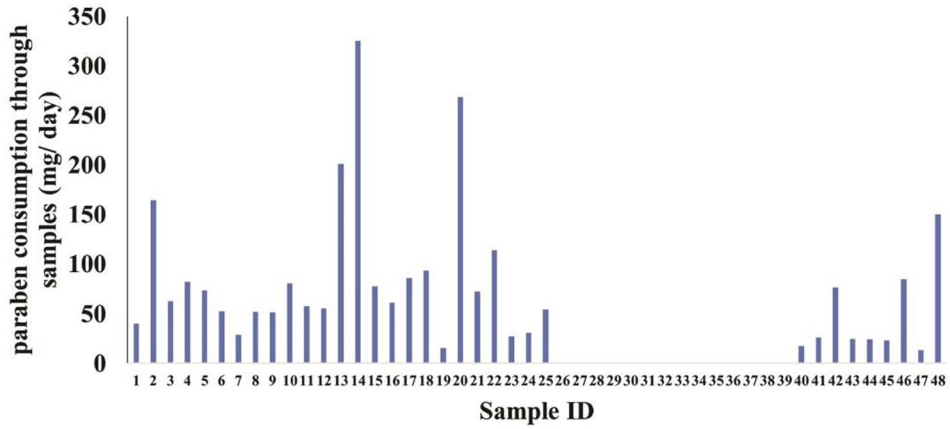

Fig. 4. Daily methylparaben consumption through anti-diabetic herbal preparations

Conclusions

As part of the safety assessment, forty-eight anti-diabetic herbal drugs from different manufacturers in Rajshahi City were screened for methylparaben using a UV-Vis spectrophotometer. Thirty-four out of forty-eight samples were found to contain the analytes but within a safe limit. As the method was found to be faster, more precise, and cheaper, the analytical procedure can be used for routine analysis of parabens in herbal drugs and other preservatives in the manufacturers' labs. More assessment of the preservatives needs to be carried out on the herbal drugs of other parts of Bangladesh to obtain the scenario of preservative content in the country and its subsequent safety.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

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COMMUNITY STRUCTURE, ECOLOGY, AND CONSERVATION ISSUES OF AVIFAUNA OF TWO HUMAN-DOMINATED LANDSCAPES IN FARIDPUR DISTRICT, BANGLADESH DURING COVID-19 PANDEMIC

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Abstract

A study was conducted on community structure, ecology, and conservation issues of the avifauna of two human-dominated landscapes in a lower Ganges Madhukhali Upazila, under the Faridpur district during the COVID-19 pandemic period. Direct field observations were made from April 2020 to March 2021. In total, 109 species of birds belong to 18 orders, and 49 families were observed, with a total count of 5,453 individuals. Resident bird species (93 species, 85.32%) dominant in the study area, whereas the migratory species were only 16 (14.68%). The highest number of bird species (93 species, 85.32%) with the highest number of individuals (n=3171, 58.15%) was observed in Beleswar (rural site) area. Shannon diversity index value (H=3.89, Ds= 0.9724) was higher in the rural site than Madhukhali municipal area (urban site). In the winter season, the highest number of bird species (95 species, 87.15%) with the highest number of individuals (n=2303, 42.23%) was observed. Among micro-habitats, trees, particularly the native tree species, were the preferable sites for bird nesting, foraging, and roosting. Among birds, *Acridotheres tristis* was the most abundant species (n=316, 5.79%) in the study area, and an uneven distribution of species in the community structure was observed. The abundance of birds shows that 59 (54.12%) species were very common, 8 (7.33%) common, 9 (8.25%) uncommon, and 33 (30.27%) rare. Among the bird species, *Ichthyophaga ichthyaetus* was categorised as Near Threatened (NT), and the rest are Least Concerned (IUCN Bangladesh 2015). Illegal hunting of birds, especially waterbirds, is the major threat in the rural site. Preparing a proper management plan based on the baseline data is essential for protecting of avian diversity in the study area.

Key words: Bird, Habitat utilization, Urban area, Rural area, Illegal hunting, Conservation.

Introduction

Birds are one of the most glorious and common components of the ecosystem that act as a bio-indicator in response to environmental pollution (Sekercioglu, 2006, Slabbekoorn and Ripmeester, 2008; Mistry *et al.*, 2008). Avifaunal diversity gives rise to the primary

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part of the natural environment, and they play a key role as agents of predators, scavengers, pollinators, seed dispersers, and ecosystem engineers in the food chain (Sekercioglu *et al.*, 2004). Birds act as pest control agents in agricultural sectors (Whelan *et al.*, 2008).

Bangladesh is a south Asian country, and the geographical location of this country expresses the transitional zone as it is situated at the junction of two enriched bio-geographic realms named Indo-Himalayas and Indo-China sub-regions of the oriental region (IUCN Bangladesh, 2015; Khan, 2018). This country is located in the Ganges-Brahmaputra delta which, makes it rich in natural resources and amazing wildlife fauna despite being the eighth-most populous country in the world (Khan, 2018). Among the faunal group in Bangladesh, birds play an important role in the ecological, environmental, and cultural sectors. Though Bangladesh is a small country, it is blessed with more than 700 bird species which is 7.64% of the total global avifauna and almost the same as the avifaunal diversity of Europe. Among them, 380 resident birds are found in our country, 209 winter visitors, 11 summer visitors, and the rest are vagrant species (Khan, 2018). Unfortunately, the populations of many bird species are declining gradually due to the increase in threats, including habitat loss, rapid urbanization, excessive use of herbicides, insecticides, and pesticides with hunting, poisoning, and trapping (Sarker and Sarker, 1988; IUCN Bangladesh, 2015; Barkat *et al.*, 2021). In Bangladesh, from 2010 to 2020 (until June), 70% of illegal bird hunting is typically conducted in wetlands areas (*Haor, Baor,* and *Beel*) and hunting mostly promoted by trapping and netting, particularly in the winter season (Datta, 2021). To monitor the threats, as well as spatial and temporal frame of bird diversity, baseline studies are needed to be conducted.

Outside protected areas of Bangladesh harbor a number of bird species, but pertinent data on those birds are absent (Mandal *et al.*, 2021). At the same time conservation initiative is also missing in that areas, and the human population is increasing. Anthropogenic activities of human in the natural habitat of birds are declining rapidly, and a number of habitat-specialized species are vanishing very rapidly (Shome *et al.*, 2022a). Local people are also unaware of the conservation of bird species, and the illegal trade of birds and bird hunting is one of the major obstacles to conserving birds, especially in rural areas (Shome and Jaman, 2021). Besides, misconceptions and superstations are present among local people not only about birds but also about other wildlife (Jaman *et al.*, 2020; Shome and Jaman, 2021).

The lower Ganges portion of Bangladesh is enriched with avian diversity because of the presence of different types of natural habitats (e.g., wetlands, forests, green patches, sandbars, etc.). Still proper data on those birds are absent, and a small amount of research work was done in that region (Shome *et al.*, 2022b; Mandal *et al.*, 2021, Shome *et al.*,

2020). The study aims to determine the avifaunal composition within the community, seasonality, habitat utilization, threats, and conservation issues of Madhukhali Upazila, Faridpur. Besides, this research will help constitute a conservation plan to protect birds and other wildlife fauna in the study area.

Materials and Methods

Study area: The study was conducted in Madhukhali Upazila (230.2 km^2) under Faridpur district (23°32'32.61"N, 89°37'51.21"E) as a part of ecological research (Fig. 1). The study was carried out from April 2020 to March 2021 and divided into three time period like summer (March-June), rainy (July-October), and winter (November-February) (Shome *et al.*, 2021b). Two study sites were selected for a better survey, a semi-urban area (Madhukhali municipality area-MMA) and a rural area (Beleswar-BW). Railway stations, playgrounds, markets, highways, small ponds, canals, rivers, homestead forests, grasslands, etc., possess MAA. In contrast, BW includes homestead forests, Bamboo patches, agricultural lands, grasslands, *beels*, ponds, ditches, canals, rivers (Kumar), etc.

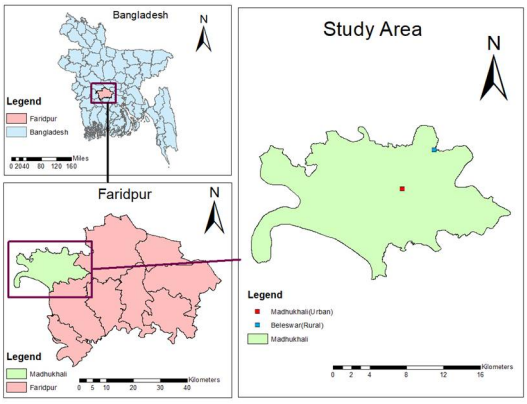

Fig. 1. Map of the study area.

Data collection: The sample was collected through direct field observations from March 2020 to February 2021 using transect line methods (Gaston, 1975). Data collection has been done at least 3 days a month in rural and urban areas. To correlate with birds' peak activity, the survey was conducted in the early morning (06:30 AM to 10:30 AM) and the afternoon (03:30 PM to 07:00 PM). To get information on nocturnal birds, sometimes survey was conducted at night. At least 9 hours of effort per day was spent to complete a field visit. The species sampling was well-adequate according to richness estimators. The calls or songs of the birds were recorded by smartphone (Realme X2) to identify those

normally hidden or camouflaged in the bushes, jungles, and branches of trees. To observe birds at night, torch light was used while performing each survey. The photograph was taken by a Canon 80D DSLR Camera with a 55-250 mm VR lens for proper identification. We also followed popular field guides for identifying bird species (Khan, 2015).

Data analysis: Data has been analyzed using PAST (version 4.07), MS Excel, R 4.0.5 (R Core Team 2020), and the ggplot2 package spreadsheets. The species accumulation curve was developed using Magurran's (2013) rarefaction approach to confirm sample completeness according to first and second-order Jackknife, Bootstrap, and Chao richness estimators, which were used to estimate the total number of species in the study area. This was done using the 'specpool' function from Vegan Package (Oksanen *et al.*, 2019). Using the mean of these four indices, the estimated number of species (x) was calculated following Fils *et al.*, (2014). Following the formula sampling completeness was calculated as: Sampling completeness = the Observed number of species (n)/ Estimated number of species (x) × 100. The relative abundance of bird species was measured by following the formula -

$$\text{Relative abundance} = \frac{\text{Number of individuals of a species}}{\text{Total number of individuals of all species}} \times 100$$

Based on the total occurrences per survey effort, Khan (2015) was applied to assess the observation status as very common (VC), 80–100%, common (C), 50–79%, uncommon (UC), 20–49%, and few (F), 10–19%. A rank abundance plot was constructed following Whittaker (1965) to describe dominance patterns. Shannon-Wiener (Shannon and Wiener, 1949) and Simpson's indices (Simpson, 1949) were used to compute the diversity indices evenness was calculated, by dividing the Shannon-Wiener index value by the natural log of species richness.

Result and Discussion

Species composition and abundance: A total of 109 species of birds were observed, and 5453 individuals were counted under 18 orders and 49 families (Table 1). The highest number of bird species (45 species, 41.28%) and individuals (n=3093, 56.72%) were under the order Passeriformes. Non-passerine birds (54 species, 58.72%) were dominant over passerine birds in the study year. Species diversity was the highest (7 species, 6.42%) and individuals (n=509, 9.33%) under the family Ardeidae. Among the observed bird diversity, 93 (85.32%) species were resident, whereas 16 (14.68%) species were migratory. Among the migratory bird species, 2 species were summer migratory (*Cuculus micropterus and Merops philippinus*).

Table 1. List of observed avifauna in the study area. (SN- Scientific name, EN- English Name, SI- Site, R- Rural area, U- Urban area, B- All areas; RA- Relative abundance; OS- Observation Status; VC- Very Common, C - Common, UC- Uncommon, Few- F; Se- Season W-Winter, S- Summer and R- Rainy, A- Year round).

Sl.No.	EN	Sl.No.	RA	Se	OS
Accipiter badius	Shikra	R	0.02	R	R
Acridotheres fuscus	Jungle Myna	B	3.21	A	VC
Acridotheres tristis	Common Myna	B	5.79	A	VC
Acrocephalus agricola	Paddy-field Warbler	R	0.11	A	C
Actitis hypoleucos	Common Sandpiper	U	0.06	W	R
Aegithina tiphia	Common Iora	B	0.26	A	VC
Alcedo atthis	Common Kingfisher	B	1.60	A	VC
Amaurornis phoenicurus	White-breasted Waterhen	B	0.90	A	VC
Anastomus oscitans	Asian Openbill	B	1.27	A	VC
Apus nipalensis	House Swift	B	1.87	A	VC
Ardea alba	Great Egret	B	0.44	A	VC
Ardea intermedia	Intermediate Egret	B	0.22	A	VC
Ardeola grayii	Indian Pond Heron	B	3.81	A	VC
Artamus fuscus	Ashy Woodswallow	U	0.04	W	R
Athene brama	Spotted Owlet	B	0.28	A	VC
Bubulcus ibis	Cattle Egret	B	3.10	A	VC
Cacomantis merulinus	Plaintive Cuckoo	U	0.02	S	R
Centropus bengalensis	Lesser Coucal	U	0.04	W	R
Centropus sinensis	Greater Coucal	B	1.47	A	VC
Ceryle rudis	Pied Kingfisher	B	0.66	A	VC
Charadrius dubius	Little Ringed Plover	R	0.09	W	R
Cisticola juncidis	Zitting Cisticola	U	0.11	S	UC
Columba livia	Rock Dove	B	2.48	A	VC
Copsychus saularis	Oriental Magpie-robin	B	2.62	A	VC
Coracias benghalensis	Indian Roller	B	0.31	A	VC
Corvus levaillantii	Jungle Crow	B	1.98	A	VC
Corvus splendens	House Crow	B	4.88	A	VC
Cuculus micropterus	Indian Cuckoo	U	0.04	R	R
Cypsiurus balasiensis	Asian Palm Swift	B	3.21	A	VC
Dendrocitta vagabunda	Rufous Treepie	B	0.42	A	VC
Dendrocopos macei	Fulvous-breasted Woodpecker	B	0.40	A	VC
Dendrocygna bicolour	Fulvous Whistling Duck	U	0.04	W	R
Dendrocygna javanica	Lesser Whistling Duck	B	2.49	A	VC
Dicrurus aeneus	Bronzed Drongo	B	0.26	A	VC

(Table 1 contd.)

Table 1 contd.

Sl.No.	EN	Sl.No.	RA	Se	OS
Dicrurus leucophaeus	Ashy Drongo	B	0.31	A	VC
Dicrurus macrocercus	Black Drongo	B	3.52	A	VC
Dinopium benghalense	Black-rumped Flameback	B	1.82	A	VC
Egretta garzetta	Little Egret	B	1.63	A	VC
Elanus caeruleus	Black-winged Kite	B	0.33	A	VC
Eudynamys scolopaceus	Western Koel	B	0.59	A	VC
Falco tinnunculus	Common Kestrel	U	0.04	R	R
Ficedula albicilla	Taiga Flycatcher	U	0.04	W	R
Gallinago gallinago	Common Snipe	R	0.04	W	R
Haliastur indus	Brahminy Kite	B	0.24	A	VC
Hierococcyx varius	Common Hawk-Cuckoo	B	0.22	A	VC
Hirundo rustica	Barn Swallow	B	2.60	A	VC
Hydrophasianus chirurgus	Pheasant-tailed Jacana	B	0.70	W	UC
Hypothymis azurea	Black-naped Monarch	R	0.04	R	R
Ichthyophaga ichthyaetus	Grey-headed Fish-eagle	R	0.02	W	R
Ixobrychus cinnamomeus	Cinnamon Bittern	R	0.11	R,W	UC
Ketupa zeylonensis	Brown Fish Owl	B	0.15	R,W	C
Lanius cristatus	Brown Shrike	U	0.04	W	R
Lanius schach	Long-tailed Shrike	B	1.03	A	VC
Lanius tephronotus	Grey-backed Shrike	R	0.04	W	R
Larus brunnicephalus	Brown-headed Gull	R	0.04	W	R
Lonchura atricapilla	Chestnut Munia	U	0.04	S	R
Lonchura malabarica	White-throated Munia	U	0.09	S	R
Lonchura malacca	Tricoloured Munia	R	0.02	W	R
Lonchura punctulata	Scaly-breasted Munia	B	0.64	A	VC
Lonchura striata	White-rumped Munia	R	0.22	S,W	UC
Malacocincla abbotti	Abbott's Babbler	B	0.18	S,W	C
Mareca strepera	Gadwall	R	0.04	W	R
Merops leschenaulti	Chestnut-headed Bee-eater	R	0.07	R,W	UC
Merops orientalis	Asian Green Bee-eater	B	0.73	A	VC
Merops philippinus	Blue-tailed Bee-eater	R	0.07	S	R
Metopidius indicus	Bronze-winged Jacana	B	0.88	A	VC
Microcarbo niger	Little Cormorant	B	1.74	A	VC
Milvus migrans	Black Kite	B	0.06	S	UC
Motacilla citreola	Citrine Wagtail	U	0.26	W	UC
Motacilla madaraspatensis	White-browed Wagtail	R	0.04	R	R
Nectarinia asiatica	Purple Sunbird	B	0.24	A	VC

(Table 1 contd.)

Table 1 contd.

Sl.No.	EN	Sl.No.	RA	Se	OS
Nectarinia zeylonica	Purple-rumped Sunbird	U	0.06	W	R
Nettapus coromandelianus	Cotton Pygmy-goose	R	0.06	W	R
Ninox scutulata	Brown Boobook	B	0.17	A	VC
Nycticorax nycticorax	Black-crowned Night Heron	R	0.02	S	R
Oriolus chinensis	Black-naped Oriole	R	0.07	A	C
Oriolus xanthornus	Black-hooded Oriole	B	1.19	A	C
Orthotomus sutorius	Common Tailorbird	B	1.08	A	VC
Passer domesticus	House Sparrow	B	5.41	A	VC
Pelargopsis capensis	Stork-billed Kingfisher	B	0.29	A	VC
Pericrocotus cinnamomeus	Small Minivet	R	0.26	R,W	UC
Pernis ptilorhyncus	Oriental Honey Buzzard	R	0.02	R	R
Phalacrocorax carbo	Great Cormorant	R	0.26	W	R
Ploceus philippinus	Baya Weaver	B	2.92	A	VC
Prinia gracilis	Graceful Prinia	R	0.07	W	R
Prinia inornata	Plain Prinia	B	0.31	A	VC
Psilopogon asiaticus	Blue-throated Barbet	B	1.25	A	VC
Psilopogon haemacephala	Coppersmith Barbet	B	0.40	A	VC
Psilopogon lineatus	Lineated Barbet	B	0.88	A	VC
Psittacula krameri	Rose-ringed Parakeet	B	0.86	A	VC
Pycnonotus cafer	Red-vented Bulbul	B	5.24	A	VC
Spilopelia chinensis	Eastern Spotted Dove	B	2.82	A	VC
Spilornis cheela	Crested Serpent Eagle	R	0.04	W	R
Streptopelia decaocto	Eurasian Collared Dove	U	0.09	R	R
Streptopelia tranquebarica	Red Turtle Dove	R	0.22	W	R
Sturnus contra	Asian Pied Starling	B	5.28	A	VC
Sturnus malabaricus	Chestnut-tailed Starling	B	1.65	A	VC
Tachybaptus ruficollis	Little Grebe	R	0.18	A	C
Tephrodornis pondicerianus	Common Woodshrike	R	0.11	W	R
Terpsiphone paradisi	Indian Paradiseflycatcher	B	0.15	R,W	C
Treron phoenicopterus	Yellow Footed Green Pigeon	R	0.20	R,W	UC
Turdoides striata	Jungle Babbler	B	3.30	A	VC
Turnix suscitator	Barred Buttonquail	B	0.11	A	C
Tyto alba	Common Barn Owl	B	0.22	A	VC
Upupa epops	Common Hoopoe	B	0.29	A	VC
Vanellus cinereus	Grey-headed Lapwing	U	0.13	W	R
Vanellus indicus	Red-wattled Lapwing	B	0.48	A	VC
Zoothera citrina	Orange-headed Thrush	B	0.26	A	VC
Zosterops palpebrosus	Oriental White-eye	B	0.35	A	VC

Table 2. Bird diversity in different areas in Bangladesh.

Location	Number of Individuals	Reference
Sreepur Upazila, Magura	84	Mandal *et al.*, 2021
Magura Sadar Upazila	140	Shome *et al.*, 2020
Kahimpur, Gazipur	72	Islam *et al.*, 2018
Keshabpur, Jessore	105	Jaman *et al.*, 2015
Ruhitpur Union, Keraniganj, Dhaka	55	Jaman *et al.*, 2014
Pashukhali and Gajdhar village, Netrokona	101	Khan *et al.*, 2015
Dharala and Brahmaputra rivers in Kurigram	105	Khan and Nahar, 2015
Padma River charland (Godagari to Charghat), Rajshahi	141	Reza *et al.*, 2014
Char-kishoreganj, Munshiganj	58	Chowdhury *et al.*, 2007
Shoipara Beel of Mohanpur Upazilla, Rajshahi	96	Hasan *et al.*, 2017
Chapadal, Shree Rampur *beel*, Mithapur, Paharpur, Jogodishpur, Kastogaree *beel* and Asranga of Joypurhat	89	Amin *et al.*, 2020
Atrai, Raninagar and Naogan Sadar, Naogaon	105	Amin and Hasan, 2019
Kashipur Union, Barishal	141	Shome and Jaman, 2021
Jamalpur Sadar Upazila	136	Shome *et al.*, 2021a
Sandwip Island, Chattogram	119	Jaman *et al.*, 2022
Dhaka megacity	161	Jaman *et al.*, 2021
Faridpur Sadar Upazila	168	Shome *et al.*, 2022a
Mymenshing City Corporetion	180	Shome *et al.*, 2022b
Madhukhali Upazila, Faridpur	109	Present study

This result indicates that the study area is the home of 16% (109) of the total bird species found in Bangladesh (Khan, 2018). This result reveals that species richness is higher in this study site then in other sites (Table 2).

