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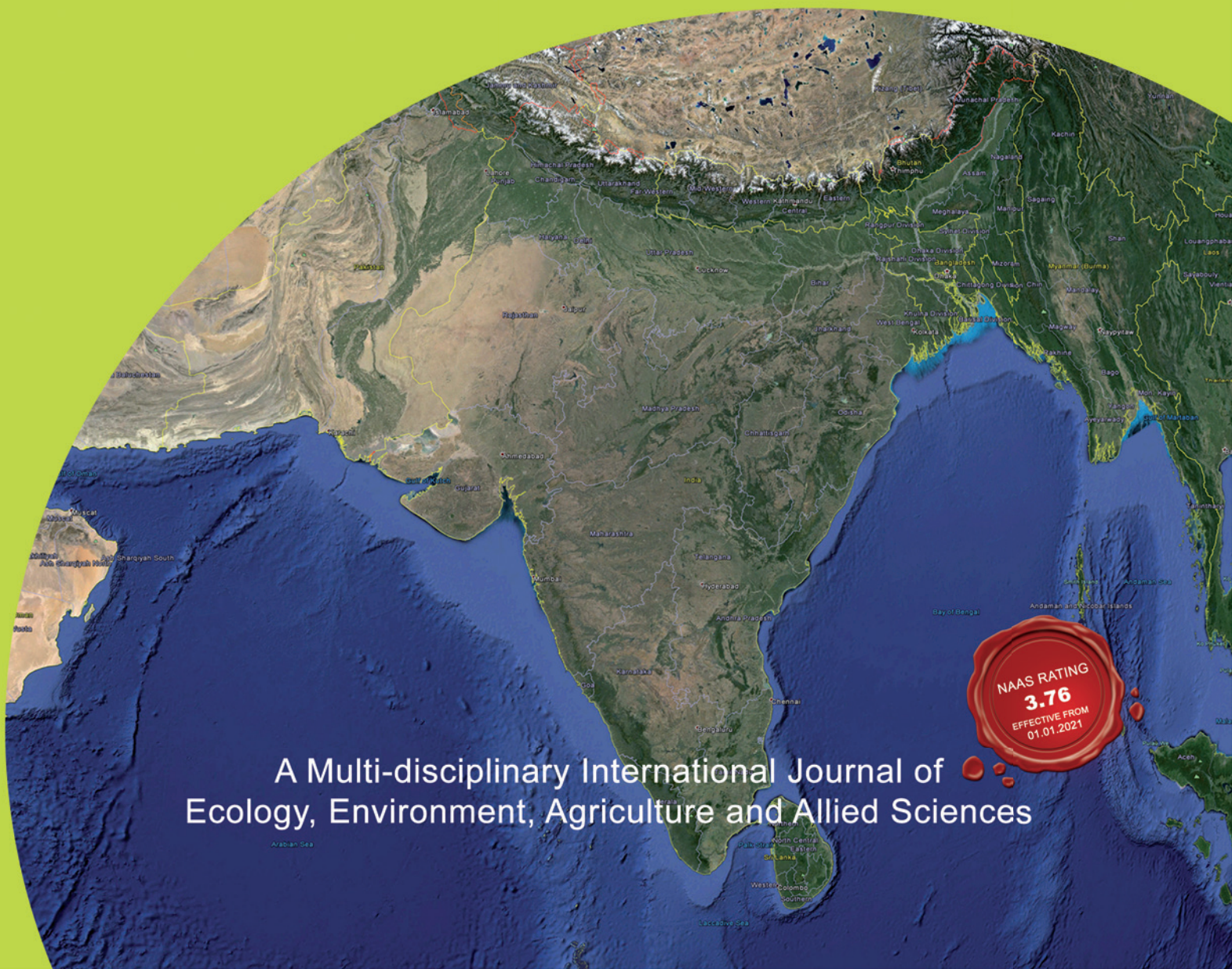