Area-wise bird diversity: Among the two study sites, the Beleswar (rural site) area showed the highest diversity of bird species (93 species, 85.32%) with a higher number of individuals (n=3171, 58.15%) than the Madhukhali Municipal Area (Urban site) (Fig. 2). Richness estimators predicted a range of 109-120 species, which is relatively close to the 109 species identified during the field visit. This is confirmed by 95.33% of the samples being completed, indicating that the study area was sampled adequately. The diversity indices also show a higher value for the Beleswar (rural site) (H=3.89, Ds= 0.9724). Species were more evenly distributed in Beleswar (rural site) (Table). Between the two study sites, variation of species richness ($\chi2 = 0.69143$, df = 1, p-value =0.4057) has not differed significantly but abundance ($\chi2 = 144.93$, df = 1, p-value <0.0001)

differed significantly. The number of species was almost similar in the two sites, the urbanization effects were not so prominent in the study area as well as the habitat structure was almost identical; thus, their evenness was also; In rural sites, disturbance to birds is lower than in the urban site, and more suitable habitats for birds are present in rural site. Thus, the number of bird species is higher in rural areas (Tryjanowski *et al.*, 2015; Shome *et al.* 2020).

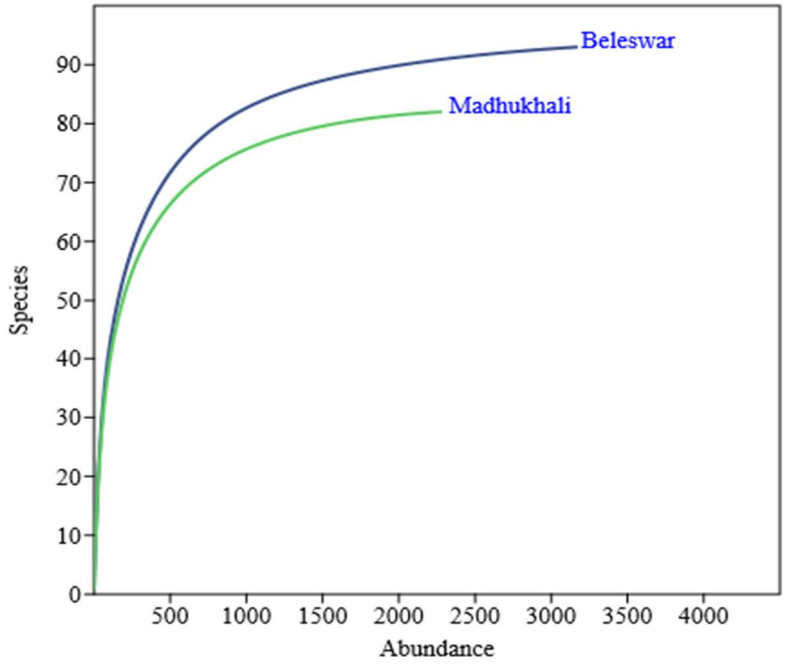

Fig. 2. Rarefaction curves in two study sites.

Seasonal variation: In the winter, the highest number of bird species (95 species, 87.15%) was observed, with the highest number of counted bird individuals (n=2303, 42.23%). In both study sites, the highest number of bird species and individuals were observed in winter. Diversity indices also show the highest (H=3.94, Ds= 0.974) value for the winter season. Species were more evenly distributed during the rainy season (E=0.5929). During the winter season, due to the presence of migratory bird species, the number of birds was higher in that period (Sandoval, 2019; Gomes *et al.*, 2017; Aung *et al.*, 2020; Shome *et al.*, 2022a).

Table 3. Species richness, abundance and diversity indices in different areas, seasons and habitats. (Species richness (S), Abundance (A), Simpson's Index (D_s), Shannon-Weiner Index (H), Evenness (E) in different areas, seasons, and habitats).

	Categories	S	A	Ds	H	E
Area	Beleswar (Rural)	**93**	**3171**	**0.9724**	**3.89**	**0.5257**
	Madhukhali (Urban)	82	2282	0.9672	3.756	0.5219
Seasonal variation	Rainy	77	1495	0.9712	3.821	**0.5929**
	Summer	73	1655	0.9624	3.628	0.5157
	Winter	**95**	**2303**	**0.974**	**3.94**	0.5411
Seasonal variationin Beleswar (Rural)	Rainy	74	874	0.9716	3.831	**0.623**
	Summer	69	953	0.9637	3.643	0.5538
	Winter	**86**	**1344**	**0.975**	**3.946**	0.6018
Seasonal variationin Madhukhali (Urban)	Rainy	64	621	0.9684	3.735	**0.6548**
	Summer	59	702	0.9575	3.494	0.5578
	Winter	**72**	**959**	**0.9683**	**3.742**	0.5856
Macro- habitat	Aquatic	21	722	0.8404	2.187	0.4241
	Arboreal	**65**	**3497**	**0.9523**	**3.378**	**0.451**
	Terrestrial	23	1234	0.8721	2.323	0.4437
Micro-habitat	Bushy area	1	14	0	0	1
	Fallow land	2	140	0.06937	0.1576	0.5854
	Floating plant	4	300	0.4788	0.8921	0.6101
	Grass land	12	309	0.672	1.638	0.4288
	Mud flat	6	33	0.7595	1.612	**0.8356**
	Tree	**65**	**3497**	**0.9523**	**3.378**	0.451
	Urban Settlements	6	754	0.7447	1.443	0.7058
	Water body	11	389	0.7609	1.674	0.4847
	Waste disposal site	2	17	0.3088	0.4954	0.8206

Habitat uses: Among the observed bird species, the highest number of bird species (65 species, 59.63%) and individuals (n=3497, 64.12%) prefer arboreal types of habitats as their macro habitat. The tree was the preferable microhabitat with the highest number of birds, and diversity indices also shows the highest value for trees (H=3.378, Ds= 0.9523). A good number of native tree species are present in the study area, which provides the proper a wide array of opportunities for livelihood to different groups of bird species in the study area; thus, number of birds species is higher in trees (Fontana *et al.*, 2011; Kaushik *et al.*, 2022).

Bird response during COVID-19 lockdown situation: The movement of bird species in the study area, especially in the urban area, was more frequent than at other times. The urban site was busy with traffic on the Dhaka-Jashore highway, industry, crowds of local and people, pollution. But those all were absent during the lockdown situation, even the movement of local people also. Movement of *Athene brama, Cacomantis merulinus, Centropus bengalensis, Centropus sinensis, Cisticola juncidis, Coracias benghalensis, Cuculus micropterus, Dendrocitta vagabunda, Dendrocygna javanica, Hydrophasianus chirurgus, Zosterops palpebrosus, Upupa epops, Terpsiphone paradise, Psittacula krameri, Psilopogon asiaticus, Psilopogon haemacephala, Ninox scutulata, Lonchura striata, Amaurornis phoenicurus* was frequent than any other time in that area. Throughout the world at that time, the freely movement of wildlife was also observed in the study site for less anthropogenic activities (Shome *et al.*, 2021a).

Relative abundances, observation status, threats and conservation issues: Among the observed 109 species of bird species, *Acridotheres tristis* was the most abundant bird species (n=316, 5.79%) in the study area. The other most abundant bird species were *Passer domesticus, Sturnus contra, Pycnonotus cafer, Corvus splendens, Ardeola grayii, Dicrurus macrocercus, Turdoides striata, Acridotheres fuscus,* and Cypsiurus balasiensis. The ten most dominant species constituted 43.40% of total individuals, whereas the 40 least dominant species held only 2.24%. This signifies an uneven distribution of species in the community, which is explained in the rank abundance plot (Fig. 5A). Between the two study sites, the urban site (Madhukhali) signifies the most uneven distribution of species compared to the rural site (Beleswar). In urban sites, the ten most dominant species constituted 48.22% of total individuals, whereas in rural sites comprised 43.66% (Fig. 5B). This result also indicates that in urban site distribution of birds is uneven than the rural area and the population of scavengers birds (e.g., *Passer domesticus, Corvus splendens, Acridotheres tristis, Sturnus contra* and *Acridotheres fuscus*) are higher than rural area (Jaman *et al.*, 2021; Jessop *et al.*, 2012; Rebolo-Ifrán *et al.*, 2017; Nepali *et al.*, 2021).

Observation status shows that among the observed 109 bird species, 59 bird species were very common (54.12%), 8 common (7.33%), 9 uncommon (8.25%), and 33 species were few (30.27%).

Among the bird species, *Ichthyophaga ichthyaetus* was categorised as Near Threatened (NT) according to IUCN Bangladesh (2015), and the rest are Least Concern (LC). Illegal bird hunting, especially water birds, is the major threat to bird species in the study area, especially in rural areas. Birds like heron, egret, bittern, wild duck and water fowl are

hunted by local people for their feeding (Shome *et al.*, 2022b). Trade of wild bird species is absent. At present urbanisation and habitat fragmentation are major threats to wild bird species in urban sites.

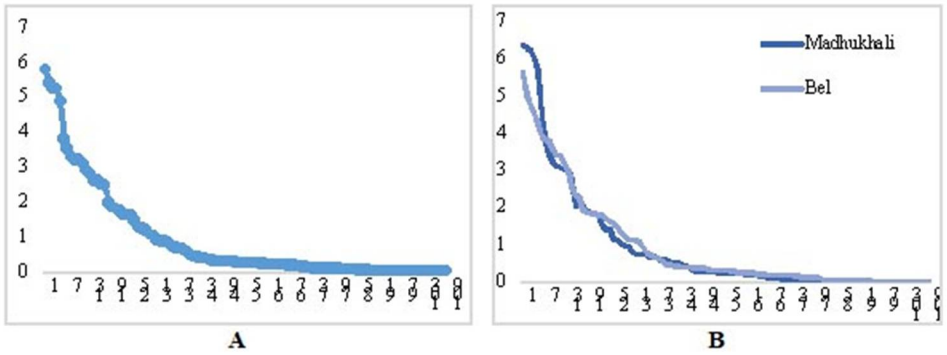

Fig. 5. Rank abundance plot for species recorded from the (A) total study area and (B) in two study sites. The y-axis shows the relative abundance, and the x-axis ranks the species in order of their abundance from the highest to the lowest.

Conclusion

Currently, the conservation of bird species in urban and rural areas of Bangladesh is important, for this, baseline information is essential. This research provides the actual scenario of status, community structure, seasonal variation, threats, and conservation issues of the study area. Although, the rich diversity of birds has indicated the suitability of the habitats for different groups of bird species, they are facing an existential crisis. Details research work is essential to know about the ecology, threats, and conservation issues. In addition to this awareness, creation is essential among local people with preparing and implementing the proper management plan and regular monitoring in the remote areas.

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MONITORING AND ECO-FRIENDLY MANAGEMENT OF CUCURBIT FRUIT FLY, *BACTROCERA CUCURBITAE* (COQUILLETT) ON BITTER GOURD

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Abstract

An experiment was conducted to assess the adequacy of distinctive management practices, viz. yellow sticky trap set at 50, 100, and 150 cm height, fruit fly bait with a mashed sweet gourd (MSG), bagging, sanitation, and Ecomec 1.8 EC at the rate of 1mL per liter of water sprayed at 7 days interval. The experiment was carried out with a randomized complete block design (RCBD) with 8 treatments and 3 replications. All the treatments significantly differed from the control. The total highest number and weight of healthy fruits were recorded in bagging (41.33 and 6.08 kg) but the lowest in control (19.00 and 2.22 kg, respectively). No infested fruits were found in bagging, meanwhile, the highest number and weight of infested fruits were counted in control (13.00 and 1.27 kg). In addition, bagging appeared to have no percent of infestation by number and weight. The highest percent of fruit infestation was found in control (40.75% and 36.48%) treatments based on number and weight. No wet reduction was found in the bagging treatment and the highest percent weight reduction per fruit was recorded in Ecomec 1.8 EC (30.17%). The highest number of larval densities were found in fruit fly bait with MSG (15.83) and the lowest in a yellow sticky trap set at 150 cm height (6.7). The highest number of fruit flies in a yellow sticky trap set at 50 cm height was captured on 21 March 2021 (4.33) and the lowest was on 29 April 2021 (1.33). The highest number of fruit flies were captured in a yellow sticky trap set at 50 cm height (43/trap) and the lowest in a bait trap with MSG (9.70/bait). The highest yield and increase of yield over control were found in bagging (12.16 tons/ha and 176.10%).

Key words: Bagging, Cucurbit fruit fly, Fruit fly bait, Yellow sticky trap, Sanitation, and Ecomec 1.8 EC.

Introduction

The cucurbitaceous vegetables are one of the largest and major groups in vegetable kingdom with their wide adaptation from arid to humid tropic environments (Nasiruddin *et al.*, 2004). Bitter gourd (*Momordica charantia*) is one of the most important cucurbitaceous vegetables in Bangladesh for its excellent market value which encouraged

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the farmers to cultivate on a large scale, while a total production of 54443 metric tons from 26491 acres of land (BBS 2020). The vegetable production in Bangladesh is very low in summer, while the major vegetables grown are cucurbits this season. Therefore, cucurbitaceous vegetables play an important role in supplementing their shortage during the lag period. But the production of bitter gourd is hindered due to several factors like disease and insect pests. The cucurbit fruit fly, *Bactrocera cucurbitae* (Coquillett), is a major pest causing yield loss in cucurbits, and infests about 15 kinds of cucurbit vegetables (Rakshit *et al.*, 2011).

It is reported that *B. cucurbitae* (Coquillett) is the most destructive pest causing yield loss ranging from 30-100% (Dhillon *et al.*, 2005) depending upon cucurbit species and the season. About 41-95% of fruit infestation by cucurbit fruit flies in bitter gourd crops has been recorded (Sapkota *et al.*, 2010). Hollingsworth *et al.* (1997) stated that cucurbit fruit fly has been reported to infest 95% of bitter gourd fruits in Papua New Guinea, 90% of snake gourd, and 60 to 87% of pumpkin fruits in the Solomon Islands. In another study, Singh *et al.* (2006) reported 31.27% damage on the bitter gourd and 28.55% on watermelon in India. The damage starts when the female fruit fly punctures the fruit with its long and sharp ovipositor. After egg hatching, the maggots feed inside the fruit (Nasiruddin *et al*., 2004; Dhillon *et al.*, 2005) and make fruits tunnels. The infested fruits become rotten, dry up and finally shed up prematurely. If not rotten, it becomes deformed and market value is severely reduced. The larvae of the pest remain inside the infested fruits, and the adults are free-living. They visit fruits only at the time of oviposition and leave immediately after egg deposition. So, the control of the pest can hardly be assured. Although several management options such as pheromone trap, different plant extracts, neem products, bagging of fruits, field sanitation, food baits, and a spray of chemical insecticides (Pawar *et al.*, 1991; Neupane, 2000; Satpathy and Rai, 2002; Dhillon *et al*., 2005; Jacob *et al.*, 2007; Mukherjee *et al.*, 2007; Islam *et al.*, 2015; Bachchu *et al.*, 2017; Siddique *et al.*, 2018, 2019) have been used for the management of cucurbit fruit fly, some of them either fail to control the pest and are uneconomic and hazardous to non-target organisms and the environment (Singh and Singh, 1998; Neupane, 2000, Dhillon *et al.*, 2005).

At present, farmers in Bangladesh solely rely on the use of toxic insecticides to control the pest in bitter gourd. Farmers spend about 25% of the cultivation cost in bitter gourd production in some areas only to buy toxic pesticides (Nasiruddin *et al.*, 2004). Moreover, repeated and long time uses of toxic insecticides have serious drawbacks such as pesticide resistance, toxic residues, increasing application costs, environmental pollution, and health hazards to human beings and domestic animals (Ahmed *et al.*, 1981;

Khanam *et al*., 1990). Therefore, it is desirable to explore alternative methods to control this pest. As a result of the recent efforts to reduce the application of harmful insecticides in vegetables, the trend has now shifted towards integrated pest management (IPM) for the control of tephritid fruit flies (Klungness *et al.,* 2005). However, information on the monitoring of insect pest species and its population is needed as a first step to address the integrated management of pests. Accurate methods for fruit fly population surveys are a prerequisite for effective decision-making in area-wide control programs aimed at pest suppression. A fruit fly species may have different preferences for trap color. The oriental fruit fly is most attracted to green, yellow, and orange colors in a laboratory experiment (Wu *et al.*, 2015).

Yellow sticky trap for field pest monitoring and control is, however, a recent approach, and it has drawn the special attention of researchers worldwide. Besides that, trap height from the ground can also affect the preference of particular species. For example, *Rhagotetis pomonella* prefers traps placed approximately 2.5m above the ground within the apple tree canopy (Reissig, 1975; Drummond *et al.*, 1984)

Islam *et al.* (2015) studied the evaluation of different management practices against cucurbit fruit fly (*B. cucurbitae* coquillett) in bitter gourd. They found that bagging of fruits showed the lowest percent infestation by number (19.49%, 7.48%, and 23.15%) and weight (11.79%, 7.18%, and 11.90%) at early mid and late fruiting stages, respectively, followed by pheromone trap treatment. To monitor the fruit fly, population pheromone trapping has been successfully used in different countries (Gillani *et al*., 2002), but, the lack of literate in regards monitoring and mass trapping of *B. cucurbitae* with yellow sticky traps. Despite this, a lot of knowledge is still lacking, and it is indispensable to understand those pests that this knowledge gap is filled. Considering the hazardous impact of chemicals on non-target organisms and the environment, it is urgent to develop a safer, cheaper, and eco-friendly management tool against the cucurbit fruit fly in bitter gourd. Therefore, the present study was undertaken for field monitoring of fruit flies by yellow sticky traps set at different heights and to investigate the effectiveness of different IPM tools for and eco-friendly management of cucurbit fruit flies on bitter gourd fields.

Materials and Methods

The present study was conducted to evaluate the monitoring and eco-friendly management of the cucurbit fruit fly, *B. cucurbitae*, on bitter gourd in the central research farm and the laboratory of Entomology Department, Hajee Mohammad Danesh Science

and Technology University (HSTU), Dinajpur during the period of October 2020 to May 2021.

Selection of treatments: Eight treatments (T), including an untreated control, were selected to monitor and manage the fruit fly infestation in bitter gourd. The treatments were T_1: Yellow sticky trap set at 50 cm height, T_2: Yellow sticky trap set at 100 cm height, T_3: Yellow sticky trap set at 150 cm height, T_4: Fruit fly bait with a mashed sweet gourd (MSG), T_5: Bagging, T_6: Sanitation, T_7: Ecomec 1.8 EC@1ml/L of water sprayed at 7 days interval, T_8: Control.

Land preparation and design of the field experiment: The tentative field was equipped with deep ploughing and harrowing followed by laddering for proper level. During land preparation, all weeds and stubbles were removed from the field. Finally, the unit plots were prepared as 10 cm raised beds and applied basal doses of Urea 1 kg, TSP 1 kg, MoP 1 kg, and cow dung 5 kg. As recommended by Rashid (1993), during land preparation, potash and other micronutrients were applied. The experiment was conducted in a randomized complete block design (RCBD) with 8 treatments and 3 replications. The whole experimental land was divided into 24 equal plots, and the size of each plot was 2.5 m × 2.0 m with an inter-plot distance of 0.50 m. Therefore, the whole experimental field was 20.0 m x 10.5 m, which was divided into 3 equal blocks. In the center of each plot, about 30 cm x 30 cm x 20 cm pits were dug for sowing seeds of bitter gourd. Each pit was considered as one replication.

Seed source, sowing, and intercultural operation: The seeds of bitter gourd (Tia) were collected from Lal Teer Seed Company, Dhaka. The seed was soaked in water for 24 hours in the Petri dishes prior to sowing to soften the seed coat for better and quick germination. Three seeds per pit were sown directly. Before sowing, the seeds were treated with Vitavax 200 @ 2 g per kg of seed. Regular irrigation was done after sowing. Finally, only one healthy plant was kept in each pit. A new one replaced damaged and virus-infected seedlings. The watering and other intercultural operations were done for each seedling in the field and a bamboo stick was used for each seedling for support.

Application of treatments

Yellow sticky trap: Yellow sticky traps were used to monitor insect populations in the bitter gourd field. The yellow sticky trap of 20×15 cm sized was hung and was adjusted vertically with the help of a wooden stick/pole. The traps were hung at three different heights (50, 100, and 150 cm) for the respective treatments of the plot. The yellow traps were replaced by fresh ones at an interval of 3 days. Each yellow trap was placed in the

middle of the randomly selected plots. Three replications were maintained for each treatment. The traps were checked regularly, and were counted the number of fruit flies captured every 3 days interval. The traps were maintained in the field from the flower initiation stage to the last harvest covering the entire reproductive stage of bitter gourd.

Fruit fly bait with a mashed sweet gourd (MSG): As standard practices, a bait trap was considered a treatment for its effectiveness with bait sprays. The trap consisted of 0.5 ml (10-15 drops) of Dynamic 40 EC (SAM Agro chemical) (Dimethoate group – systemic and contact insecticide**)** mixed with 100 g of sweet gourd mash and 100 ml of water. The bait was kept in a small earthen pot placed within three split bamboo sticks, 50 cm above the ground. Fresh ones replaced the old bait materials at 2 to 3 days. Each set of bait traps was placed in the middle of the randomly selected plots. Three replications were maintained. Bait traps were checked regularly, and the number of fruit flies captured in each 3 days interval was counted. The traps were maintained in the field from the flower initiation stage to the last harvest covering the entire reproductive stage of bitter gourd.

Bagging: The bagging of fruits was applied using a transparent polythene bag with a few holes made by an ordinary pin. These tiny holes provided for aeration. The size of the perforated polythene bag was large (30 cm × 20 cm) enough for normal growth and provide a sufficient aeration. All the full-bloomed female flowers of the plant under treatment were visually checked every day and tagged. In the morning hours (8.00 AM to 9.30 AM) before the beginning of frequent visits of fruit flies, the tagged female flowers were bagged individually with perforated polythene bags at 3 days after anthesis (DAA) and were left for five days. The open mouth of the bags was wrapped and closed by thread near the fruit peduncle. After 5 days, the polythene bags were removed. Regular observation was done to check the fruit fly infestation on these tagged fruits, and the operations were continued till the last harvesting.

Sanitation: For sanitation treatment, fallen and rotten fruits were removed and disposed of every 3 days interval. This sanitation was done from flower initiation to the final harvest.

Ecomec 1.8 EC: Ecomec 1.8 EC (Neem-based biopesticide from Ispahani Agro Limited) was applied at the rate of 1ml/L of water sprayed at 7 days intervals starting from the flower initiation stage of bitter gourd. The application of Ecomec 1.8 EC was continued till the late fruiting stage. This was uniformly sprayed to ensure complete coverage of the plants.

Control treatment: Three plots were selected as control. No other control measures were taken in these plots. All other intercultural operations were similar to those done for other treatment.

Data collection: Data was recorded on the number of healthy fruit, the number of infested fruit**,** the weight of healthy fruit, and the weight of infested fruit harvested at different fruiting stages. The numbers of fruit fly trapped in different traps (Yellow sticky and bait trap) at different fruiting stages of the crop were also counted. After harvesting, the weight of healthy and infested fruit was separately recorded. The laboratory also measured and recorded the larval density of infested fruits. The total production of each treatment was calculated, and determined the yield (t/ha).

Percent fruit infestation as a number: After harvesting, the healthy fruit (HF) and the infested fruit (IF) were separated by visual observation. The percent fruit infestation for each treatment was calculated by using the following formula:

% Fruit infestation by number = Number of IF/ (Number of HF + Number of IF) × 100

Percent fruit infestation as weight: After sorting of the healthy and the infested fruit, the weight was taken for healthy, infested and total ones separately. The percent infested fruit as weight for each treatment was calculated by using the following formula:

% Fruit infestation by weight = Weight of IF / (Weight of HF + Weight of IF) × 100

Percent weight reduction per fruit: Fruit infestation by number and weight for each treatment of reproductive stages was used to determine the average weight of single healthy and infested fruits. The percent weight reduction per fruit for each treatment was then calculated using the following formula:

% weight reduction per fruit= (Weight of single HF - Weight of single IF) / Weight of single HF × 100

Percent yield increase over control: After harvesting, the weights of healthy and infested fruit were separately recorded. The total production of each treatment was calculated and was determined the yield (t/ha). The percent yield increase over control was computed by using the following formula:

% Increase of yield over control = (Yield of treated plot – yield of control plot) / Yield of control plot × 100

Statistical analysis: All the collected data were analyzed following the analysis of variance (ANOVA) technique with the help of the MSTAT-C Computer Package. The mean differences were adjusted by Duncan's Multiple Range Test (DMRT) techniques.

Results and Discussion

The results of the experiment are presented and discussed under the following subheadings:

Effect of different management practices on the fruit infestation (by number) of bitter gourd: Number of healthy fruit per plot, number of infested fruit, and percent fruit infestation are shown in Table 1. The result indicated that the number of healthy fruit were significantly different (p>0.001, F=20.36, df=7) among the treatments. The highest number of healthy fruits per plot was recorded in bagging (41.33), followed by a yellow sticky trap set at 150 cm height (39.00), bait trap with a mashed sweet gourd (38.00), Ecomec 1.8 EC (38.00) and yellow sticky trap set at 100cm height (37.33) which were statistically identical. But the lowest number of healthy fruits per plot (19.00) was recorded in the control treatment. On the other hand, the highest infested fruit was recorded in the control (13.00) treatment. However, no fruit infestation occurred in bagging. The percent infestation was found 9.48, 15.69, 18.24, 18.49, 18.84, and 21.36% in bait trap with mashed sweet gourd, Ecomec 1.8EC, yellow sticky trap set at 100cm height, sanitation, yellow sticky trap set at 50cm height, and yellow sticky trap set at 150cm height, respectively. On the other hand, the highest fruit infestation by number was recorded in the control (40.75%) treatment.

From the above findings, it was revealed that the lowest fruit infestation by number was recorded in bagging in the field, whereas the highest fruit infestation (40.75%) by number was recorded in the control treatment. The present study agrees with those of Islam *et al.* (2015). They studied the evaluation of different management practices against cucurbit fruit flies (*B. cucurbitae* coquillett) in bitter gourd. They found that bagging of fruits showed the lowest percent infestation by number (19.49%, 7.48%, and 23.15%) at the early mid and late fruiting stages, respectively.

Effect of different management practices on the fruit infestation (by weight) of bitter gourd: The effects of different management practices on fruit infestation by weight are displayed in Table 2. Significant variations (p>0.001, F=37.19, df=7) were found among the treatments regarding of fruit fly infestation on bitter gourd. The weight of healthy fruits ranged from 2.22 to 6.08 kg/plot. The highest weight of healthy fruit per plot was recorded in bagging (6.08), followed by Ecomec 1.8 EC (5.94), which is statistically similar. But the lowest weight of healthy fruit per plot was recorded in control (2.22). The highest weight of infested fruit per plot (1.27) was recorded in control followed by a yellow sticky trap set at 150cm height (1.12) which was statistically similar. Considering the percent fruit infestation by weight, no percent fruit infestation by weight was recorded

in bagging. On the other hand, the highest percent fruit infestation by weight was recorded in the control (36.48%), which is statistically different from all other treatment applied in this study.

Table 1. Percent fruit infestation by a number of bitter gourd fruit affected by different treatments.

Treatments	Number of healthy fruit	Number of infested fruit	% infestation
Yellow sticky trap set at 50 cm height	30.33^{b}	7.00^{c}	18.84^{bc}
Yellow sticky trap set at 100 cm height	37.33^{a}	8.33^{c}	18.24^{bc}
Yellow sticky trap set at 150 cm height	39.00^{a}	10.67^{b}	21.36^{b}
Bait trap with mashed sweet gourd	38.00^{a}	4.00^{d}	9.48^{d}
Bagging	41.33^{a}	0.00^{e}	0.00^{e}
Sanitation	28.00^{b}	6.33^{c}	18.49^{bc}
Ecomec 1.8 EC	38.00^{a}	7.00^{c}	15.69^{c}
Control	19.00^{c}	13.00^{a}	40.75^{a}
LSD	5.06	1.91	4.22
CV %	8.52	15.49	13.51

In a column, means followed by the same letter(s) are not significantly different at a 5% probability level by DMRT.

It is clear from the study that in the bagging treatment, no infestation was found; accordingly, bagging was very effective to control fruit flies. The present study is supported by Islam *et al.* (2015). They found that bagging of fruits showed the lowest percent infestation by weight (11.79%, 7.18%and 11.90%) at early mid and late fruiting stages, respectively. Amin (1995) found lowest infestation significantly (4.61%) in bagged cucumbers compared to other chemical and botanical control treatment. Bagging of cucumbers with perforated polythene bags at the immature stage significantly reduced the fruit fly infestation (Akhtaruzzaman *et al*., 1999).

Weight reduction: The weight of single healthy fruit, single infested fruit, and the percent weight reduction per fruit are presented in Figure 1. The result specified that the weight of single healthy fruit among the treatment was significantly different (p>0.001, F=68.97, df=7). The percent weight reduction per fruit ranged from 0.0 to 30.17%. Results also revealed that there were no wet reduction was found in the bagging treatment, and the highest percent weight reduction per fruit was recorded in Ecomec 1.8 EC (30.17%),

followed by a yellow sticky trap set at 100 cm height (26.69%), control (16.51%), yellow sticky trap set at 150 cm height (15.76), fruit fly bait with MSG (13.02%). The present study was similar to the study of Amin (1995). He significantly obtained the lowest weight reduction (24.45 %) when the fruits were bagged at fruit initiation stage.

Table 2. Percent fruit infestation by weight (Kg) of bitter gourd fruit affected by different treatments.

Treatments	Weight of healthy fruit (Kg)	Weight of infested fruit (Kg)	% infestation
Yellow sticky trap set at 50 cm height	3.66^{c}	0.73^{b}	16.71^{bc}
Yellow sticky trap set at 100 cm height	5.17^{b}	0.83^{b}	13.86^{cd}
Yellow sticky trap set at 150 cm height	4.86^{b}	1.12^{a}	18.64^{b}
Bait trap with mashed sweet gourd	4.96^{b}	0.45^{c}	8.34^{e}
Bagging	6.08^{a}	0.00^{d}	0.00^{f}
Sanitation	3.37^{c}	0.68^{b}	16.94^{bc}
Ecomec 1.8 EC	5.94^{a}	0.77^{b}	11.52^{de}
Control	2.22^{d}	1.27^{a}	36.48^{a}
LSD	0.67	0.19	3.77
CV %	8.39	15.00	14.07

In a column, means followed by the same letter(s) are not significantly different at a 5% probability level by DMRT.

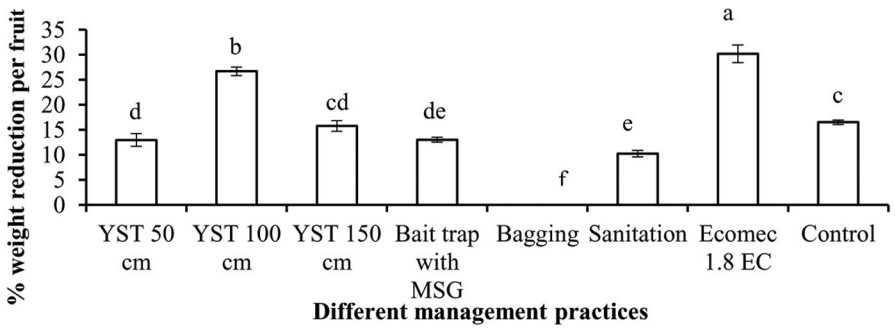

Fig 1. Effect of weight reduction per fruit on different management practices. Where, YST 50 cm=Yellow Sticky Trap set at 50 cm height, YST 100 cm=Yellow Sticky Trap set at 100 cm height, YST 150 cm=Yellow Sticky Trap set at 150 cm height, MSG=Mashed Sweet Gourd.

Larval density: Figure 2 represent the number of larvae per infested fruit in different management practices. Among the treatments, the number of larvae per infested fruit was significantly different (p>0.001, F=13.80, df=7). The highest number of larval density were found in fruit fly bait with MSG (15.83) followed by sanitation (10.60), yellow sticky trap set at 50 cm height(10.48), Ecomec 1.8 EC (10.60), control (8.85), yellow sticky trap set at 100 cm height (7.70). But the lowest number of larval densities was found in a yellow sticky trap set at 150 cm height (6.7).

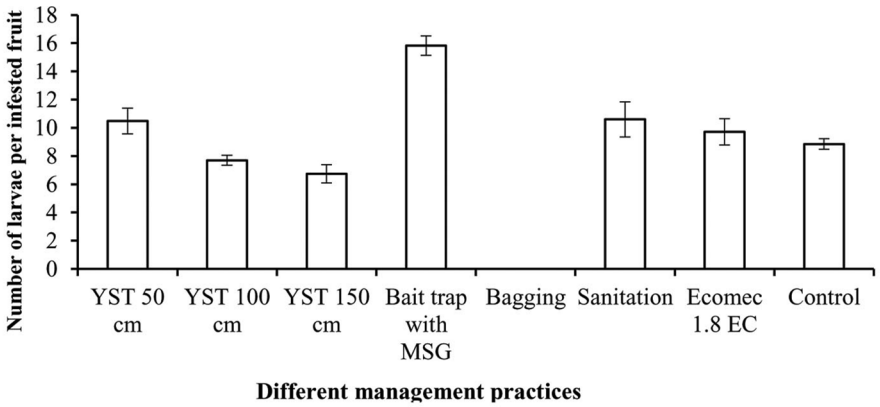

Fig 2. Larval density in infested fruit in different management practices. Where, YST 50 cm= Yellow Sticky Trap set at 50 cm height, YST 100 cm=Yellow Sticky Trap set at 100 cm height, YST 150 cm=Yellow Sticky Trap set at 150 cm height, MSG=Mashed Sweet Gourd.

Monitoring of fruit flies by yellow sticky traps and poison bait traps: To monitor fruit fly abundance at the different dates of the study period, four type of traps viz yellow sticky trap set at 50 cm, 100 cm, 150 cm height and bait trap with mashed sweet gourd was used to capture fruit fly which is shown in Figure 3. The highest number of fruit flies in a yellow sticky trap set at 50 cm height was captured on 21 March 2021 (4.33) and the lowest on 29 April 2021 (1.33). The highest number of fruit flies in a yellow sticky trap set at 100 cm height was captured on 14 February 2021 (2.00) and the lowest on 05 April 2021 (1.00). Consequently, the highest number of fruit flies in a yellow sticky trap set at 150 cm height were captured on 4 March 2021 (2.00) and the lowest on 29 April 2021 (0.67). The highest number of fruit flies in the bait trap with mashed sweet gourd was captured on 4 February 2021 (1.00), but no fruit flies were captured on 29 April 2021.

The present results showed that the yellow sticky trap set at 50 cm height performed the best among the traps studied. Figure 3 revealed that the fruit fly abundance was higher on

early February 2021. After that decreased to 21.03.2021 and again increased for a week, followed by a decreased in population found with slight increase at the end of April 2021. Other than a yellow sticky trap set at 50 cm height, it showed a steady increase and decrease due to weather parameters and various biotic and abiotic factors.

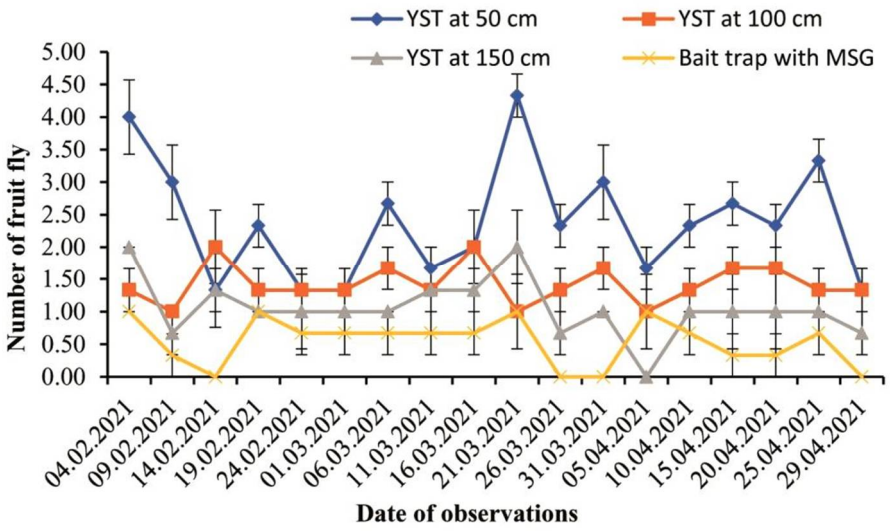

Fig 3. Fruit flies were captured in different yellow sticky traps and poison bait of various dates in the experiment. Where, YST 50 cm=Yellow Sticky Trap set at 50 cm height, YST 100 cm=Yellow Sticky Trap set at 100 cm height, YST 150 cm=Yellow Sticky Trap set at 150 cm height, MSG=Mashed Sweet Gourd.

Performance of yellow sticky trap and poison bait in capturing fruit flies during the study period: Total numbers of fruit flies captured in the study period in four types of traps are represented in Figure 4. The highest number of fruit flies were captured in a yellow sticky trap set at 50 cm height (43/trap) followed by a yellow sticky trap set at 100 cm height (26/trap) and a yellow sticky trap set at 150 cm height (19.70/trap) which are gradually decreased. Consequently, the lowest numbers of fruit flies were found in a bait trap with a mashed sweet gourd (9.70/bait).

The present results agreed with those of Said *et al.* (2016), who studied the effect of sticky trap color and height on the capture of adult oriental fruit fly, *B. dorsalis* (Hendel) (Diptera: Tephritidae) on chili pepper in Indonesia and found that yellow trap was consistently the most attractive trap amongst the other trap colors tested with an overall average of 62.6 adults per trap during the study where the second most attractive trap

were white and green traps with overall averages of 45.2 and 40 adults per traps respectively. Besides that, trap height from the ground can also affect the preference of particular species. In addition, traps set up at 25 and 50 cm above the ground captured significantly more adults (187.8 and 171.9 per trap, respectively) compared to those set up at 75 and 100 cm above the ground (60.8 and 37.1 per trap, respectively).

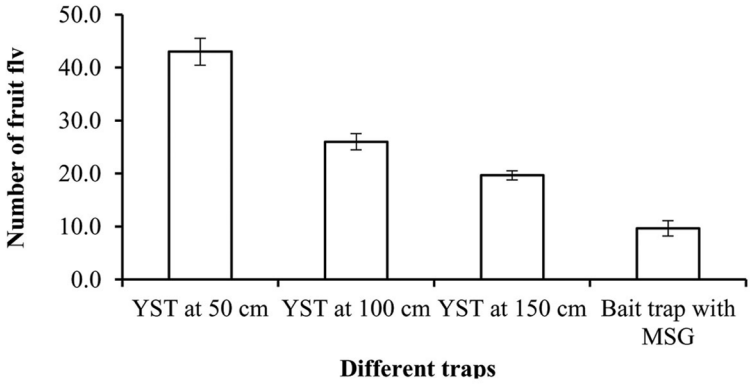

Fig 4. The number of captured fruit fly at the yellow sticky trap and poison bait. Where, YST 50 cm= Yellow Sticky Trap set at 50 cm height, YST 100 cm=Yellow Sticky Trap set at 100 cm height, YST 100 cm=Yellow Sticky Trap set at 100 cm height, MSG=Mashed Sweet Gourd.

The relationship between the number of captured fruit flies per trap and the percent fruit infestation by number is shown in Figure 5(a). The highest percent fruit infestation by number was found in a yellow sticky trap set at the height of 150 cm (21.36%), and the lowest number of fruit flies were captured in a bait trap with a mashed sweet gourd (9.70/bait). On the other hand, the lowest percent fruit infestation by number was found in a bait trap with a mashed sweet gourd (9.48%), and the highest number of fruit flies were captured in a yellow sticky trap height at 50 cm (43/trap). Percent fruit infestation by weight and the number of captured fruit flies per trap is presented in Figure 5(b). The highest percent of fruit infestation by weight and captured fruit flies were found in yellow sticky trap height at 150cm (18.64%) and yellow sticky trap height at 50cm (43/trap), respectively. But the lowest percent fruit infestation by weight and the number of captured fruit flies was found in bait traps with MSG (8.34%, 9.70/bait, respectively).

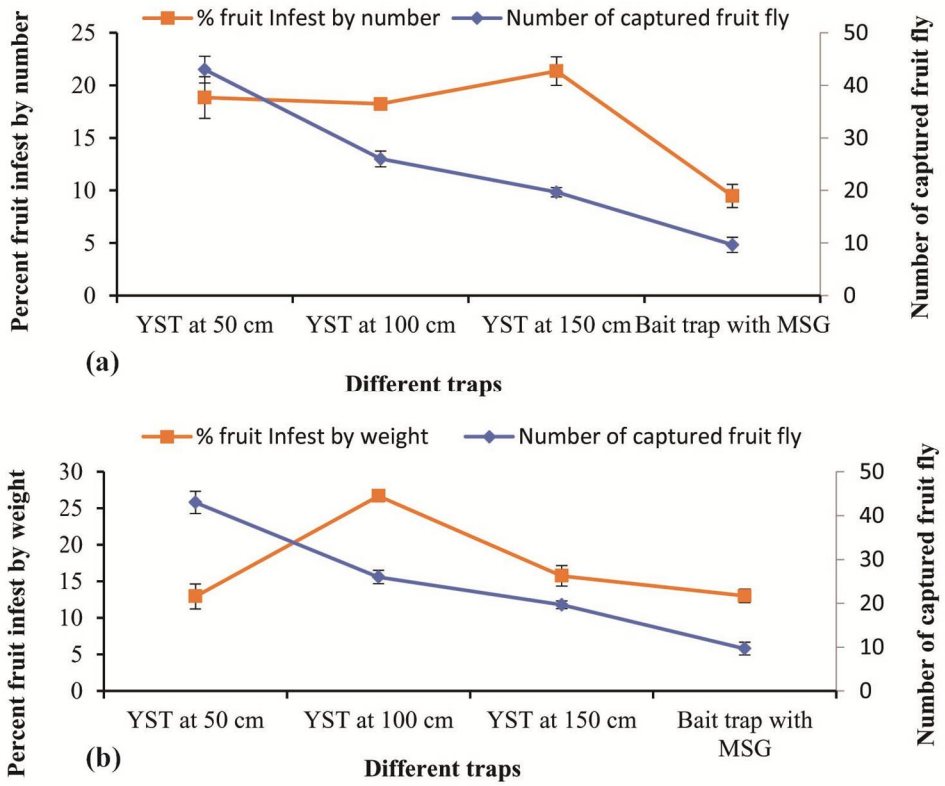

Fig 5 (a) Relationship between percent fruit infestation by number and number of captured fruit flies (up) (b) Relationship between percent fruit infestation by weight and number of captured fruit flies (down). Where, YST 50 cm=Yellow Sticky Trap set at 50 cm height, YST 100 cm=Yellow Sticky Trap set at 100 cm height, YST 150 cm=Yellow Sticky Trap set at 150 cm height, MSG=Mashed Sweet Gourd.

Effect on yield of bitter gourd: The effect of different management practices such as yellow sticky trap set at 50 cm height, yellow sticky trap set at 100 cm height, yellow sticky trap set at 150 cm height, fruit fly bait with mashed sweet gourd, bagging, sanitation, Ecomec 1.8 EC and control on the yield and percent increase of yield over control are shown in Table 3. Significant variations (p>0.001, F=37.21, df=7) of bitter gourd yield were observed among the treatments. The highest yield per plot was recorded in bagging (12.16 ton/ha), followed by Ecomec 1.8 EC (11.89 ton/ha), which was statistically similar. But the lowest yield per plot was recorded in control (4.43 ton/ha). On the other hand, the highest increased yield over control was recorded in bagging (2.75

times) followed by Ecomec 1.8 EC (2.68 times). In contrast, the minimum yield increase over control was recorded in sanitation (1.52 times).

Table 3. Percent increase of yield over control (ton/ha).