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Drip irrigation technology, originated from Odisha province of India : A review

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ABSTRACT

Water resources are becoming scarce day-by-day, reducing their availability at various levels throughout the world. The per capita per annum water availability of India has also reduced from 5177 m^3 in 1951 to 1450 m^3 in 2022. To ensure water security for all, the water use efficiency (WUE) must be increased in every field by reducing wastage. WUE is the single most measurable parameter, which indicates whether there is judicious use or wastage of water. For enhancing WUE scientific, efforts have been made in various parts of the world in different time spans. Evidence has suggested that first attempt in the direction of enhancing WUE was made in India during *Satya Yuga*. Mythological evidence and religious traditions have proved that first invention of drip irrigation was made by Odia people in the Odisha province of India during the same period.

Key words: Drip irrigation, India, Odisha, originated, water use efficiency

INTRODUCTION

Water is the most important natural resource of the world, which supports life directly. Out of the total water resources, fresh water supports most of the living components of the ecosystem like human beings, animals, plants, microbes, and freshwater aquaculture. However, the share of freshwater in the total water resources of the world is only 2.7 per cent, compared to the 97.3 per cent saline water (Vision, 2030). It is again ironic that whatever fresh water we have, only 12% are useful to us, and the rest 88% remain in nature without having any use to the living world (Vision, 2025). It is estimated that about 77% of total fresh water is locked in permanent snow and glaciers and 11% of fresh water remains at a depth below 800 meters in the earth's crust, which is difficult to extract by using the present-day extraction technology. Another 11% of the freshwater remains at a depth within 800 m in the earth crust, which is extracted by wells and submersible pumps and put to use. Approximately 1% of the total freshwater resources remain as surface water, in rivers, ponds, freshwater lakes, dams and ditches. The per capita freshwater availability in the world has been constantly decreasing over the decades due to rise in population. To meet various requirements of the burgeoning population, subsidiary sectors, like industry, transport, housing, domestic etc. are growing very fast and consuming huge quantities of fresh water. Among various sectors that utilize fresh water, the agriculture sector utilizes the lion's share world over. An estimate shows that by the year 2050, total freshwater requirement of India would reach to 1447 Billion Cubic Meter (BCM) against the present utilizable water resources of 1123 BCM. Since the total demand would exceed the total supply by 324 BCM, multiple challenges will emerge from various sides. During the same period, the agriculture sector will require a total of 1074 BCM fresh water which

is 74% of the total requirement (Kumar et al., 2013). It would be very difficult for India to spare this much for a single sector leaving the demands of all other sectors unfulfilled. However, to meet the requirements of the ever-increasing population, we must produce the required quantities of food and other materials. As the agriculture sector utilizes the highest quantities of freshwater compared to other sectors, there is an urgent need to enhance its use efficiency. The answer is to switch to the most efficient irrigation method, where wastage of water can be reduced for enhancing its productivity. Among various irrigation methods such as flooding, furrow, ring, pitcher, sprinkler, and drip, the last one is found to be the most efficient method. Drip irrigation saves huge quantities of water, enhances production, and enhances fertilizer use efficiency. Studies show that drip irrigation saves 50 to 70 % water as compared to furrow irrigation (Mandal and Jana, 2017). In the evolutionary process, uses or methods of water wastage reduction or efficient uses of water might have been developed for general purposes first and then those principles might have been applied in irrigation sector to save water. Efficient irrigation methods might have been invented much later dealing with watering crop plants.

CONCEPT OF WATER USE EFFICIENCY

Water is a vital resource which supports life, and its use efficiency has been calculated at different points of time by various workers for quantifying the exact loss to provide desired policy support for its sustainable use. Water use efficiency can be best described as the amount of water really used in the production process compared to total amount of water diverted for that production process. In other words, the higher the efficiency, the lower is the wastage component and vice versa. In agricultural research, some people calculate it as ratio of quantity of biomass produced over amount of water used (kg per m^3) in that production process. In recent times the term water productivity is used which denotes quantity of biomass production per unit of water used (kg per m^3 of water used). More recently, system water productivity comprising different enterprises is calculated and expressed as total monetary value obtained per unit of water consumed (Rs m^{-3}) which is also known as economic water productivity.