Treatments	Yield of treated plot	Yield increased in times
Yellow sticky trap set at 50 cm height	7.32[c]	1.65
Yellow sticky trap set at 100 cm height	10.36[b]	2.33
Yellow sticky trap set at 150 cm height	9.72[b]	2.19
Fruit fly bait with mashed sweet gourd	9.92[b]	2.24
Bagging	12.16[a]	2.75
Sanitation	6.75[c]	1.52
Ecomec 1.8 EC	11.89[a]	2.68
Control	4.43[d]	-
LSD	1.33	-

In a column, means followed by the same letter(s) are not significantly different at a 5% probability level by Duncan's Multiple Range Test (DMRT).

Conclusion

It is concluded that bagging, fruit fly bait with mashed sweet gourd and yellow sticky trap set at 50 cm height may be used for monitoring and eco-friendly management of cucurbit fruit fly for the cultivation of bitter gourd in Bangladesh.

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USE OF BAGASSE TO REMOVE 2-CHLOROPHENOL IN AQUEOUS SYSTEM

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Abstract

The adsorption method using waste bagasse has been examined to remove 2-chlorophenol (2-CP) from aqueous solutions at room temperature. The adsorption of 2-chlorophenol by bagasse carbon could be studied in batches by changing the contact time, operating temperature, pH of the solution, initial concentration, adsorbent dose, and particle size. It took three hours to reach equilibrium. The Langmuir model correctly predicted the adsorption equilibrium data for 2-chlorophenol-sorbent systems in the concentration range that was examined. When the pH was lower, getting rid of 2-CP from surfaces was easier. Studies of desorption show that chemisorption is an important part of the adsorption process.

Key words: 2-Chlorophenol, Bagasse, Adsorption, Equilibrium, Desorption

Introduction

Cancer and mutations caused by halogenated aromatics can lead to incurable diseases (Zada *et al.,* 2021). 2-Chlorophenol is a halogenated aromatic molecule that has found utility in several fields, including herbicides, polymers, pharmaceuticals, petroleum, different chemicals, etc. (Huong *et al.,* 2016). The USEPA has designated 2-CP as a priority organic pollutant and established a limit of 0.1 ppb for its presence in potable water supplies (Pera-Titus *et al.,* 2004). Industrial waste, agricultural runoff, and landfill leachate are all pathways via which 2-CP enters the environment (Shen *et al.,* 2021). Given its resistance to degradation, 2-CP tends to accumulate in natural settings. Because of its potency as a mutagen and carcinogen, 2-CP is disastrous for aquatic ecosystems (Barakat *et al.,* 2021). This means that before discharge, 2-CP must be removed from industrial effluent.

Technologies such as chemically induced precipitation, reverse osmosis, oxidation-reduction, and adsorption have all been used to successfully remove 2-CP from

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wastewater (Enoyh and Isiuku, 2021; Liu *et al.,* 2021; Kusmierek *et al.,* 2021). Adsorption has recently replaced all other wastewater treatment methods as the most well-known and widely used option due to its low cost and high efficiency. To offset the high expense of synthetic adsorbents, researchers have been working overtime to find cheaper alternatives. There has been a rise in interest in using adsorbents made from waste biomass to detoxify the environment of contaminants like 2-CP (Kusmierek *et al.,* 2021; Garba *et al.,* 2019).

It is prohibitively expensive for factories in developing nations like Bangladesh to use chemicals, including alum, ferric chloride, polymer flocculants, and activated carbon derived from coal which has been used for decades in conventional wastewater treatment. The bagasse by-product is a low-cost material for waste management in the ongoing search for novel, widely used agricultural wastes (Mandal *et al.,* 2004; Barraclough *et al.,* 2005; Singh *et al.,* 2008). Sugarcane is cultivated on over 425,000 hectares of land in Bangladesh. Every year, we generate almost 800,000 metric tons of discarded bagasse (Mahamud and Gomes, 2012). Using this biomass source would reduce costs associated with cleaning water systems of harmful pollutants (Williams and Nugranad, 2000). More research, however, need to be done to determine whether or not untreated bagasse can efficiently eliminate 2-CP. This research aimed to determine if waste bagasse (specifically, sugarcane industry bagasse) might be used as a substitute adsorbent. To accomplish this, a simple, highly effective adsorption method for removing 2-CP from wastewater was developed and implemented.

Materials and Methods

Chemicals and apparatus: The chemicals and reagents utilized were of the highest quality (BDH). Both of the solutions were prepared by using double-distilled water as starting material. As a stock solution, 0.5 g of 2-CP was dissolved in 500 ml of deionized water. This was a 2-CP stock solution that had 1,000 milligrams per milliliter. A double-beam spectrophotometer, the Shimadzu UV-160, and the 4-aminoantipyrene technique were used to quantify the concentration of 2-CP (APHA 1985). Buffer solutions from the German company E. Merk are used to maintain a constant pH level. A microprocessor-based bench pH meter was used to measure the substrate's pH (HANNA pH 300) (Amin *et al.,* 2012).

Phenol concentration measurement: Results from the 4-aminoantipyrine technique (APHA, 1985) were deemed insufficient, prompting researchers to seek alternate approaches. Neither 2-CP nor 4-aminoantipyrine showed any significant color change in

early testing with potassium ferricyanide in an acidic medium. In contrast, 2-CP has a dark reddish hue at a pH of 6.0. This hue becomes more vibrant as the pH increases, to a point where it suddenly fades at a pH of 10.0. This color scheme was developed after researchers found that they could more accurately measure low concentrations of 2-CP by shifting their spectrophotometric measurements to a buffer with a pH of 10.0 (instead of the more often used of pH 8.0). Color fully develops during 25 minutes; the absorption peak has been measured to be at 500 nm using blank reagents. As shown experimentally, the maximal color intensity in a 2-CP solution system with a 5 mgl^{-1} concentration can be achieved using just 0.4 ml of a 2.0% (w/v) 4-aminoantipyrine solution. For a system containing 5 mgl^{-1} 2-CP, the optimal amount of potassium ferricyanide is 0.5 ml of an 8.0% (w/v) solution. The color intensity was also shown to drop gradually after this range. The modified strategy was proven more effective (Amin *et al.*, 2012).

Preparation of adsorbent : This initiative made use of bagasse from a regional sugar mill. The waste bagasse was exposed to the elements for ten days until its moisture content stabilized. The bagasse was chopped and then sieved to remove the fibrous parts. Dust and particles were removed from the gathered items by washing them multiple times in clean water. To ensure that the washed water was completely clear, the washing operation was repeated several times. Materials were washed and dried in a hot-air oven at 60 ^{o}C for 24 hours. After drying, the substance was sieved into five different particle sizes (75, 95, 125, 175, and 400 µm). The materials were used to remove 2-CP from the environment without adding any additional physical or chemical treatment.

Table 1. Sugarcane bagasse characterization (Figueroa *et al.*, 2014).

Proximate analysis (wt%)		**Ultimate analysis (wt% free water)**		**Lignocellulosic analysis (wt% free water)**	
Moisture	7.80±0.50	C	44.52±1.59	Cellulose	40.99±0.72
Fixed carbon	10.81±0.33	H	5.90±0.22	Hemicellulose	25.45±0.85
Volatile matter	83.97±0.36	N	0.32±0.08	Acid insoluble lignin	14.47±0.45
Ash	5.22±0.68	S	0.10	Acid soluble lignin	5.26±0.04
		Cl	0.29	Extractives	4.86±1.09
		O*	43.65		
Bagasse particle density			1.49±0.01		

* by difference.

Bagasse characteristics: The sturdy stem of the sugarcane plant sets apart from other types of grass. Table 1 shows the characteristics of sugarcane bagasse.

Adsorption of 2-CP from aqueous solutions: Each batch experiment used a 250-ml sealed bottle containing 100 ml of 2-CP solution and 2.0 g of adsorbent. The pH of all the tests was set at 8.0. The texture of the adsorbent shifts when the pH rises. Next, an electric shaker was used to agitate the bottles at the same rate as they warmed to room temperature. The higher temperatures required the use of beakers in a water bath with a thermostat and an electric stirrer. The contents were centrifuged at predetermined intervals, and spectrophotometry was used to compare the amount of 2-CP in the supernatant to a reagent blank. The bottles containing the various concentrations of 2-CP and the appropriate pH were shaken for 5 hours to ensure that the concentration of the residual 2-CP remained constant. The capacity to absorb was determined by comparing the initial and final 2-CP concentrations.

The effectiveness of removal (adsorption) was determined using the following equation:

$$\text{Removal (adsorption) efficiency} = ((C_o - C_e)/C_o) \times 100 \quad (1)$$

where C_o and C_e are the 2-CP concentrations in the sample solution before and after the treatment. The uptake of 2-CP by bagasse was also measured in batch tests conducted at 30, 35, 40, and 50^{o}C. Subsequent experiments were conducted at a temperature of 30^{o}C (room temperature), although the concentration is somewhat higher at 50^{o}C. Beer's law predicts that the 2-CP concentration in the research may range from 0 to 10 mg/l.

Results and Discussion

First, nine adsorbents, including coconut shell, motorsuti straw, maize husk leaf, bagasse, mustard straw, rice straw, maize cob, and newspaper, were tested on their ability to remove 2-CP from aqueous solutions. At an initial 2-CP concentration of 5 mg/l, a working temperature of 25^{o}C, an amount of adsorbent of 2.0 g, a contact period of 1 hour, and a solution pH of 6, 2-CP was successfully removed. According to preliminary research, coconut shell, motorsuti straw, maize husk leaf, baggage, mustard straw, rice straw, maize cob, and newspaper were each 10.3, 11.2, 28.7, 13.9, 9.4, 17.3, 16.8, and 14.3% effective at removing 2-CP. Baggage was more effective at removing the substance than these eight adsorbents. Therefore, they were disregarded and ignored in the follow-up research.

Effect of adsorbent dosages: How bagasse carbon removed 2-CP as adsorbent amounts varied from 0.5 to 5.0 g is depicted in Fig. 1. It was also ensured that the pH level remained constant at 6.0. The amount of 2-CP eliminated increased as the amount of adsorbent increased, up to a maximum of 2.0 g. After that point, there was slight variation in the total amount of 2-CP that was eliminated. The adsorption rate increases with the amount of adsorbent since there are more adsorption sites and surface area to absorb. Scientists have found that most phenols function similarly (Mustafa *et al.*, 2008).

Effect of contact time: Finding the equilibrium point involved studying how time affects adsorption. This effect of agitation time on bagasse's 2-chlorophenol elimination is shown experimentally in Fig. 2. According to the results, 2-CP takes nearly an hour to establish equilibrium on bagasse. There was little to no noticeable difference in the rate of 2-CP removal between 1-3 h. Adsorption data indicates that adsorbate species are rapidly ingested at the outset of the contract duration. These findings also demonstrate the rapidity of the sorption process, as the major portion of 2-CP was adsorbed to the sorbent in the first 60 minutes. The same sort of outcome was reported by Esma *et al.* in 1998.

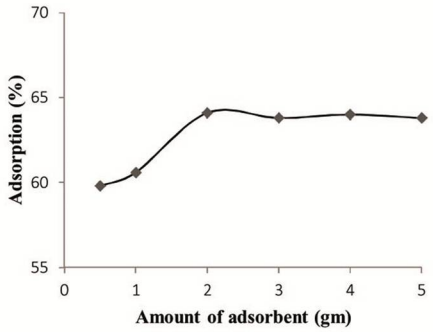

Fig. 1. Effect of bagasse amount on 2-CP adsorption (contact time, 60 min; avg. particle size, 150 µm; initial 2-CP concentration, 5 mg/l; pH, 6.0; operational temperature, 25 ^{o}C).

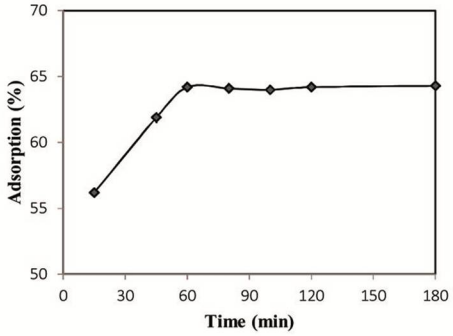

Fig. 2. Effect of contact time on the adsorption of 2-CP (adsorbent dosage, 2.0 min; avg. particle size, 150 µm; initial 2-CP concentration, 5 mgl^{-1}; pH, 6.0; operational temperature, 25 ^{o}C).

Effect of particle size: Taking 2-CP out of an aqueous solution, batch adsorption studies were conducted using a range of five average particle diameters of 60, 100, 150, 200, and 250 µm. The outcomes are depicted in Fig. 3. The percentage of 2-CP eliminated increased from 56% to 72% as particle size decreased. When rice husk is used, similar results are observed (Amin *et al.*, 2006). These occurrences may occur because smaller particles have more surface area and more sites for things to attach to them.

Effect of initial concentration: 2-CP removal efficiency highly depends on the concentration of 2-CP in the starting sample solution. The effectiveness of bagasse in removing 2-CP varies with the starting concentration of the sample, as shown in Fig. 4. According to the data, when 2-CP concentration increased, so did the sorbents' sorption capabilities, but the adsorption yields decreased. The amount of 2-CP that could be adsorbed by bagasse increased from 63 to 68% when the phenol concentration was raised from 1 to 6 mg/l. It's easy to see that the driving force for mass transfer is greater and that there are more adsorption sites. Because of this, the adsorbate can easily access the adsorption site. More adsorbent crowds into the particles, reducing the number of active sites and making it more difficult for the adsorbate to migrate. Because of this, the initial concentration plays a pivotal role in promoting the transformation of 2-CP from the liquid to the solid state by boosting the force responsible for mass transfer. More 2-CP would be taken up if this happened. However, the amount of 2-CP present at the outset reduces adsorbed. Due to its greater specific surface area and microporous nature, bagasse was predicted to have the highest equilibrium uptake and adsorption yield. Rao et al. 2003 also observed similar results.

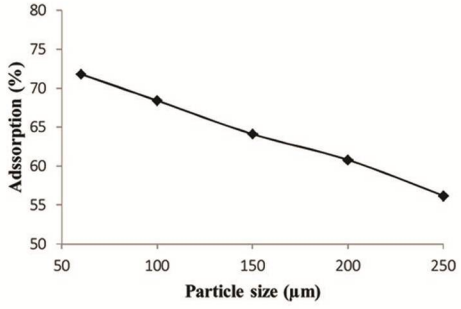

Fig. 3. Effect of particle size on the adsorption of 2-CP (adsorbent dosage, 2.0 min; contact time, 60 min; initial 2-CP concentration, 5 mg/l; pH, 6.0; operational temperature, 25°C).

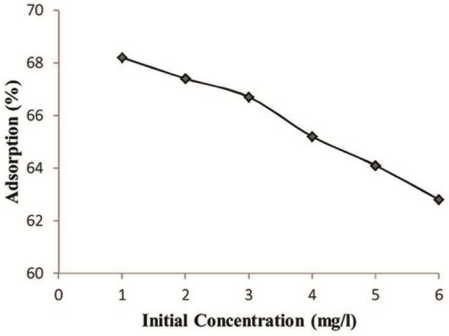

Fig. 4. Effect of initial concentration on 2-CP adsorption (adsorbent dosage, 2.0 min; contact time, 60 min; avg. particle size, 150 µm; pH, 6.0; operational temperature, 25°C).

Effect of pH: The surface charge, ionization level, and separation efficiency of an adsorbent all change when the pH of the solution changes. At varying pH values (from 4 to 12), bagasse uptake of 2-CP was studied. Results are depicted in Fig. 5. There is a positive pH-dependent linear relationship between the amount of 2-CP adsorbed and the pH up to 6. Adsorption of 2-CP decreases sharply when pH increases beyond pH 6 and remains low at higher pH levels. The solution's pH has an impact on the protonation or

deprotonation of functional groups in both the adsorbent and the adsorbate. The decrease in CP adsorption as pH increases is presumably due to the charge characteristics of both the adsorbate and the adsorbent (Ghaffari *et al.*, 2014). Since 2-CP has a pKa of 8.44, it is a weak acid. Since the neutral form of 2-CP exists at pH values below 6, nearly no anionic species exist at these lower pH values (Fig. 5). When the pH is higher than its pKa value, more of the anionic CP is present. At a pH of 6, the anionic form of CP predominates, and the neutral form disappears entirely. Adsorbents have positively charged surfaces, but the presence of mostly neutral 2-CP means that electrostatic interactions are negligible. Electrostatic attraction is one of many factors in determining neutral 2-CP adsorption. When a lot of non-positive and anionic 2-CP accumulates on the adsorbent's surface, electrostatic repulsion becomes the dominant force. At pH values greater than its pKa, which is anionic, 2-CP is more common than neutral. At pH >6, the electrostatic repulsion between the adsorbate and the ashes was greater due to the concomitant generation of negative net charges on the ash surfaces. This made it so that 2-CP had much more trouble remaining in the ashes. A similar uptake of 2-CP was seen in the ash produced from rice straw, as reported by Chang *et al.* (2011). According to the results, pH 6 is optimal for absorbing 64%. This led to the conclusion that a pH of 6.0 was the best, and all further studies were conducted in this buffer.

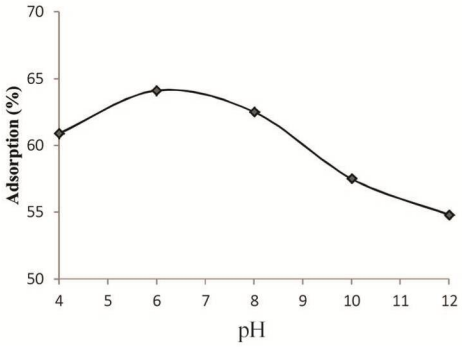

Fig. 5. Effect of solution pH on 2-CP adsorption (adsorbent dosage, 2.0 min; contact time, 60 min; avg. particle size, 150 µm; initial 2-CP concentration, 5 mg/l; operational temperature, 25°C).

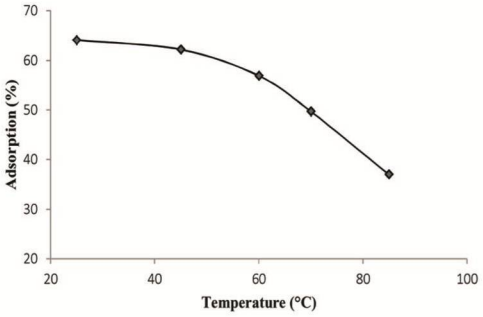

Fig. 6. Effect of temperature on 2-CP adsorption (adsorbent dosage, 2.0 min; contact time, 60 min; avg. particle size, 150 µm; initial 2-CP concentration, 5 mg/l; pH, 6.0).

Effect of temperature: To separate substances, temperature plays a crucial role. Bagasse was heated to various levels (25, 45, 60, 75, and 85°C) to determine how effectively it eliminated 2-CP. The temperature dependence of adsorption capacity is depicted in Fig.

6. The 2-CP's ability to adhere to bagasse is diminished when the temperature rises from 25 to 85°C. This means that 2-CP is more likely to adhere to a surface at a cooler temperature. Adsorption decreases as temperatures increase. This occurs when the absorbate's thermal energy increases and the 2-CP's attractive force towards the adsorbent decreases. To put it another way, the molecular attraction between the adsorbent and the 2-CP isn't strong enough to keep the molecules bound. Numerous sources have reached the same conclusion (Ofomaja and Ho, 2007; Jadhav and Vanjara, 2004; Senturk *et al.*, (2009). The efficiency with which adsorbents take in molecules is significantly affected by temperature. Adsorption is an exothermic process, and it stands to reason that as the adsorbate-adsorbent system temperature increases, the sorption capacity decreases. All of the data points in this direction, demonstrating that sorption is an exothermic process (Bazrafshan *et al.*, 2016).

Adsorption isotherm: An adsorption experiment is set up in the lab by dispersing 150 ml of the solution into five flasks, each containing a known amount of bagasse. Initial 2-CP concentrations in the solutions were 5 mg/l (pH 6), and the amounts of adsorbent ranged from 0.5 to 2.0 gm. Afterward, the bottles were sealed and stirred in a rotary shaker for an hour at 25°C.

Many different models have been proposed to account for experimental results. Two of the most prevalent isotherm models are the Langmuir isotherm (Langmuir, 1918) and the Freundlich isotherm (Freundlich, 1906). Both models described the correlation between 2-chlorophenol uptake by the bagasse and equilibrium concentration. In its linear form, the Freundlich isotherm model is expressed as follows (Rengaraj *et al.*, 2002; Banat *et al.*, 2000; Aksu and Yener, 2001; Khalid *et al.*, 2000):

$$\ln q_e = \ln K + 1/n \ln C_e$$

where K and 1/n are the Freundlich constants for the sorbent's adsorption capacity and intensity, respectively, and q_e is the amount of adsorbate at equilibrium (mg/mg) and Ce is the concentration of adsorbate at equilibrium (mg/l). Linear regression of q_e data versus Ce can be used to determine K and 1/n by analyzing the intercept and slope of the plot (Rengaraj *et al.*, 2002; Banat *et al.*, 2000; Aksu and Yener, 2001; Khalid *et al.*, 2000). The following is a linear representation of the Langmuir isotherm model (Rengaraj *et al*., 2002; Banat *et al.*, 2000; Aksu and Yener, 2001; Khalid *et al.* 2000):

$$1/q_e = 1/Q^o + 1/bQ^o \; 1/C_e$$

where maximal adsorption capacity (Qo) and adsorption energy (b) are given in milligrams per milligram of solid (mg/mg). The intercept and slope of a line plot of

experimental data for $1/q_e$ against $1/Ce$ (Rengaraj *et al.,* 2002; Banat *et al.,* 2000; Aksu and Yener, 2001; Khalid *et al.,* 2000) can be used to figure out these constants.

Table 2. Isotherm model parameters.

Adsorbents	Freundlich			Langmuir			Reference
	K	*1/n*	R^2	Q^o	*b*	R^2	
Barley straw	0.032	0.389	0.99	0.067	1.017	0.98	Maleki *et al.,* 2010
Bagasse	0.11	0.62	0.98	0.41	0.46	0.99	Present study

Table 2, Fig. 7, and Fig. 8 demonstrate the values of the isotherm constants and the correlation coefficients. In comparing the Freundlich equation with the Langmuir isotherm equation, the latter is more accurate in capturing the equilibrium data. The sorption equilibrium data is almost perfectly described by the Langmuir and Freundlich equations, with R^2 values of 0.99 and 0.98, respectively. Increases in the Freundlich constant *k* indicate that phenol can be readily absorbed from an aqueous solution (Rengaraj *et al.,* 2002, Aksu and Yener, 2001). Compared to the phenol-barley straw

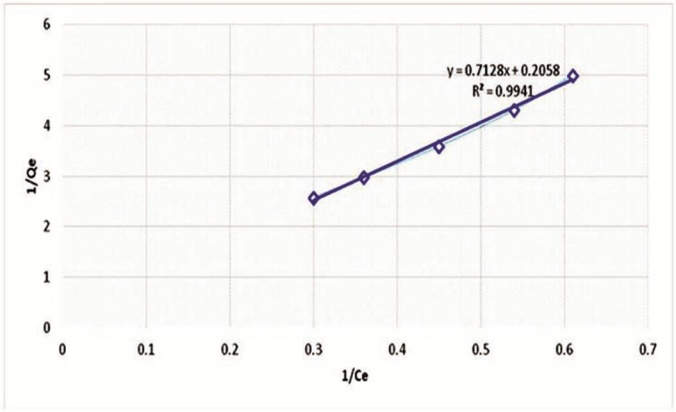

Fig. 7. Langmuir isotherm curve for removing 2-CP by the adsorption onto the bagasse.

system, the 2-CP-bagsse system (this study) had a greater *k* value for adsorption (Maleki *et al.,* 2010). The *n* value, which represents the sorption intensity, decreases. However, as demonstrated in Table 3, both the sorbents and the pollutants have *n* values that are sufficiently high to separate. When *1/n* is not exactly 0, the sorbent's surface is not

perfectly smooth and uniform (Khalid *et al.*, 2000). The agro-waste bagasse's maximal sorption capacity under a uniform covering was calculated using the Langmuir isotherm model. As shown in Table 2, the 2-CP-bagasse system has a maximum sorption capacity of 0.41 mg/g, which is more than that of barley straw (0.067 mg/g) and carbonized beet pulp (0.064 mg/g), but less than that of activated rice husk (75.15 mg/g) and burned water hyacinth (30.49 mg/g) (Uddin *et al.*, 2008).

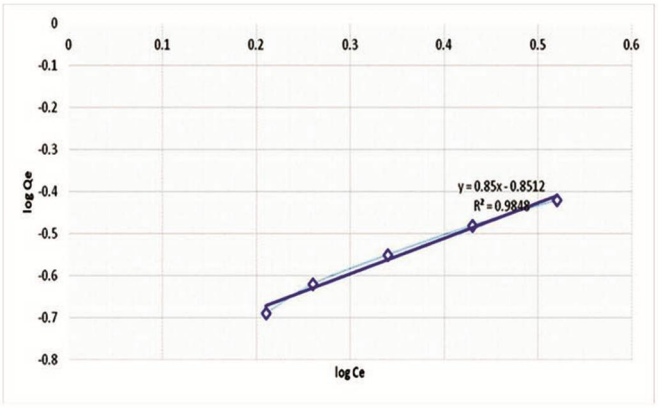

Fig. 8. Freundlich isotherm curve for removing 2-CP the adsorption onto the bagasse.

Desorption studies

Recovering the adsorbed material and regenerating the adsorbent are crucial steps in wastewater treatment. Hydrochloric acid, nitric acid, and sodium hydroxide solutions were used as eluents on trial to dissolve the 2-CP from the bagasse's surface. This desorption procedure was carried out using a batch procedure. 1.0 g of spent adsorbent after adsorption at pH 6 was shaken with 1M NaOH, 1M HCl, and 1M HNO_3 in a volume of 100 ml. It takes roughly 60 minutes to replenish the adsorbent. Single-step desorptive evaporation removed approximately 88.17%, 85.02%, and 77.30% of the adsorbed 2-CP from samples with an initial concentration of 5 mgl^{-1}. In the current study, alkaline solutions were shown to be more effective for desorption. The observed influence of pH is consistent with these considerations. The effectiveness of phenol desorption generally increases with increased desorption duration. So, the 2-CP was removed from the bagasse's surface with a sodium hydroxide solution.

The desorption (after biosorption) efficiency of 2-CP is calculated using the equation,

$$\text{Per cent desorption of 2-CP} = \frac{\text{Amount desorbed after desorption}}{\text{Amount sorbed before desorption}} \times 100$$

Conclusion

The current investigation demonstrated that waste bagasse an excellent approach to eliminating 2-CP from wastewater by absorbing it. Bagasse's adsorbent potential also represents a new frontier in discovering novel applications for biomaterial. The pH range of aqueous solutions that can be treated with bagasse is from 4.0 to 12.0. The Langmuir and Freundlich adsorption isotherms fit the experimental adsorption equilibrium data very well. Reviving adsorbed 2-CP with 1M NaOH allowed it to be used again. Because they are simple, easy to use, and easy to handle, the batch treatment systems proposed by our research group will be appropriate and suitable for removing organic compounds containing 2-CP from industrial wastewater. Bagasse, after desorbed, is clean enough to be utilized as fuel and contains no noxious substances.

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EVALUATION OF PANT CHARACTERISTICS AND PHYSIOLOGICAL PERFORMANCE OF MUNGBEAN (*VIGNA RADIATA* (L.) WILCZEK) GENOTYPES UNDER SALT STRESS

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Abstract

Mungbean is a delicious popular pulse crop whose yield is hampered by biotic and abiotic factors. Salinity is considered serious abiotic stress that hinders growth and yield drastically. To find out the response features of salinity tolerance in mungbean, a series of experiments were carried out in the Department of Crop Botany of BSMRAU. The experiment was performed in the greenhouse of the Crop Botany department using nutrient solution under hydroponics conditions. Initially, fifty-two mungbean genotypes were used in the experiment to screen out a susceptible and tolerant genotype. The results indicated that salinity affected the plants at various morphological characteristics namely plant height, and dry matter of root, stem, and fruit. The genotypes were placed in four groups based on their performance in salinity. A higher quantity of proline with a lower amount of Malon-dialdehyde was observed with the increase in salinity. Chlorophyll content increased initially and after that declined sharply. The susceptible genotype resulted in a sharp decline of chlorophyll and increased proline content which reflected the [1]accumulation of root and shoot dry matter, and consequently, the total dry matter content compared to that of the tolerant genotype.

Key words Mungbean, Salinity, Resistant, Susceptible, Dry matter

Introduction

The coastal region of Bangladesh comprises nearly 30% of the cultivable land. Around 1.056 million ha of land in 2086 million ha of coastal and off-shore lands are subject to a wide range of salinity (Moslehuddin *et al.*, 2015). Due to the presence of brackish soil, most of the lands remain fallow during the dry period (February-May) because of the scarcity of quality fresh water for irrigation and the poor drainage system (Karim *et al*., 1990; SRDI, 2000). Saline-tolerant pulse crops would have a good perspective to be

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cultivated in this area during winter. Among the pulses, mungbean is a vital crop of Bangladesh with multipurpose usages. It has the clear benefit of having a small life span and can be grown in different types of soil and environment (Rao *et al*., 2016). A high-value crop like mungbean is a potential candidate for this area. However, it is sensitive to salinity, and salt stress harms plant growth and development as saline stress leads to low osmotic potential (osmotic stress), specific ion impacts (ionic stress), and nutritional imbalance (Parida and Das, 2005).

The morphology of salt-induced plants is severely affected, thereby changing the tissue structures and system. High salt stress harms plants, while moderate to low levels negatively influence the plant development rate and sequentially show symptoms related to morphological, physiological, or biochemical change (Hasegawa, 2013).

The altered anatomical features change the physiological, namely photosynthetic pigments, such as, chlorophyll, and amino acid synthesis is hampered by saline conditions (Rahman *et al.,* 2002). Anatomical and physiological strategies could foster the development and endurance of the plant under stressful conditions. Deposition of Na^+ and Cl^- in plant tissues can lead to plant growth reduction under high saline conditions. At the seedling stage, plant growth is severely affected by the distribution of Na^+, Cl^-, and K^+ in root and shoot. In saline conditions, Na^+ and Cl^- concentrations increased in roots and shoots, but this escalation is less in tolerant than sensitive ones (Singh *et al.*, 2017). One of the most important salinity tolerance strategies of the crop is thought to be the dilution of the excessive amounts of deposited Na+ and Cl-ions from the plant body (Sultana *et al*., 2021).

A high-yielding saline-tolerant mungbean variety can find a place in the existing cropping pattern in the salty soil, which is yet to be developed. To fit this crop in the existing cropping pattern, it is imperative to develop a saline-tolerant mungbean variety. Aiming to this criterion, the department of Crop Botany of BSMRAU has conducted a series of experiments to study the salinity-induced responses in different plants, including Mungbean. And in this sequence, fifty-two mungbean genotypes were procured from the Plant Genetic Resources Centre (PGRC), Bangladesh Agricultural Research Institute (BARI), Gazipur. They were used in the quest of evaluating the response of mungbean plants in saline conditions.

Materials and Methods

A glass house and laboratory experiments were carried out at the Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur.

Initially, the procured genotypes were screened against salinity, and later screened genotypes were evaluated from different physiological perspectives.

Screening of genotypes against salinity: Two treatments, namely salinity of 8 dSm^{-1} and control, were imposed in the experiment. The NaCl salt was applied in the container through irrigation water starting at 7 days old seedlings continued up to 40 days.

The collected genotypes were used for screening against salt stress. Watering on the pot was performed regularly, maintaining a scheduled routine during the experiment period. Plants were kept in plastic pots, which were put in a bigger plastic container (10 L) containing Hoagland nutrient solution (Hoagland and Arnon, 1950). Intercultural operations were performed regularly to ensure the typical growth and development of the mungbean plant.

Plants were harvested 45 days after germination. Roots and shoots were separated, scrubbed, and weighed after drying in an oven at 81^{o} C for 72 hours. Data were recorded on common growth parameters (plant height, root and shoot length, root and shoot dry weight). The characters were studied in percent relative values for a proper understanding of the salinity tolerance in mungbean genotypes. The following formula was used to calculate the relative value:

$$\text{Relative value} = \frac{\text{Value of saline treated genotype}}{\text{Value of control genotype}} \times 100$$

The mungbean genotypes were grouped based on their shoot dry matter. A tolerance scale was made to categorize the genotypes in different group orders based on dry shoot matter (Ashraf and Wahid, 2000).

Physiological and Yield Attributes of Salinity Tolerance in Mungbean: After initial screening, the selected two genotypes were tested in terms of physiological and yield response. The experiment was planned in a factorial, a completely randomized design where factor one is mungbean genotypes (BD 6895 and BD 6905) and factor two is salinity levels (control, 6, 8, 10, and 12 dSm^{-1}).

Estimation of Proline Content: To estimate the proline, the top-most completely extended leaf samples were used according to the process of Bates *et al.* (1973). The standard curve was used to determine proline concentration and calculated on a fresh weight basis as follows:

Proline content (µmole/g fresh wt.) = {µg proline/ml × vol. of extra. sol. (ml) × toluene used (ml)} / {115.13 µg/mole × g sample}

Estimation of Chlorophyll Content: Chlorophyll contents were appraised using the youngest and top-most completely extended leaf samples with the following method described by Porra et al. (1989). The formula for measuring the chlorophyll a, b, and total chlorophyll are:

Chlorophyll a (mg^{-1} fresh weight) = [12.21 (A_{663}) – 2.81 (A_{646})] × [V/1000 × W]

Chlorophyll b (mg^{-1} fresh weight) = [20.13 (A_{646}) – 5.03 (A_{663})] × [V/1000 × W]

Total Chlorophyll (mg^{-1} fresh weight) = [20.2 (D_{646}) + 8.02 (D_{663})] × [V/1000 × W]

Where, V = Volume of acetone used (mL) W = Weight of fresh leaf sample in (g).

Determination of lipid peroxidation: The thio-barbituric acid (TBA) method described by Tayebi-meigooni *et al.* (2012) was used to determine the level of Malon-dialdehyde (MDA). An extinction coefficient of 155 ηm $^{-1}$ cm^{-1} was adapted to calculate the MDA concentrations using the following formula:

MDA (µmolg^{-1} fresh weight) = [(A_{532} – A_{600})/155] ×10^3×Dilution factor

The data were analyzed using statistical software (Statistix10) and comparisons with *P*-values<0.05 were considered significantly different by using honestly significant difference (HSD) values (Tukey's Test).

Results and Discussion

Screening of genotypes against salinity: Fifty-two mungbean genotypes were used for screening against one salt stress (8 dSm^{-1}). The characters were studied in percent relative values for a proper understanding of the salinity tolerance in mungbean genotypes. Relative plant height ranged from 45.6 to 82.0, with a mean of 63.3. In mungbean genotypes, there was remarkable variation in root dry weight which ranged from 14.5 to 99.0 with a mean of 56.4. Relative shoot dry matter of mungbean genotypes fluctuated from 27.3 to 100.0 with a mean of 58.1, which were significantly different among the genotypes with a corresponding mean of 39.16.

Table 1. Variation in quantitative plant characters of 52 mungbean genotypes under salinity stress.

Plant characters	Minimum	Maximum	Mean	LSD	CV (%)
Relative plant height	45.6	82.0	63.32	17.01	13.4
Relative root dry weight	14.5	99.0	56.43	17.58	15.5
Relative shoot dry weight	27.3	100.0	58.10	39.16	33.5

The genotypes BD-6888, BD-6895, BD-6906, BD-10028, and BD-10585 performed better in relative plant height in salinity-stressed conditions (Fig. 1). Salinity-induced stunted plant growth and necrosis of leaf were observed among the different pulse crop varieties where the growth of aerial shoot was more affected compared to that of the root. Sehrawat et al. (2015) reported a reduction in height and other growth parameters in mungbean in response to salinity stress. Greenway and Gibbs (2003) mentioned the destruction of energy in a saline condition caused growth retardation in the plant.

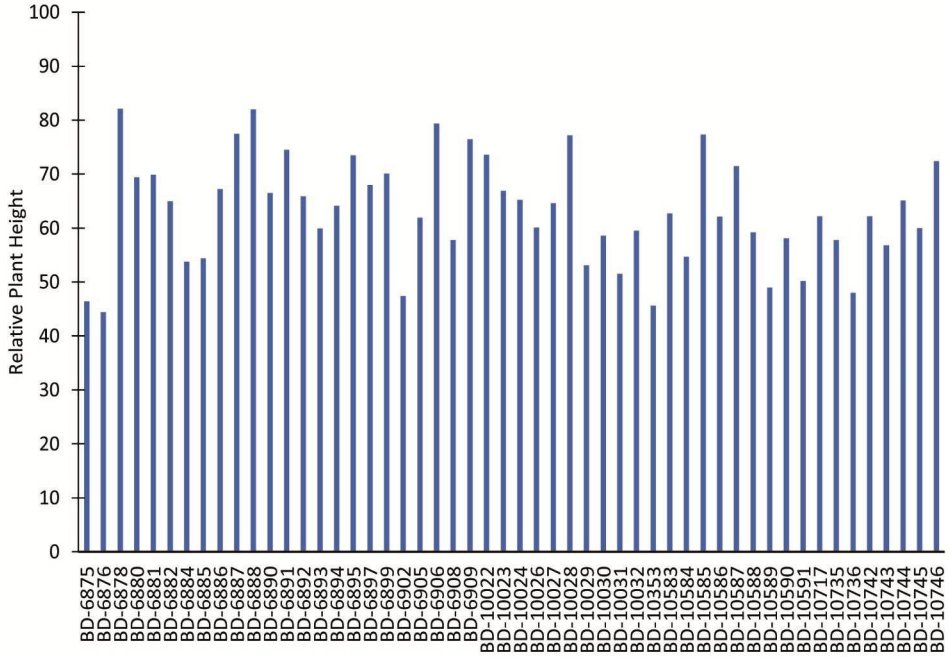

Fig. 1. Relative plant height (%) of 52 mungbean genotypes under salt stress at 8 dSm^{-1}

Relative root dry matter

The genotypes cv. BD-6878, BD-6887, BD-6888, BD-6897, and BD-6908 performed better in salinity-stressed conditions (Fig. 2). The increase in salt concentration has been reported to reduce the partitioning of dry matter significantly in *Glycine max* and *Phaseolus vulgaris* (Taffouo *et al.*, 2009). Dai et al. (2009) reported that in perennial ryegrass, root dry matter is reduced with increasing salinity. The findings of the present results are also similar to some researchers' reports claiming that root weight decreased as increment with salt concentration for some plants (Kaya *et al.*, 2005).

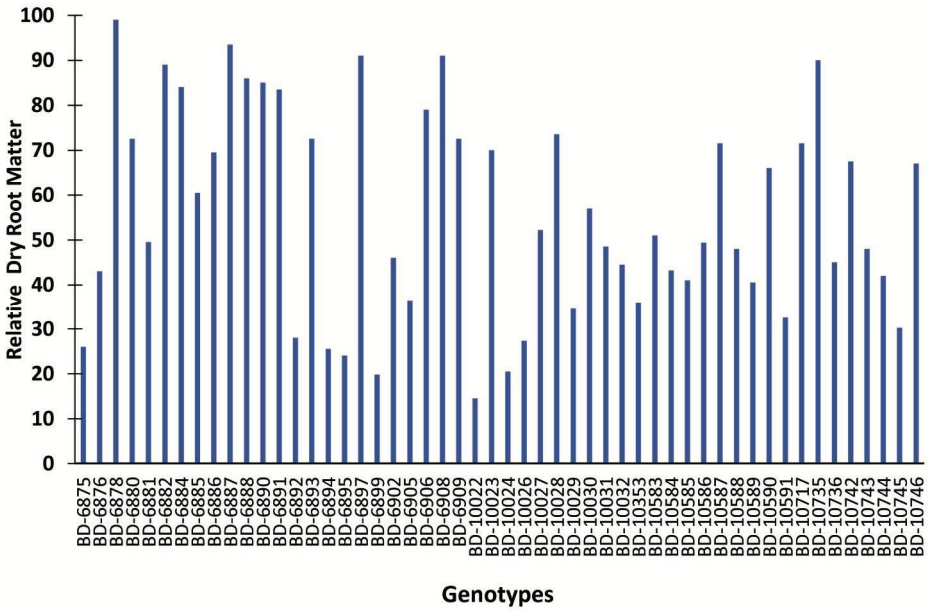

Fig. 2. Relative dry root matter (%) of 52 mungbean genotypes under salt stress at 8 dSm^{-1}

Relative shoot dry matter

Relative shoot dry matter (%) was severely affected by the salinity in 52 mungbean genotypes under saline conditions (Fig. 3). However, some genotypes cv. BD-6878, BD-6885, BD-6887, BD-6888, BD-6895, BD-6906, BD-10585, BD-10587, BD-10588, and BD-10717 performed better under saline stress conditions compared to that of the others.

The plant height of mungbean was reported to be reduced drastically in saline conditions (Ullah *et al.*, 2016). Salt stress was reported to reduce the growth of most legumes, as well as mungbean (Kabir *et al.*, 2004). These reductions in growth often resulted in reductions in tissue water potential, eventually reducing water availability to the cells (Garg and Bhandari, 2016), which leads to stomatal closing, less photosynthesis, and ultimately stunted growth (Garg and Manchanda, 2009).

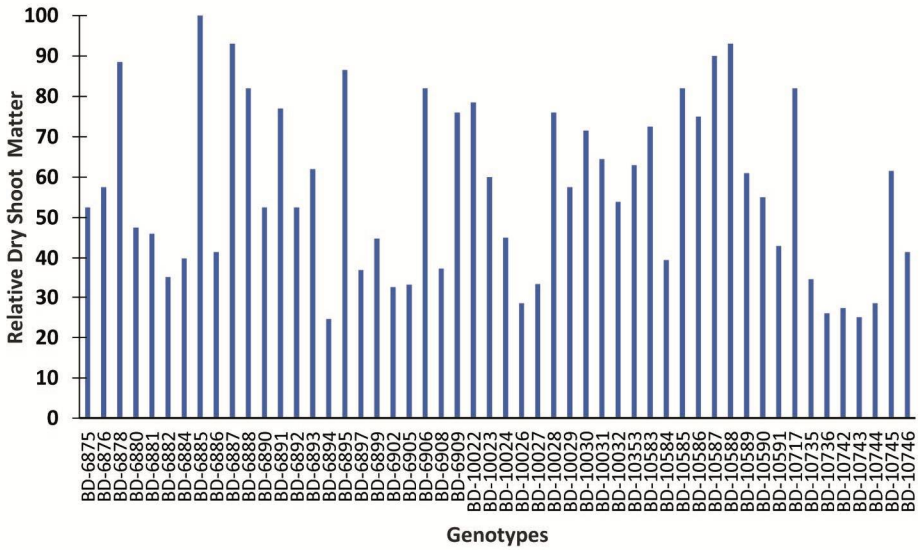

Fig. 3. Relative shoot dry matter (%) of 52 mungbean genotypes under salt stress at 8 dSm^{-1}

Grouping of the mungbean genotypes based on dry shoot matter

Grouping of the mungbean genotypes based on dry shoot matter under salinity stress (Ashraf and Wahid, 2000) (Table 2).

Table 2. Grouping of mungbean genotypes based on dry shoot matter under salinity stress.

Scale	RSDW (%)	Frequency	Genotypes
Group I	>80	10	BD-6878, BD-6885, BD-6887, BD-6888, BD-6895, BD-6906, BD-10585, BD-10587, BD-10588, BD-10717
Group II	60-80	13	BD-6891, BD-6893, BD-6909, BD-10022, BD-10023, BD-10028, BD-10030, BD-10031, BD-10353, BD-10583, BD-10586, BD-10589, BD-10745
Group III	40-60	14	BD-6875, BD-6876, BD-6881, BD-6886, BD-6890, BD-6892, BD-6899, BD-10024, BD-10029, BD-10032, BD-10590, BD-10591, BD-10746, BD-6880
Group IV	< 40	15	BD-6882, BD-6884, BD- 6894, BD- 6897, BD-6902, BD-6905, BD-BD-6108, BD-10026, BD-10027, BD-10584, BD-10735, BD-10736, BD-10742, BD-10743, BD-10744

Genotypes were categorized into four groups, viz. group I, group II, group III, and group IV. Group I included ten genotypes viz. BD-6878, BD-6885, BD-6887, BD-6888, BD-6895, BD-6906, BD-10585, BD-10587, BD-10588 and BD-10717 in which RSDM more than 80%. This group produced the highest relative shoot dry matter.