MYTHOLOGICAL ASPECT OF WATER USE EFFICIENCY

In Hindu religion, during churning of ocean of milk by the *Gods and Asuras* (demons), many valued things were discovered. Among them *Amrit* (nectar of immortality) *Parijat flower, Airabat elephant, Goddess Laxmi, Kumadhenu* (cow of plenty), *Madira* (Goddess of wine), *Kalpabriksha* (wish fulfilling tree), *Apsara* (celestial dancer), *Uchhaishrava* (celestial horse), *Panchajanya*, Vishnu's mace and magic bow, various gems, *Dhanwantari* (Physician of Gods), *Halahal* (poison) and many other items. (www.britanica.com). The valued items were distributed by Gods and Asuras. Neither of them was interested in taking the poison. The impact of the poison was so high that could have killed the entire universe. So, it was decided that all will approach *Mahadev*, the supreme Lord of the universe to get rid of this problem. *Mahadev* agreed and drank the poison and kept it in his throat. The poison was so powerful that it burnt his throat and changed the colour of his throat to blue. After this incident, Lord *Mahadev's* name changed to *Neelkanth* (blue throat). It generated high intensity heat and to nullify the impact of heat, he kept Goddess Ganga on his head to pour water on his throat for long time. To reduce the heat of his body and relieve pain, water is required to be continuously dropped to his body (www.hindunismfacts.org).

WHY WATER DROPS MADE TO DRIP ON *SHIVALINGA*

To reduce the impact of intense heat generated from the *Halahal* (deadly poison) in the throat of Lord *Shiva*, water needs to be continuously poured into the head of Lord Shiva. During those days water conveyance through pipe was not discovered. At the same time, it was difficult to keep *Shivalinga* wet by continuously putting water for 24 hours. Initially devotees might have to bring thousands of pots filled with water for this purpose. Later, they might have thought, if pots filled with water were hung over the *Shivalinga* and a tine hole was made on the pot, then it will allow water to tickle for long period. When

the water in the pot disappears, again they will refill it. Later, to reduce the flow further, they might have used Kusa grass or Doob grass to plug the hole, which could have lasted for hours together. Even today, the popular belief is that if one does *Jalavisek* worship (pouring water on *Shivalinga*), Lord Mahadev is pleased and fulfills the desire and Hindus are doing it. The same practice is seen in Shiva mandir (Fig. 1). This was in *Satya Yuga*, the oldest *Yuga*, as per mythological calculation. As per mythology, the entire time spam of this universe is divided into 4 Yugs such as *Satya Yuga* (38,91,102 BCE to 21, 63, 102 BCE), *Tretaya Yuga* (21,63,102 BCE to 8, 67, 102 BCE), *Dwapara Yuga* (8, 67,102 BCE to 3102 BCE) and *Kali Yuga* (3102 BCE to 4,28,899 CE) (www.wikipedia.org). Perhaps this is the first attempt in the history of human civilization where attempts have been made for prolonging water delivery from a limited supply and became successful. We can say that this was the first attempt in the world to prolong water release from a limited supply. In other words, this attempt can be considered as the first invented step or technology for enhancing water use efficiency by reducing wastage for a specific purpose.