Thirteen genotypes were found in group II namely, BD-6891, BD-6893, BD-6909, BD-10022, BD-10023, BD-10028, BD-10030, BD-10031, BD-10353, BD-10583, BD-10586, BD-10589, and BD-10745 with an RSDM range from 60 to 80%. This group was the second-highest shoot dry matter producer than other related traits. Fourteen genotypes viz. BD-6875, BD-6876, BD-6881, BD-6886, BD-6890, BD-6892, BD-6899, BD-10024, BD-10029, BD-10032, BD-10590, BD-10591, BD-10746, and BD-6880 were grouped in group III which was the (40-60) % RSDM. Fifteen genotypes BD-6882, BD-6884, BD-6894, BD- 6897, BD-6902, BD-6905, BD-BD-6108, BD-10026, BD-10027, BD-10584, BD-10735, BD-10736, BD-10742, BD-10743, and BD-10744 were grouped in group IV which was less than 40% of relative shoot dry matter.

Among the genotypes, one tolerant (BD-6895) and one susceptible (BD-6905) were selected for physiological evaluation. The selection was based on this screening and another experiment conducted in the department of Crop Botany (Khan *et al.,* 2022).

Screening and grouping based on responses were done at the seedling stages in chickpeas (Mustafa *et al.,* 2020). The shoot biomass under saline conditions and the shoot biomass production ratio under salt stress to that of the control showed notable differences at all sampling stages when 41 chickpea genotypes were screened against salinity stress (Serraj *et al.,* 2004). Generally, stunted growth of the plant is one of the most common salinity effects. A gradual reduction in plant height, root and shoot length, and root and stem dry matter with a progressive increase in salinity were found in several reports (Khan *et al.,* 2010).

Physiological and Yield Attributes of Salinity Tolerance in Mungbean

Chlorophyll content was increased with lower doses of salinity up to 6 dSm^{-1}, and after that declined with the increase of salinity (Fig. 4). The chlorophyll content of genotype BD-6905 declined sharply after 6 dSm^{-1} (Fig. 4 A). In the case of chlorophyll b, the genotype BD-6895 showed an increasing trend up to 8 dSm^{-1}, and after that declined sharply. On the other hand, genotype BD-6905 gradually declined after 6 dSm^{-1} (Fig. 4 B). In the case of total chlorophyll, both genotypes showed an initial increasing trend, and after that declined. However, the genotype BD-6895 showed better performance in total chlorophyll content.

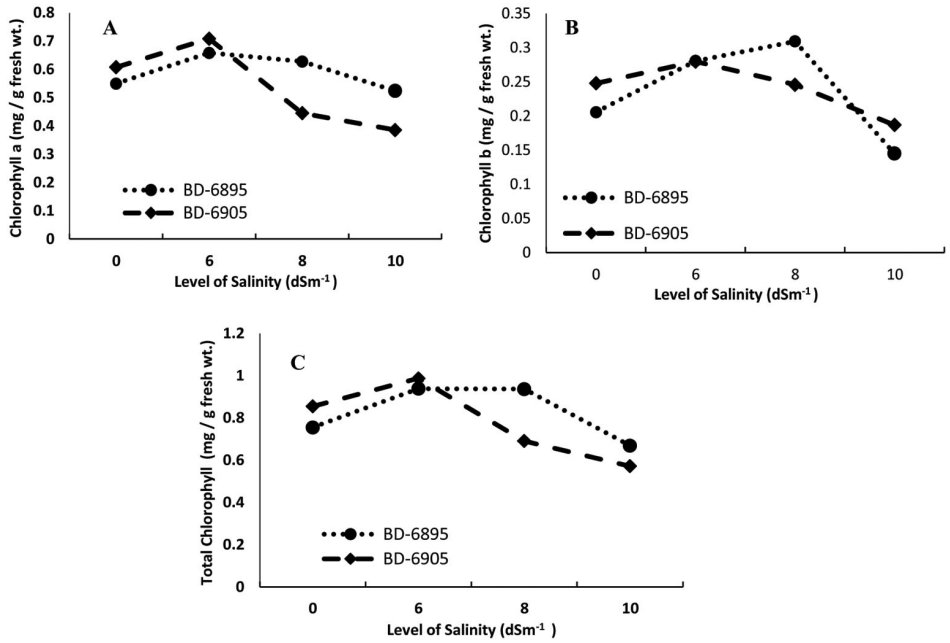

Fig. 4. Effect of varying salinity levels on mungbean genotypes' chlorophyll content (A-Chlorophyll a, B-Chlorophyll b, and C-Total chlorophyll content).

The reduction of chlorophyll content in mungbean genotypes in saline conditions was reported by Sehrawat *et al.* (2015). Salinity-induced reduction in chlorophyll level of the leaf was reported up to three-fold of control (Subashree *et al.*, 2021). With the increase in the salinity, Roychoudhury and Ghosh (2013) have reported a similar drop in the chlorophyll content.

Lipid Per Oxidase (MDA) Activity

The MDA (Malon-dialdehyde) contents of both the genotypes showed a decreasing trend with the increase of salinity levels where BD-6905 showed higher values (Fig. 5). Lipid peroxidation is an important membrane-damaging agent under abiotic stresses (Yang *et al.*, 2010) and MDA act as a marker to observe the ROS (reactive oxygen species)-induced membrane injury under stresses (Gong *et al.*, 2008). Oxidative stress markers such as malondialdehyde (MDA) contents are amplified due to a continuous upsurge in salt stress (Ghosh *et al.*, 2015). The salt tolerance mechanism can be achieved by decreasing malondialdehyde levels (Mahmood *et al.*, 2022).

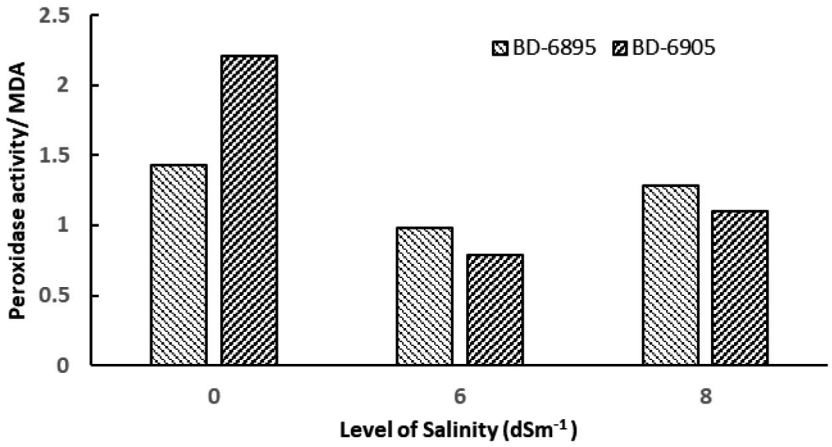

Fig. 5. Effect of varying salinity levels on the lipid peroxidase (MDA) accumulation in mungbean.

Proline Content

Higher proline was accumulated in both genotypes with the upsurge of salt levels. In this experiment, clear proline augmentation was observed with the increase in salinity. The genotype BD-6895 showed higher accumulation than BD-6905 (Fig. 6).

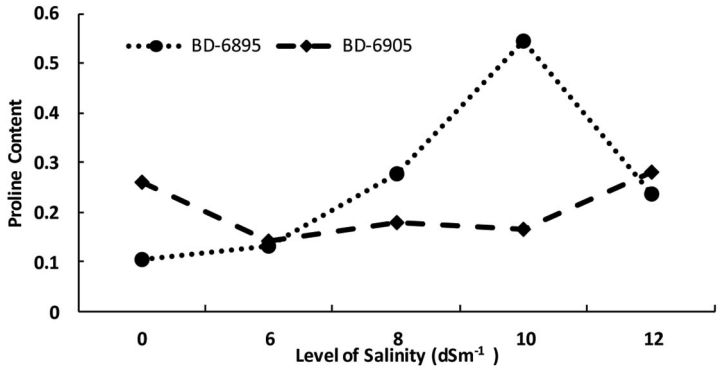

Fig. 6. Effect of salinity on the accumulation of proline in mungbean

Different osmolytes, like proline, induces physiological process favorably by maintaining the cellular equilibrium under salinity stress through the regulation of diffusion (Yang and Guo, 2018). The present experiment revealed that the leaf proline compound

increased with the salinity. A similarly increased accumulation of proline content under salt stress in mungbean was reported by Ghosh *et al.* (2015). Reddy *et al.* (2015) suggested that a higher proline accumulation may reduce saline-imposed stress and shield photosynthetic and antioxidant enzyme activities.

Dry Matter Accumulation

Shoot dry matters were seriously affected due to the imposement of salinity, where BD-6895 showed better performance than BD-6905 (Fig. 7). Similar trend was detected for the root dry matter and finally, the whole plant dry matter. Dry matter is one of the key aspects to evaluate plant performance. The highest dry matter was recorded in the control condition, and dry weight was reduced with the increasing salinity treatment (Benlioglu and Ozkan, 2020).

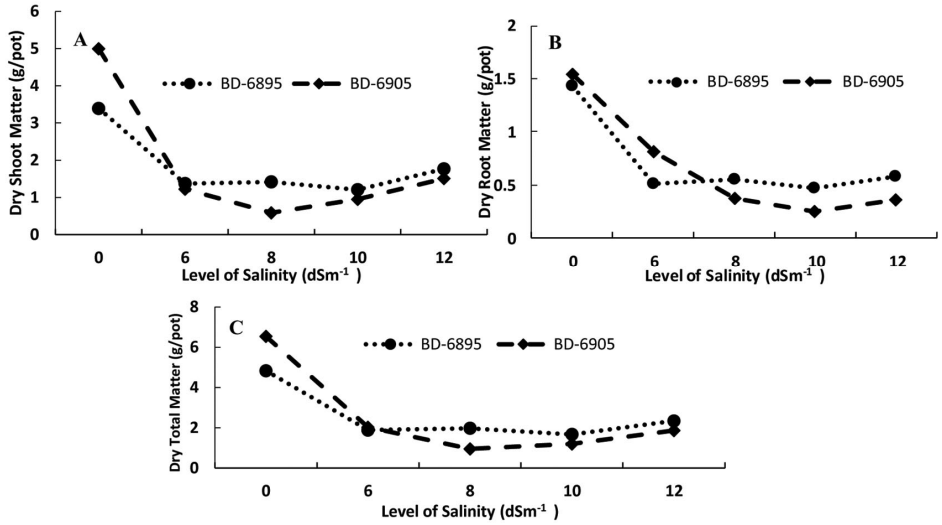

Fig. 7. Effect of varying levels of salinities on the dry matter accumulation in mungbean (A- Dry Shoot Matter, B-Dry Root Matter, C- Dry Total Matter).

Ercan (2008), in an experiment on lentils, reported a significant reduction in plant dry weights under saline stress and reported that reduction as a sign of susceptibility. Drought stress in sunflowers resulted in a drop in the whole plant's fresh and dry weight (Manivannan *et al.*, 2007). Several reports which showed decreases in the fresh and dry

weight of plants in salt shock (Baloğlu *et al*., 2012; Munir and Aftab, 2009; Mohammed, 2007) are similar to the inferences of the current experiment.

Among the legumes, mungbean bears an acceptable genetically inherent tolerance system, and many physiological traits are yet to be explored. It has the clear benefit of being a short-duration crop and can be grown in different types of soils and environments (Rao *et al*., 2016). The current series of the experiment focuses on the saline stress and the ability of mungbean to find a place in the saline coastal area of Bangladesh as a fill crop (mungbean-rice-wheat to replace fallow-rice-fallow) or a relay crop in the existing cropping pattern. Among the procured genotypes, BD-6895 was screened as tolerant, and BD-6905 as susceptible from the initial screening and findings of a previous experiment (Khan *et al*., 2022). Considering the physiological parameters, mungbean genotypes showed high proline and low MDA deposition in tissues under salt stress. Chlorophyll content also increased initially and declined sharply with the increase in the saline treatment. Of the two selected genotypes, the susceptible genotype BD-6905 resulted in a sharp deterioration of chlorophyll content and augmentation of the proline content compared to that of the tolerant one BD-6895. And that change is eventually reflected in the accumulation of root and shoot dry matter content, and ultimately, the total dry matter content of the mungbean plant.

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EFFECTS OF FEEDS ON SELECTED SHELLFISH (*MACROBRACHIUM ROSENBERGII*) AND FINFISHES (*PLANILIZA PERSIA* AND *RHINOMUGIL CORSULA*) IN POLYCULTURE SYSTEM: PROFITABILITY AND VIABILITY

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Abstract

The experiment was conducted at earthen ponds in the Bagerhat sadar upazila of Bagerhat to examine the growth, production capacity, and economic return of freshwater prawn (*Macrobrachium rosenbergii*) with persa (*Planiliza persia*) and corsula mullet (*Rhinomugil corsula*) under polyculture system. Four treatments, designated as T_1, T_2, T_3, and T_4 were used in the study, each with three replicates. The final weight of the prawns after 120 days of culture was highest in T_1 (72.07 g) and lowest in T_4 (50.23 g), but there was no significant difference in prawn growth between T_1 and T_2. In T_4, where artificial feed was not employed, prawn, persa, and corsula mullet growth and survival rates were lower. T_1 produced more prawns (173.27 kg ha^{-1}), while T_4 produced less (617.83 kg ha^{-1}). Significantly ($p<0.05$) higher production of persa was found in T_1 (295.97 kg ha^{-1}) and lower in T_4 (152.28 kg ha^{-1}). Corsula production was also observed to be higher in T_1 (275.70 kg ha^{-1}) and lower in T_4 (155.36 kg ha^{-1}). However, T_1 had much higher total production and net profit from prawn and fish farming (1744.94 kg ha^{-1}, BDT 244694.75 ha^{-1}), whereas T_4 had a significantly lower total production and net profit (925.46 kg ha^{-1}, BDT 115894.42 ha^{-1}). According to the study, quality feed (T_1) outperforms other commercial feeds in terms of growth, production, and net profit. In order to increase productivity and get a high return on investment in a short period of time. The quality feed can be recommended.

Key words: Polyculture, Survival rate, Economic return, Water quality.

Introduction

The nation's socioeconomic development is significantly influenced by the resources of the fishing industry. The fisheries sector provides 3.52% of the national GDP and 26.37% of the entire agricultural GDP. More than 12% of Bangladesh's almost 170 million people rely on fisheries and aquaculture-related activities for their livelihoods, either full- or part-time. Bangladesh produced enough fish to meet its own needs, consuming 63.01 g of fish per capita per day as opposed to the goal of 60 g (DoF, 2020).

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Both "golda" (*Macrobrachium rosenbergii*) and "bagda," (*Penaeus monodon*), often known as "white gold" or "dollar," are significant fisheries products. 42,749 metric tons of shrimp were harvested from the sea in 2018-2019. In the 2018-2019 fiscal year, frozen shrimp and prawns earned 3088.85 crore BDT as foreign exchange (DoF, 2020).

One of the most significant current industries of Bangladesh's national economy is freshwater prawn fishing. The gigantic freshwater prawn, *M. rosenbergii*, is primarily used for aquaculture in Bangladesh's current freshwater prawn fishery. The fisheries sector has drawn much attention due to its enormous export potential. Unfortunately, since shrimp and prawns are not differentiated in the fisheries sector survey, freshwater prawn fishery statistics continue to be inconsistent, fragmentary, and frequently erroneous (Ahmed *et al.*, 2008).

Because prawns are more disease resistant than shrimp and have a higher production rate and higher profitability than other shrimp, farmers in the southwestern region of Bangladesh are gradually focusing more on prawn farming (Huque, 2007). In their shrimp and prawn farms, farmers are now more frequently involved in the unplanned and unmanaged culture of tilapia (*Oreochromis* sp.), rui (*Labeo rohita*), grass carp (*Ctenopharyngodon idella*), persa (*Planiliza persia*), and khorsula (*Rhinomugil corsula*) with prawns. However, they are ignorant of the scientific methods used to handle prawns and other species (Islam and Mahmud, 2011).

P. persia is one of the most popular and well-liked finfish among the locals because of its taste and demand. These non-carnivorous species are also easily cultivated in shrimp and prawn production zones, which are essential for maintaining healthy conditions for both shrimp/prawn and finfish (Ali *et al.*, 2000). They consume mostly debris, diatoms, algae, and minute invertebrates as filter feeders (McDonough and Wenner, 2003).

Due to its high nutritional content, consumer choice, and market price, *R. corsula*, also called the "corsula mullet" and "domra" in southern Asia and Australia, is a commercially significant fish. It is also known as the "false four-eyed fish" in tropical Americas. It is extensively dispersed across Australia, Bangladesh, Nepal, and Myanmar in the eastern Indian Ocean. *R. corsula* is an economically significant possibility for polyculture with shrimp and prawns and is simple to cultivate in shrimp and prawn growing regions (Menon, 1999; Shofiquzzoha *et al.*, 2001; Sultana, 2013). It can withstand a wide range of environmental changes and is found at depths of 10-15 m in freshwater, brackish estuaries, and marine water (Riede, 2004).

The survival rate of juveniles of *M. rosenbergii* is unaffected by finfish stocking because *P. persia* and *R. corsula* do not eat them. On the other hand, raising prawns in

environmentally friendly environments may aid in increasing productivity in shrimp/prawn ponds (Shofiquzzoha and Alam, 2008). The current study was carried out to evaluate the impacts of feeds on growth, production, and financial returns in a polyculture system of *M. rosenbergii*, *P. persia*, and *R. corsula* in light of the aforementioned facts.

Materials and Methods

Study area and design: The experiment was conducted in twelve earthen ponds of brackish water in the sadar upazila of Bagerhat (Fig. 1). The average pond measured 400 m^2 in the area and ranged in depth from 0.8 to 1.6 m. The experiment lasted 120 days, from March to July 2019 and was divided into four treatments (T_1, T_2, T_3, and T_4), each of which had three replications. Ponds were chosen at random for each treatment. In all treatments, *M. rosenbergii* (prawn), *P. persia* (persa), and *R. corsula* (corsula mullet) were stocked at the same density (3:1:1).

Pond preparation and management: Ponds treated with agricultural lime ($CaCO_3$) at a rate of 250 kg ha^{-1} dependent on soil pH and gradually filled with tidal water up to a depth of 0.9 m from the adjacent tidal canal through a screen net. Rotenone at a concentration of 3 ppm was used to eradicate all undesirable species, and its effect was neutralized by lime at 125 kg ha^{-1}. Ponds were fertilized with urea and Triple Super Phosphate (TSP) at 50 and 100 kg ha^{-1}, respectively, after 5 days of cleaning. To keep out potential disease vectors like snails, snakes and others, pond dikes were covered with nylon nets with a fine mesh size.

Stocking and feed management: For all treatments, uniform-sized fingerlings of persa, corsula, and juvenile prawns were released in experimental ponds at 3 m^{-2}, 1 m^{-2}, and 1 m^{-2}, respectively. The polythene bags were held in a few selected ponds after the juvenile and fingerling prawn, persa, and corsula were collected for around 45 minutes to acclimate before being released into all the ponds. Commercial fish feed, including quality feed, mega feed, and nourish feed, was applied to the ponds six days a week at a rate of 10% of the total biomass of prawn, persa, and mullet for the first month, 6% for the second month, and subsequently fell to 3% until the end of the study.

Water quality monitoring: Temperature, salinity, transparency, dissolved oxygen (DO) concentration, pH, total alkalinity, and ammonia concentrations of the water were measured between 9.00 and 10:00 am after ten days. A portable refractometer, a standard centigrade thermometer, a Secchi disc, and a DO meter were used to test salinity, water

temperature, transparency, and dissolved oxygen, respectively. A pH meter was used to record the water's pH. Titrimetric analysis was used to determine the total alkalinity. Using an ammonia test kit, ammonia nitrogen was determined.

Sampling of prawn and finfishes: The biomass of the stocked species was estimated, the feeding rations were adjusted, and physical conditions of the stocked species were also observed through a fortnightly sampling of 10-15% of the prawn, persa, and corsula. Cast nets were used to sample prawns and fish, and each species' weight and length were recorded for growth evaluation. Sampling persisted until the harvest.

Estimation of growth, survival, and production of prawn and finfishes: Water was removed from ponds after 120 days of culture, and all prawns and fish were taken by repeatedly netting them (cast net and surrounding net). Each individual was numbered, measured, and weighted to calculate the survival rate, growth, and production of each pond's harvested prawn and fish. Specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (%) was calculated following the equation as cited by Pechsiri and Yakupitiyage (2005). The following are the equations:

Weight gain (g) = Mean final weight (g) - mean initial weight (g).

Specific growth rate (SGR) (%/day)

= {Ln (final body weight) – Ln (initial body weight) ×100}/cultured period (days).

Feed conversion ratio (FCR)

= Feed consumed (g dry weight)/live weight gain (g wet weight) of prawn/fish

Survival rate (%)

= (Number of prawn/fish harvested ÷ total number of prawn/fish stocked) × 100

Production of prawn/fish= No. of prawn/fish caught × average final weight of prawn/fish.

***Production and economic analysis*:** The following equations used to calculate production and profitability (Chowdhury *et al.* 2020, Dillon and Hardaker 1993).

Gross return (GRi) = $\Sigma iPiQi$

Net return (π) = $\Sigma i(PiQi)$ $-TFC$ $-TVC$

Benefit cost ratio (BCR) = GRi/TC

Here, Pi = market value of harvested prawn and finfish in BDT, Qi = production (kg ha^{-1}), i = treatments, TFC = total fixed cost, TVC = total variable cost and TC = total cost (TFC + TVC). Total net return divided by total input cost was used to calculate the

benefit cost ratio (BCR). Prices for a number of inputs, including prawn, persa, and corsula, were consistent with those of the Bagerhat wholesale market in 2019. Persa and corsula were sold at rates of BDT 100.00-120.00 kg^{-1} and BDT 500.00-550.00 kg^{-1}, respectively, for prawn.

***Statistical analysis*:** The information was presented as mean and standard deviation (SD). Utilizing IBM SPSS Statistics version 23, all data were examined. Before performing a one-way analysis of variance (ANOVA), data were first evaluated for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test for equality of variance). The variance was roughly equal across the board, and all data were normally distributed. Both requirements were met, so ANOVA was used to test economic, production, and growth data. Tukey's HSD test was used to compare significant mean differences at $p < 0.05$. To determine how different feeds could predict growth metrics, linear regression was used. It was also determined how well the regression model fit the observed data using the coefficient of determination (r^2).

Results and Discussion

Growth, FCR and survival of prawn and finfishes: The prawn's final weight was highest in T_1 (72.07 g) and lowest in T_4 (50.23 g). For finfish, T_1 had the highest final weight of persa (51.03 g) and the lowest (33.84 g), whereas T_1 had the highest final weight of corsula (53.02 g) and the lowest (38.84 g) (Table 1). In a 150-day prawn and tilapia mixed culture in Bagerhat farmers' shrimp ponds, Islam *et al.* (2016) reported that the mean final weight of prawn and tilapia was 58-63 and 149-199 g, respectively. Islam and Mahmud (2012) determined the final weights of prawns and tilapia that were raised in mixed culture for 180 days at various stocking densities in the Shrimp Research Station (SRS) pond complex, Bagerhat. They pointed out that the weights were 63–73 g and 163.5–168.5 g, respectively. The final weight of prawn and tilapia in brackishwater ponds at various stocking densities for 180 days in the SRS pond complex was similarly pointed out by Islam and Mahmud (2011) to be between 74 and 85 g and 99 and 149 g, respectively. These results differ slightly from those of the most recent study. Shofiquzzoha and Alam (2008) reported the final shrimp and silver barb weights to be 23.77 and 69.75 g, respectively, after 120 days of continuous cultivation in the Brackishwater Station (BS) pond complex in Khulna. These outcomes are less favorable than those from the current investigation. The final weights of the shrimp and tilapia, measured after 120 days in the same pond complex, were 24.93 and 161.83 g, respectively. Additionally, this is lower than the most recent findings.

For 120 days, the weight gain per day for the prawn, persa, and corsula was 0.31 to 0.57, 0.27 to 0.89, and 0.31 to 0.43 g, respectively (Table 1). In shrimp ponds, the daily weight of prawn and tilapia as 0.39-0.42 and 0.99–1.33 g, respectively, by Islam *et al*. (2016). The daily weights of tilapia and prawn at various stockings were 0.35 to 0.41 and 0.91 to 0.94 g, respectively, according to Islam and Mahmud (2012). Islam and Mahmud (2011) also observed that the daily weight of prawns and tilapia in brackishwater ponds was 0.41 to 0.47 and 0.55 to 0.83 g, respectively. Shofiquzzoha and Alam (2008) stated that the daily weight of shrimp and silver barb in concurrent cultivation was 0.20 and 0.55 g, respectively, for 120 days, which is less than the current findings. In the same pond complex, they also observed the daily weights of shrimp and tilapia for 120 days as 0.21 and 1.34 g, respectively. These values are similarly lower than the current findings.

The prawn's specific growth rate (SGR) varied from 1.26 to 1.66%. On the other hand, T_1 had the highest SGR of persa (2.81%), while T_4 had the lowest (2.47%). T_1 had a greater SGR of corsula (2.80%), while T_4 had a lower SGR (2.54%) (Table 1). The ranges of shrimp SGR reported by Akter *et al.* (2019) for 120 days at Bagerhat sadar upazila of Bagerhat are higher than the results of the current study. In shrimp ponds, Islam *et al*. (2016) found that the SGR for prawn and tilapia was 1.52-1.65 and 3.98-4.13%, respectively. According to reports from Islam and Mahmud, 2012, the SGR for prawns varied between 1.71 and 1.80 and 3.13 and 3.15%, respectively. The results for prawn in the research described above are lower; however, the results for tilapia are higher than those of this study. In contrast to the present findings, Shofiquzzoha and Alam (2008) showed that the SGR of shrimp and tilapia was 6.94 and 4.26%, respectively, during 120 days.

The feed conversion ratio (FCR) of shrimp and fish was much greater in T_4 (3.7) and significantly lower in T_1 (2.40). The results of Islam *et al*. (2016), who recorded the FCR of prawn and tilapia as 2.70-3.60, are consistent with these observations. FCR changes with stocking density, feed quality, and the size at which shrimp, prawns, and fish are harvested, according to Chanratchakool *et al.* (1995). Additionally, it depends on the population dynamics and the production cycle. Depending on the quality of the additional feed and the mean weight of the prawn, shrimp, or fish as it grew, FCR increased or decreased, claims Hasan (2001). Due to the prawn and finfish's efficient utilization of the maximum ratio, T_1 in this study had the lowest FCR.

There was no statistically significant difference between T_1 and T_2, however the survival rate of prawns was substantially ($p<0.05$) higher in T_1 (54.002±52%) and T_2 (52.002±08%) than in T_3 (49.003±51%) and T_4 (41.003±06%). On the other hand, T_1 had

the highest persa survival rate (58.01±53%), and T_4 had the lowest (45.002±52%). Additionally, T_1 had the highest corsula survival (52.03±06%), and T_4 had the lowest (40.002±08%). Under four treatments, a significant difference in persa and corsula survival was seen (Table 1). Similarly to this, Islam *et al*. (2016) showed that prawn and tilapia survived for 150 days at rates of 66-72 and 56.2-65.5%, respectively, while Islam and Mahmud (2012) found that prawn and tilapia survived for 180 days at rates of 62-70%, and 68-71.5%, respectively. Islam and Mahmud (2011) noted that these species had 58-65%, 67%, and 73% survival rates, respectively. The intra- and inter-specific rivalry among the stocked animals may cause the significantly reduced SGR and prawn survival seen in T_4 in the current investigation. According to Garcia-Perez *et al*. (2000), various parameters, such as environmental stress, water level, the amount of feed needed, stocking ratio, cannibalism, bird predation, predator fish, etc., affect the survival of prawns and shrimp. Cannibalism often occurs during the molting phase and may be to blame for the 4% monthly death rate (AQUACOP 1990).

Production of prawn and finfishes: T_1 had the largest prawn output (1173.27 kg ha^{-1}), whereas T_4 had the lowest (617.83 kg ha^{-1}) (Table 1). Additionally, it increased the polyculture system's overall production and financial gain from raising shrimp and fish. T_1 had a higher persa production (295.97 kg ha^{-1}), while T_4 had a lower persa production (152.28 kg ha^{-1}). T_1 produced more corsula (275.70 kg ha^{-1}) than T_2 (239.18 kg ha^{-1}), T_3 (193.80 kg ha^{-1}), and T_4 (155.36 kg ha^{-1}), respectively. This difference was statistically significant ($p<0.05$). In T_1, combined production was significantly greater (1744.94 kg ha^{-1}); in T_4, it was lower (925.46 kg ha^{-1}) ($p<0.05$) (Table 1). The combined output of prawn and tilapia was found to be 2491.80-2510.60 kg ha^{-1} in 150 days and 2191.39-2441.47 kg ha^{-1} in 150-180 days, respectively, by Islam *et al*. (2016) and Islam and Mahmud (2012). The observed production was lower. In contrast, the shrimp output reported by Islam and Mahmud (2010) and Shofiquzzoha and Alam (2008) was 416.9-641.7 kg ha^{-1} and 402.73 kg ha^{-1}, respectively, which is less than the production recorded in the current study. Because three different species were stocked, the provided feed had greater protein content, the prawn and finfish fry were larger, and the water quality was well-managed, the total production of the current study was higher than that of Islam and Mahmud (2010). According to Asaduzzaman *et al*. (2009), the combined yield of prawn and tilapia was 1,763.0 kg ha^{-1}/120 days, which is nearly identical to the current data. The highest total production, 1,691 kg ha^{-1}, was reported by Uddin *et al*. (2006) in ponds stocked with 75% tilapia and 25% prawns, which is also less than the current study.

The overall net profit from prawn and fin fish farming in the current study was significantly greater in T_1 (BDT 244694.75 ha^{-1}) and lower in T_4 (BDT 115894.42 ha^{-1})

(Fig. 2). Additionally, the benefit-cost ratio (BCR) was higher in T_1 (1.56) and lower in T_4 (1.48). The observed profit was somewhat greater than Islam *et al.* (2016)'s findings, which estimated that brackishwater ponds' prawn and tilapia aquaculture generated BDT 147, 819.00-238,923.00 ha^{-1} in profit. According to Islam and Mahmud (2011), the profit from prawn and tilapia culture ranges from BDT 137,021.00 to 236,797.00 ha^{-1}, which is also less than the results from the current study. Islam and Mahmud (2010) reported BDT 45,086.33-181,182.35 ha^{-1} as the net profit of shrimp farming, which is significantly less than the current study's findings. This shows that treating quality feed (T_1) yielded higher net profits and BCR than other methods.

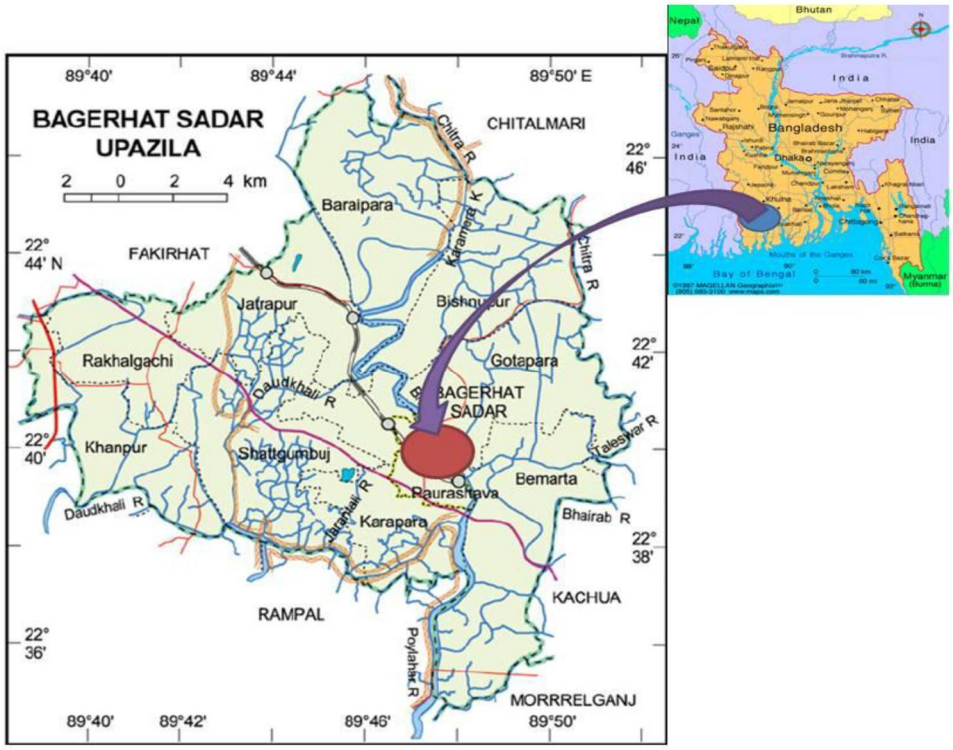

Fig.1. Map of Bagerhat sadar upazila showing the experimental area (Source: Modified from Google Maps).

Water quality parameters: The range of the water temperature was 28.95 to 32.98°C, which is consistent with the results of Islam *et al.* (2016), and Islam and Mahmud (2012), who reported that the water temperature ranges were 27.0-32.3°C and 28.0-35.5°C,

respectively. The concentration of dissolved oxygen (DO) was 4.34 to 5.25 mg l^{-1}, which is comparable to the findings of Islam *et al.* (2016), who recorded that the DO ranges from 4.0 to 5.1 mg l^{-1} in shrimp ponds.

Table 1. Growth, survival rate, and production (mean±SD) of *Macrobrachium rosenbergii*, *Planiliza persia* and *Rhinomugil corsula* in different treatments.

Parameters	Treatments			
	T_1 (Quality feed)	T_2 (Mega feed)	T_3 (Nourish feed)	T_4 (Control)
Macrobrachium rosenbergii				
Stocking density (nos. m^{-2})	3	3	3	3
Average initial weight (g)	11.01±1.38	11.05±1.16	11.11±2.30	11.21±1.78
Average final weight (g)	72.07^{a} ±2.95	70.24^{a} ±1.28	65.21^{b} ±1.25	50.23^{c} ±2.28
Daily weight gain (g)	0.57^{a} ±.0.02	0.49^{b} ±0.02	0.45^{b} ±0.02	0.32^{c} ±0.02
Specific growth rate (% day^{-1})	1.66^{a} ±0.11	1.54^{a} ±0.10	1.49^{b} ±0.16	1.26^{c} ±0.14
Survival rate (%)	54.00^{a} ±2.52	52.00^{a} ±2.08	49.00^{b} ±3.51	41.00^{c} ±3.06
Production (kg ha^{-1})	1173.27^{a} ±8.24	1095.74^{b} ±79.70	958.59^{c} ±64.74	617.83^{d} ±31.15
Planiliza persia				
Stocking density (nos. m^{-2})	1	1	1	1
Average initial weight (g)	1.75±0.04	1.75±0.03	1.75±0.03	1.75±0.04
Average final weight (g)	51.03^{a} ±1.82	47.74^{b} ±1.46	42.76^{c} ±2.72	33.84^{d} ±1.68
Daily weight gain (g)	0.41^{b} ±0.02	0.38^{b} ±0.01	0.89^{a} ±0.03	0.27^{c} ±0.01
Specific growth rate (% day^{-1})	2.81^{a} ±0.05	2.76^{b} ±0.04	2.66^{c} ±0.07	2.47^{d} ±0.05
Survival rate (%)	58.00^{a} ±1.53	53.00^{b} ±2.52	50.00^{b} ±2.65	45.00^{c} ±2.52
Production (kg ha^{-1})	295.97^{a} ±11.04	253.02^{b} ±15.09	213.80^{c} ±20.55	152.28^{d} ±11.75
Rhinomugil corsula				
Stocking density (nos. m^{-2})	1	1	1	1
Average initial weight (g)	1.83±0.03	1.83±0.05	1.83±0.04	1.83±0.04
Average final weight (g)	53.02^{a} ±5.35	50.89^{b} ±3.41	45.07^{c} ±3.73	38.84^{d} ±2.70
Daily weight gain (g)	0.43^{a} ±0.05	0.41^{b} ±0.03	0.36^{c} ±0.03	0.31^{d} ±0.02
Specific growth rate (% day^{-1})	2.80^{a} ±0.08	2.77^{b} ±0.05	2.67^{c} ±0.08	2.54^{d} ±0.07
Survival rate (%)	52.00^{a} ±3.06	47.00^{b} ±2.00	43.00^{c} ±2.65	40.00^{d} ±2.08
Production (kg ha^{-1})	275.70^{a} ±13.84	239.18^{b} ±12.63	193.80^{c} ±11.52	155.36^{d} ±14.34
FCR (all species)	2.4^{c} ±0.06	3.0^{b} ±0.15	3.5^{a} ±0.20	3.7^{a} ±0.21
Combined production (kg ha^{-1})	1744.94^{a} ± 5.52	1587.93^{b} ± 79.61	1366.18^{c} ± 93.19	925.46^{d} ± 48.83

Mean values in the same row with the same superscript letters are not significantly different ($p>0.05$).

Water salinity ranged from 3.45 to 6.97 ppt, supporting the findings of Islam *et al.* (2016), who found that shrimp ponds had water with a salinity of 1.5 to 6.5 ppt. According to Akter *et al*. (2019), which is more recent than the current investigation results, the salinity ranges for *Penaeus monodon* and tilapia ranged between 3.50 and 6.14 ppt. Ammonia nitrogen (NH_3-N) concentrations ranged from 0.002 to 0.090 mg l^{-1}, which is consistent with Meade's (1985) and Islam *et al*. (2016)'s findings that it was within an acceptable level (>0.012 mg l^{-1}) for prawn/shrimp aquaculture.

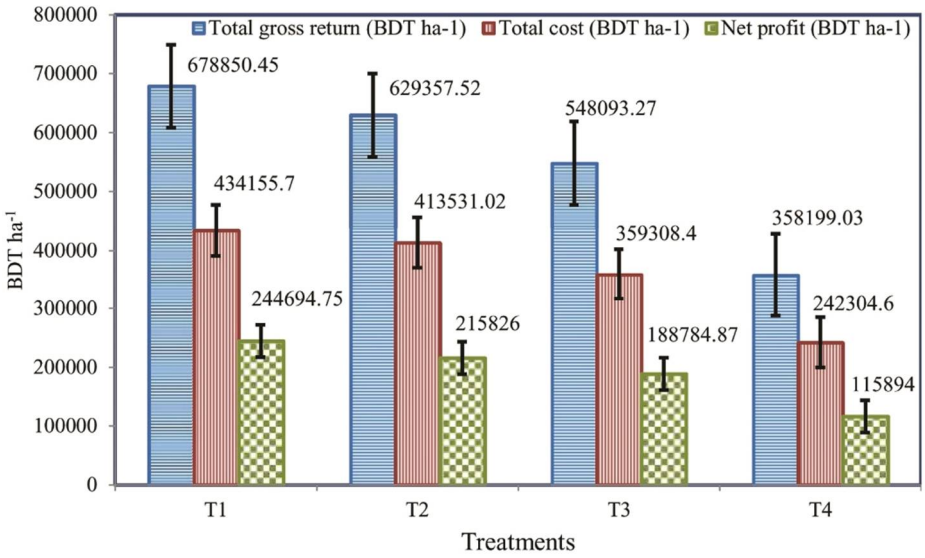

Fig.2. Cost and economic return of *M. rosenbergii, P. persia* and *R. corsula* farming during the study period.

Due to its role in increasing global prawn, shrimp, and fish production, polyculture systems are currently more popular than monoculture. The quality feed (treatment 1) is the best treatment among all treatments in terms of growth, productivity, and net profit, according to current study's findings. Finfish inclusion had no adverse effects on prawn growth or productivity. This commercial feed could be employed in prawn and finfish polyculture systems in the coastal area to increase prawn and finfish production, which would be financially rewarding, technically feasible, and socially acceptable.

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INCIDENCE OF CHILLI MITE ON CHILLI VARIETIES UNDER FIELD CONDITIONS

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ABSTRACT

The current experiment was carried out to evaluate the occurrence of mites on chilli (*Capsicum* spp.) leaves. The trial was conducted in the experimental field and laboratory of RSRC, BARI, from December 2020 to April 2021. Three released varieties (viz. BARI Morich 1, BARI Morich 2, and BARI Morich 3) and six genotypes (Viz. G10, G13, G25, G27, G30, and G31) were used as the test crops of the experiment. The findings showed a considerable amount of variation among the treatments. Regarding the number of mites/leaf, the G25 had the highest mean number (7.24) whereas the G31 had the lowest (0.69). The G30 was the most productive in terms of yield (512.27 g/plant). The weight of each fruit and the number of fruits per plant were directly related to yield. On the contrary, the G10 and the G13 supplied an optimum yield per plant, whereas the G25 produced the least yield (21.02/plant). The correlation between yield and mite infestation was negative, and the results revealed that the BARI Morich-1, the G27, and the G31 were highly resistant to chilli mite infestation, and the G13, the BARI Morich-3, and the G30 were resistant, while the BARI Morich-2 was only moderately resistant. The G10 was tolerant and the G25 was susceptible to chilli mite infestation. The results concluded that the tested genotypes G13, G27, G30 and G31 showed remarkable resistance to mite infestation.

Key words: Chilli, Chilli mite, Infestation, Yield, Resistant.

Introduction

Nowadays, Chilli (*Capsicum* spp.) has become a major economic crop in the world. It is widely cultivated in the warm temperate, tropical, and subtropical parts of the world. It has a great potential demand with versatile uses, such as spice and vegetables. The use of

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chilli has expanded in culinary, medicinal, and many food and beverage industries worldwide (Tatagar *et al*., 2011, Jangra *et al.,* 2017). As an essential spice, chilli is rich in vitamins C, A, and B, oleoresins, and red pigment and is used to add color and spiciness to dishes (Tatagar *et al*., 2011). The most serious pest of chilli is *Polyphagotarsonemus latus* (Banks), commonly recognized as a broad mite, or yellow mite or chilli mite (de Coss-Romero and Peña, 1998) and caused severe yield loss (Keerthana *et al*., 2022). Mites are a significant issue in growing chillies. They mainly occur on the young shoots at the tips of the chilli plant. Adults and nymphs appear especially on the underside of leaves to suck cells (Kumar *et al*., 2019).

Downward curling along with the brittleness of leaves, extension of petioles of elder mature leaves, and bunching of young leaves at the tip of the branches are common symptoms of mite infestation in plants. Flowers are distorted and do not open properly. During a heavy infestation, shortened internodes and premature fruit drops may occur in most infested hosts (Aarwe *et al*., 2019). In severe conditions, defoliation, shedding of buds and drying of growing points may occur. Toxic mite saliva is responsible for stunted or dead shoot growth (Kumar *et al*., 2019).

The plants of the genus *Capsicum* are extremely vulnerable to injuries triggered by these mites, and 10 individuals per plant are enough to cause significant damage and reduce crop yields (de Coss-Romero and Peña, 1998, Rodrı´guez-Cruz, 2014). It can cause about 96.39% yield loss in chilli (Borah, 1987). The prevalence of leaf curl disease complex up to 80.23% has been reported in Karnataka (Venkatesh *et al*., 1998). About 21.29% of crop damage was reported due to mite invasion (Jeyarani and Chandrasekaran, 2006).

Due to the minute size of chilli mites, farmers need help understanding the incidence of chilli mites on the crop. They use different insecticides in their field to prevent other insects, and the wide exposure to them helps chilli mites become resistant to insecticides. And that makes such pests challenging to control chemically, and eventually, growing costs have increased so much that chilli cultivation has become less profitable. Moreover, the repeated application of insecticides results in increased pesticide residues on produce and the environment. It poses a threat to the ecosystem, apart from destroying natural predators and resurgence of chilli mites, the main threat to chilli production (David, 1987).