DRIP IRRIGATION

As water resources are scarce, its efficiency must be increased at every user point. Various scientists have tried to enhance water use efficiency in irrigation methods in past centuries which led to invention of furrow irrigation, paired row irrigation, ring irrigation, pitcher irrigation, sprinkler irrigation and drip or trickle irrigation over wild flooding. Among all the methods of irrigation, water saving in drip irrigation has been calculated to be highest. Gustafson (1975) from Senegal reported 30% water saving in drip while Singh et al. (1978) reported 50% water saving in drip irrigation. Mandal and Jena (2017) reported that drip irrigation resulted in 40-50% water saving compared to furrow irrigation and 50-70% water saving compared to flood irrigation. Water saving approach of drip irrigation has also been found across all the soil types. In heavy soil units, 20-40% water saving has been noticed whereas in case of light permeable soil units, water saving varied from 50-70% (Goyal, 2013; Mandal and Jena, 2017).

HISTORY AND EVOLUTION OF DRIP IRRIGATION

Around 100 BCE, people of China used to burry clay pots filled with water called *ollas* in soil for growing plants, which is similar to '*Matka Sichai*' of desert state of Rajasthan in India and '*Pitcher Irrigation*' elsewhere. During nineteenth century in Afghanistan, unglazed clay pipes were used for growing crops. In Germany, during the year 1860, researchers started experimenting sub-surface irrigation using clay pipe to create a system which will cater both irrigation and drainage. Harris Thill of Australia developed the use of plastic to hold and distribute water in drip irrigation. Meanwhile, the researchers have tried to develop a trickle system for water delivery to plants through use of perforated pipes. The demerits of this system were that the water flow was choked due to deposition tiny particles. Simcha Blass and his son Yeshayahu of Israel in the year 1959 used plastic emitter in drip irrigation. Here the holes of the pipe were not blocked, and results were very good. Simcha Blass and Kibbutz Hatzerim created an irrigation company in 1964 named it Natafim and got patent for surface drip irrigation emitter. During 1960, the first drip tape called Dew Hose was developed by Richard Chapin of Chapin Watermatics in the United States. In the year 1987, Plasto irrigation developed T-tape in drip irrigation with slit outlet and a laminar flow track which later evolved into a turbulent flow regulating flow track. In the year 2006, Chapin Watermatics was acquired by Jain Irrigation (www.bluejayirrigation.com; www.dripworks.com;www.irrigation.learnabout.info; US Patent, 2017, Brain bridge, 2001). In India, systematic experimentation on drip irrigation was started in early seventies of 20th century by All India Coordinated Research Project on Irrigation Water Management of ICAR at MPKV, Rahuri Centre followed by TNAU, Coimbatore Centre. Later, research on drip irrigation on various crops was undertaken by other AICRP centers and state agricultural universities (SAU). In the year 1989, Jain Irrigation started commercial micro-irrigation including drip irrigation. ICAR centers and SAUs supported the research back up and Jain irrigation extended material support

Fig. 1(a). Water trickle on Shivling in Ujjain

Fig. 1(b). Water trickle on Shivling in Shyameswara temple of Bhadrak

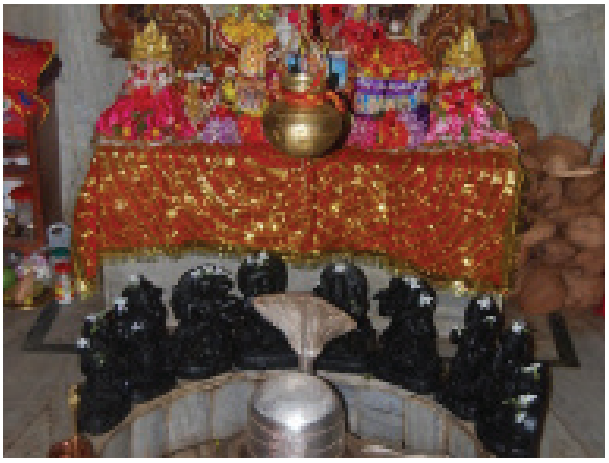

Fig. 1(c). Water trickle on Shivling in outskrit of Bhubaneswar

Fig. 1(d). Water trickle on Shivling in Jamujhari temple in Khordha district

Fig. 1(e). Water trickle on Shivling in Faneswar Mahadev of Gurunagarsasana

Fig. 1(f). Water trickle on Shivling in Mahadev temple of Chhataber

Fig. 1(a-f) Photographs showing water dripping through traditional method of Jalavisek (controlled bathing)

for micro-irrigation spread in India including drip irrigation. The research support of ICAR and SAUs, financial support of government of India and various state governments, material and installation support of Jain irrigation and other private players, resulted in expansion of micro-irrigation in India and its spread reached to 13.78 million hectares (Anonymous, 2022).