Considering the beneficial role of chilli discovered in the new scientific research emphasizing its adaptation in many areas of the world is increasing. To meet the demand,

chilli production needs to be increased, and the selection of a desirable mite-tolerant or resistant line for commercial cultivation in Bangladesh is imperative. The main purpose of this work is to screen advanced chilli lines with registered varieties, aiming for tolerance to chilli mites, and high-yielding varietal development in mind. Given the information mentioned above, studies were conducted with the following goals in mind:

(i) to reveal the occurrence of mites on the leaf of chilli varieties/advanced lines and
(ii) to evaluate the chilli varieties/advanced lines in terms of resistance to chilli mites.

Materials and Methods

The research trial was conducted in the experimental field and laboratory of the Regional Spices Research Centre (RSRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, during the period from December 2020 to April 2021. The experiment was executed with three replications in a randomized complete block design (RCBD). The total land area was divided into small plots at the experimental site. Each plot area was 3 m × 1 m with a spacing of 60 cm × 45 cm. Three released varieties (BARI Morich-1, BARI Morich-2, and BARI Morich-3), and six genotypes (G10, G13, G25, G27, G30, and G31) were used as the test crop of the experiments. Data on plant height, canopy diameter, number of branches, number of leaves, leaf surface area, fruit number, fruit diameter, fruit length, and yield (g/plant) were recorded.

The incidence of chilli mite was recorded at an interval of seven days and randomly five top leaves (third leaf) were collected for each of the three replications of each treatment to observe the incidence of mite in the plant following (Naituku *et al*., 2017). The leaves thus collected from the field were put in separate zip lock polypropylene bags according to replication of each treatment for observation of the mite population under a stereo-zoom binocular microscope (Olympus SZ2-ILST) following Samanta et al. (2017). From both surfaces (dorsal and ventral), the number of mites per leaf was counted and recorded for each replicate. The study was continuous till the termination of the crop. The Selected varieties and genotypes of chilli were classified based on the number of mites, as Girish et al. (2019) suggested.

For statistical analysis, recorded data were collated and organized. Analysis of variance (ANOVA) was performed using the computer package STATISTIX 10 program. The mean differences of the treatments were observed by Tukey's Highest Significant Difference (HSD) test at a 5% level of probability for the explanation of the findings.

Results and Discussion

Effects of chilli mite infestation on the growth parameters: Table 1 represents the effect of chilli mite infestation on the selected varieties and genotypes of chilli. The number of mites per leaf was ranked 7.24, 4.54, 2.89, 1.76, 1.40, 1.21, 0.98, 0.85, and 0.69 in G25, G10, BARI Morich-2, G30, G13, BARI Morich-3, BARI Morich-1, G27, and G31, respectively.

The growth and yield parameters of particular chilli varieties and genotypes differed significantly ($p \leq 0.05$) (Table 1). The plant's maximum height (55.11 cm/plant) was noted in the G10. The BARI Morich-2 had the second tall height (43.00 cm/plant), followed by the G30 (42.44 cm/plant) and the G27 (37.67 cm/plant). On the contrary, the G31 had the least plant height (16.22 cm/plant), followed by the BARI Morich-3 (23.33 cm/plant) and the G13 (27.67 cm/plant).

Accounting for the development of the chilli plant, Genotype G10 has the highest canopy diameter (46.22 cm/plant) followed by the G30 (42.06 cm/plant), the G27 (41.39 cm/plant), and the G13 (31.56 cm/plant). The lowest canopy diameter (17.61 cm/plant) occurred in the BARI Morich-3. Among the screened genotypes, the G25 had the highest number of branches (9.33), followed by the G30 (8.67) and the G13 (8.00). The least number of branches (5.33) was recorded in the BARI Morich-3. The distribution of the number of other treatments' branches was more or less similar.

Although the frequency of mite attacks did not correlate with plant height in several studies (Borah,1987; Hosamani, 2007; Nasrin, *et al.* 2021), current results showed a positive and significant correlation between plant height and mite attack. Pest attacks can affect the plant height in different crops. Zeeshan and Kudada (2019) reported significant variations in chilli plant height in response to different management treatments to control pest infestation. Jangra *et al.* (2017) reported the stunted growth of hybrid chilli. The decrease in plant height depends on the pest's severity and the infestation stage. The infestation of bell paper by mites has been reported to reduce plant height by 50 percent (Vichitbandha and Chandrapatya, 2011). Manjunatha (1982) reported that plant height decreased with the severity of the mite infestation at the seedling stage. Toxins injected by the mites in the chilli plant resulted in shorter internodes, producing a restricted or tufted presence (Pal and Karmakar, 2017)

The G30 had 1256.0 leaves per plant, which was the highest number. The second highest (1222 leaves/plant), which was statistically comparable to the G30, was found in the G10. The BARI Morich-2 (742.3 leaves/plant), the G25 (654.3 leaves/plant), and the BARI

Table 1. Growth and yield parameters of selected chilli varieties and genotypes.

Treatments	Height/ plant (cm)	Canopy diameter (cm)	Branch/ plant	Leaf/ plant	Leaf surface area (mm^2)	Fruit/ plant	Fruit length (mm)	Fruit diameter (mm)	Yield/plant (g)	No. of mites/leaf
BARI Morich-1	28.33 cd	20.89 d	7.67 abc	622.3 bc	84.27 d	28.33 d	75.93 b	9.64 bcd	62.62 cde	0.98
G10	55.11 a	46.22 a	7.33 abc	1222.0 a	144.89 ab	49.67 c	76.67 b	11.70 ab	153.92 b	4.54
G13	27.67 cd	31.56 bc	8.00 abc	470.0 cd	86.77 d	76.67 b	47.20 c	10.77 bc	101.94 c	1.40
BARI Morich-2	43.00 b	24.22 cd	6.00 bc	742.3 b	132.59 bc	21.33 de	90.27 b	9.30 cd	49.63 def	2.89
BARI Morich-3	23.33 de	17.61 d	5.33 c	545.0 cd	121.92 bcd	20.33 de	85.00 b	7.75 de	36.18 ef	1.21
G25	35.11 bc	25.50 cd	9.33 a	654.3 bc	178.01 a	8.67 e	53.53 c	13.05 a	21.02 f	7.24
G27	37.67 bc	41.39 ab	7.00 abc	480.0 cd	109.12 bcd	52.67 c	58.00 c	7.08 e	65.39 cde	0.85
G30	42.44 b	42.06 a	8.67 ab	1256.0 a	103.12 cd	108.33 a	125.00 a	10.67 bc	512.27 a	1.76
G31	16.22 e	22.94 cd	7.33 abc	391.0 d	106.07 bcd	76.00 b	44.33 c	9.65 bcd	80.19 cd	0.69
CV	10.14	11.35	14.11	9.34	11.50	11.55	7.26	7.18	11.80	-

Application data are the average of 9 observations from 3 replications. In a column, means followed by the same letter(s) are non-significantly different by Tukey's HSD Test at $p \leq 0.05$.

Morich-1 (622.3/plant) was ranked in that order. The plant with the least number of leaves (391.0/plant) was in the G31.

Among the chilli varieties/genotypes, Genotype 25 (G25) was found with the highest leaf surface area (178.01 mm^2/leaf). Following BARI Morich-2 (132.59 mm^2/leaf) and BARI Morich-3 (121.92 mm^2/leaf), G10 had the second-highest leaf surface area (144.89 mm^2/leaf). In BARI Morich-1, the smallest leaf surface area (84.27 mm^2/leaf) was observed.

Mite populations per leaf increased with the increasing number of branches per plant. New growth is stunted or suspended by the mite infestation, forcing additional shoots to develop and causing more branching (Sarmento *et al.*, 2011). In the presence of mites, plant development has been testified to be halted (Vichitbandha and Chandrapatya, 2011). It has been reported mites attack the young tender leaves. The stems caused severe growth losses, particularly ceasing the growth of young branches (Jangra *et al.*, 2017) and eventually resulting in less fruit production (Kamruzzaman *et al.*, 2013). Interestingly, adequate growth of plants was observed even with mite infestation when alternate food was provided for the mites (Duarte *et al.,* 2015), indicating the damage efficiency of mites on chilli plant growth and development.

The toxic components in mites' saliva result in deformed and curly leaves with less leaf area (Kotresh *et al.,* 2020). The mite pest infestation also causes leaf shading and ultimately reduces the number of leaves in the plant (Jangra *et al.,* 2017). In a quest to screen the mite-resistant genotypes, tolerant ones were found to have more leaf and higher leaf area under mite infestation than the susceptible genotypes (Sarwar 2014; Satpathy *et al.,* 2008). Apart from the number, the area of the mite-infested plant leaves has been reported to be reduced (Nasrin *et al.*, 2021). While studying the population dynamics, the mite presence was found to reduce the number of leaves, and the area of leaves managed to sustain against the severe infestation (Kumar *et al*., 2019).

The leaf number and area reduction may be related to the egg-laying pattern of the mite, as the adult female usually lay their eggs on the ventral surface preferably on the young unfolded ones (Pal and Karmakar, 2017). And eventually, the extreme infestations of mites result in the withering and dropping off of young leaves, flowers and fruits, and may cause more than 60% yield loss in the chilli plant (Srinivasan *et al.*, 2003).

Effects of chilli mite infestation on the yield parameters: Genotype 30 (G30) produced the maximum number of fruits (108.33/plant), followed by Genotype 13 (76.67/plant), Genotype 31 (76.00/plant), and Genotype 27 (52.67/plant). On the contrary, the G25 had

the lowest number of fruits (8.67 per plant), followed by the BARI Morich-3 (20.33 per plant), the BARI Morich-2 (21.33/plant), and BARI Morich-1 (28.33 per plant).

The G25 had the largest fruit diameter (13.05 mm/fruit), followed by the G10 (11.70 mm/fruit), the G13 (10.77 mm/fruit), and the G30 (10.67 mm/fruit). In the G27, the lowest fruit diameter (7.08 mm/fruit) was noted. The G30 variety had the longest total fruit length (125.00 mm/fruit), followed by the BARI Morich-2 (90.27 mm/fruit), the BARI Morich-3 (85.00 mm/fruit), and the G10 (76.67 mm/fruit). The G31 had the shortest fruit length (44.33 mm/fruit). The G30 yielded the highest amount of crop per plant (512.27 g), followed by the G10 (153.92 g/plant), the G13 (101.94 g/plant), and the G31 (80.19 g/plant). The yield reported at the lowest rate (21.02 g/plant) was from the BARI Morich-3, followed by the BARI Morich-2, the BARI Morich-1, and 36.18 g/plant from the BARI Morich-3.

The number of fruits is negatively correlated with the number of mites, and the severity of the mite affected the amount of fruit formed. The fruit becomes smaller as the plant's ability to meet nutrient requirements during fruit development is reduced. The curling of leaves due to heavy infestation of mites also causes flowers and fruit to drop, thereby reducing the number of fruits formed and yielded (Kamruzzaman *et al*., 2013). The severe infestation of mites results in fruit discoloring and premature fruit dropping (Kumar *et al.*, 2019) The dented fruit that is not presentable in the local market can be used for processing but it’s quite troublesome (Pena and Campbell, 2005). The results are consistent with the findings of Reddy and Baskaran (1991). The findings of van Maanen et al. (2010) further confirmed that chilli mites attack young leaves and shoots, causing significant losses including stunted growth of branches and flower drops leading to a marked reduction in the number of fruits formed in the chilli plant.

Calculating the data between mite infestation and fruit length of chilli varieties/genotypes, the correlation on the prevalence of *P. latus* infection in chilli plants showed that an increase in the mite population resulted in a correspondingly significant reduction in fruit length (Jangra *et al.*, 2017).

The highest yield was recorded in the G30 with lower mite infestation, and the lowest yield was obtained from the G25 with the highest mite infestation. The individual yield of each plant was negatively correlated with mite infestation (-0.13). A study by Reddy and Puttaswamy (1984) yielded similar results. The greater the damage to the crop, the lower the chilli yield. Mites caused leaf curl damage, resulting in reduced fruit and overall yield. Varieties (BARI Morich 1, 3) that showed the lowest flower and fruit infestation levels were categorized as moderately resistant to mites (Nasrin *et al.*, 2021). Reddy and

Puttaswamy (1984) suggested that crop losses due to *P. latus* varied between 23.87 and 73.29%. Severe infestation of mites damages the leaf flower and fruiting seriously and may reduce the yield by 60% (Srinivasan *et al.*, 2003). Latha and Hunumanthraya (2018) screened chilli genotypes, sorted them on the leaf curl index and found the tolerant group with the most fruit and eventually the highest yield. Fruit number is imperative to study the tolerance against mites. Vichitbandha and Chandrapatya (2011) reported dropping chilli fruit production and fruit weight when mites damaged plant.

Weekly mite infestation of selected chilli varieties and genotypes: Knowing pest populations is of utmost importance to an effective pest control system. The number of chilli mites on the leaf of chilli plants was counted at weekly intervals (Fig. 1). This study exposed that mites started to grow at the beginning of January. Initially, in January, the growth was controlled on a small scale. On 1st of February, we found the least number of mites in all genotypes. After that, the number started to increase, and the peak abundance of mites on all the chilli varieties was observed on 9th March, when the plants were juicy, succulent and green and remained in the flowering stage or green fruits. The mite abundance continued till the final harvesting in all the genotypes. High temperature and low relative humidity favored the development of the mite population to the peak during the study period.

In another study in Bangladesh, the peak of the mite was recorded in the second week of April (Nasrin *et al.,* 2021). The development and reproduction of mites depend on the temperature and rainfall pattern (Gotosh *et al.*, 2014). Kethran *et al.* (2014) studied the prevalence of mites on chilli plants in Pakistan and found the highest frequency of mites in the fourth week of March because of the differences within the geographical position, the ambient weather situations, and the chilli varieties. Our conclusions are different. Although our results showed slight variation, temperature and humidity played an important role in all the findings. In every study, the peak was observed in hot and dry conditions, and the mite population sharply declined after the rainfall. Many authors have reported a positive correlation between temperatures on the population of *P. latus* and a negative correlation with relative humidity and rainfall (Chakrabarti and Sarkar, 2014).

The average mite infestation of the genotypes over the study period is presented in Fig. 2. the G25 had the highest number of mites (36.21 mites/5 leaves), followed by the G10 (22.70 mites/5 leaves), the BARI Morich-2 (14.45 mites/5 leaves), and the G30 (8.82 mites/5 leaves). Contrarily, the G31 had the least amount of mite infestation (3.45 mites/5 leaves), followed by the G27 (4.24 mites/5 leaves), the BARI Morich-1 (4.91 mites/5 leaves), and the BARI Morich-3 (6.06 mites/5 leaves).

The average number of mites during the study period helps to reveal the endurance capacity of chilli genotypes. At the ultimate infestation of mites, Patil and Nandihalli (2009) observed 6.4 mites/leaf of chilli plants in the fourth week of April in Karnataka, India. Kethran *et al.* (2014) observed the maximum abundance of mites from the first week of March to the fourth week of August. A mean abundance of 0.52 mites/leaf was recorded by Kethran *et al*. (2014). The screening of 14 chilli hybrids was introduced to select the mite-tolerant one. Though none was found completely immune to mites, 2.53 mites/ leaf were observed in the best hybrid chilli genotype (Jangra *et al.*, 2022). They ended up grouping the hybrids based on susceptibility, mainly based on the number of mites per leaf. In our study, the prevalence of mites over the study period could be a good indicator for selecting tolerant genotypes.

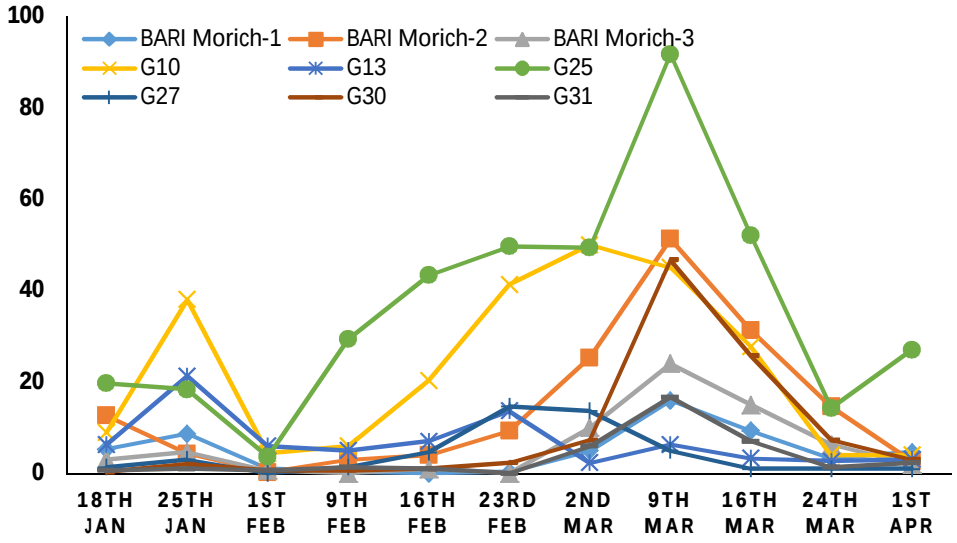

Fig. 1. Weekly mite infestation of three varieties and six genotypes of chilli during the experimental period.

Categorization of selected chilli varieties and genotypes against mite infestation: Based on the mite population (number of mites/5 leaves), three varieties and six genotypes of chilli were grouped (Table 2). The data for assessing mite damage was inconsistent. In this case, average mite populations were considered to assess the response of different chilli varieties and genotypes to mite infestation.

Thus, three varieties and six genotypes were differentiated into 5 major response groups based on the average mite swarming (recorded from weekly intervals). One variety and 2 genotypes which harbored <5 mites/5 leaves, namely BARI Morich-1, Genotype 27, and Genotype 31, were designated as highly resistant; Genotype 13, BARI Morich-3, and Genotype 30 with 5 to 10 mites/5 leaves as resistant; BARI Morich-2 as moderately resistant with 11 to 20 mites/5 leaves; Genotype 10 was recorded with 21 to 30 mites/5 leaves as tolerant and Genotype 25 was found as susceptible with >30 mites/5 leaves (Table 3).

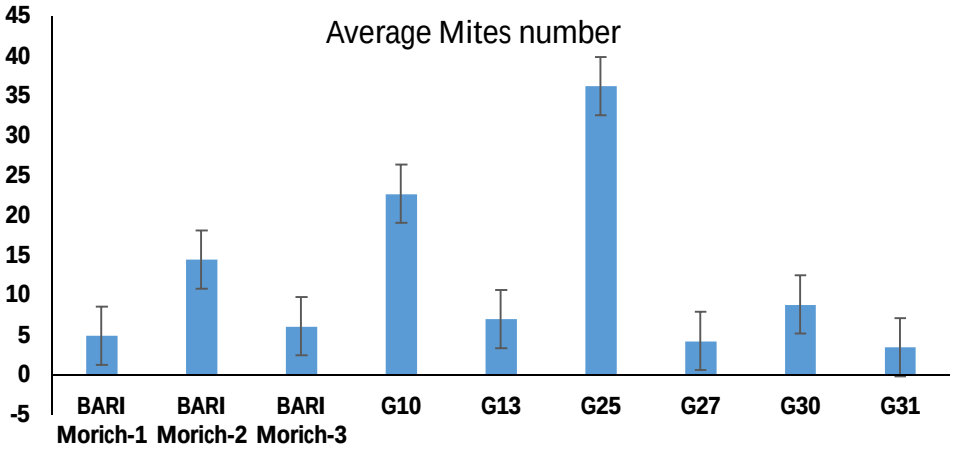

Fig. 2. Average number of mites in chilli plant during the experiment period.

Table 2. Category of chilli varieties and genotypes based on mean mite population.

Category	No. of mites/5 leaves	Varieties/genotypes
Highly resistant	<5 mites	BARI Morich-1, Genotype 27, Genotype 31
Resistant	5-10 mites	Genotype 13, BARI Morich-3, Genotype 30
Moderately resistant	10-20 mites	BARI Morich-2
Tolerant	20-30 mites	Genotype 10
Susceptible	>30 mites	Genotype 25

Mites are tough to manage because of their polyphagous nature and high reproduction percentage and resistance to the host plant plays a key role in alternate pest management approaches. Several workers in India reported a series of screening of chilli genotypes in

the quest to find the resistant chilli (Singh and Pandey, 2015; Bala *et al*., 2016). The current study resembles the results of Gillis *et al*. (2019) examined the genotypes of 30 hot peppers and ranked the genotypes based on mite populations. The existence of mite resistance to chilli cultivars was observed by Samanta *et al*. (2017), who revealed tolerant and the most susceptible hybrids to yellow mites. Latha and Hunumanthraya (2018) screened 30 chilli genotypes and grouped them as resistant, moderately resistant, susceptible and highly susceptible. Nasrin *et al.* (2021) studied five chilli varieties and concluded BARI Morich 1, and BARI Morich 2 were moderately tolerant and others as susceptible to mites. The findings are similar to our results.

Conclusions

Genotype 25 was found to have the highest number of 7.24 mites/leaf, while the least number of 0.69 mites/leaf was revealed in Genotype 31. The yield was negatively correlated with a mite infestation. The yield was proportional to the number of fruits per plant and the weight of each fruit. Genotype 30 was the most prolific (512.27 g/plant) in production. On the other hand, the relative fruit number per plant in G10 and G13 provided an optimum yield and the lowest 21.02 g/plant from G25 with the highest mite infestation per plant.

Except for Genotype 25, which was vulnerable and had the lowest yield, all evaluated varieties and genotypes were tolerant to highly resistant to chilli mite infestation. Genotype 30 produced the highest (512.27 g/plant) and had fewer chilli mite infestations per plant. This genotype could be grown in regions where the chilli mite is a major pest and used as a starting point for creating resistant varieties. The findings of the current experiment could be beneficial for breeders to select appropriate genotypes resistant to mites. It opens a scope for the breeders to take the opportunity to develop a mite-resistant variety of chilli plants.

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