WHY *TULSI* PLANT

Tulsi or holy *Basil* / sacred *Basil*, whose scientific name is *Ocimum tenuiflorum* is a sacred plant in Hindu religion. The Centre of origin of this plant is India. It is considered as Goddess *Laxmi* and without her presence, the worship of Lord *Vishnu* is incomplete. Various interesting stories are mentioned in different *Puranas* regarding the origin of this sacred plant *Tulsi*. According to one legend, during *Samudra Manthan* (Churning of cosmic ocean) by Demons and Gods, *Dhanvantari* rose from ocean with *Amrit* (the elixir of immortality). Lord *Vishnu* took it for Gods. When demons tried to steal it, Vishnu shed happy tears which when fell on *Amrit* gave birth to *Tulsi* (Deshpande, 2005; Wikipedia. org; Anonymous, 2019). According to another legend, *Vrinda* was the beautiful daughter of demon king Kalanemi. She was a great Vishnu devotee. *Jalandhar*, a demon king was borne out of water and became powerful after getting the blessings of Lord *Shiva. Jalandhar* fell in love with *Vrinda*, a pious, devoted and extremely chaste lady and married her. *Jalandhar* became very powerful due to the chastity and devotion of his wife and multiplied his strength. His arrogance multiplied further, and he thought he would be the supreme power of the universe by defeating all gods. Even Lord *Vishnu* couldn't defeat him. All gods went to Lord *Vishnu* for help. Lord *Vishnu* caught himself in dilemma, as *Vrinda* was his ardent devotee and *Jalandhar* is posing a great threat to deities. When *Jalandhar* was fighting with Lord *Shiva, Vrinda* was in chastity. Lord *Vishnu* knew that if *Vrinda's* chastity will not be broken, then *Jalandhara* cannot be killed. Lord *Vishnu* decided to play a trick. He went to *Vrinda* in disguising attire of *Jalandhar* while real *Jalandhar* was fighting with Lord *Shiva. Vrinda* couldn't recognize Lord *Vishnu* and thought he was the real *Jalandhar* and greeted him. When she touched Lord *Vishnu*, she realized that he was not her husband *Jalandhar*. Her chastity was shattered, and her husband *Jalandhar* became vulnerable. When she realized her mistake, she asked Lord Vishnu to show his original form. When she saw Lord *Vishnu*, she was shattered to know that her own Lord had cheated her. She cursed Lord *Vishnu* to become a stone with anger for playing tricks to dilute her chastity. Lord *Vishnu* accepted her curse and turned into a stone known as *Shaligrama* stone which is available near Gandak river and is worshiped as Lord *Vishnu* even today in Hindu religion. *Jalandhar* was not killed as he was under protection of his wife's purity and chastity. When *Vrinda's* chastity was broken, *Jalandhar* became powerless, and he was killed by Lord Shiva. *Vrinda's* heart was broken down and she decided to kill herself. *Vrinda* took the head of her husband and immolated herself in the in the pyre. From the ashes, one plant emerged, and Lord *Vishnu* named it *Tulsi* and blessed the plant that she would be worshipped along with Lord *Vishnu*. She will be always in the needs of Lord Vishnu and without her; the worship of Lord Vishnu will be incomplete.

SPIRITUAL AND MEDICINAL PROPERTY OF *TULSI*

The significance and importance of *Tulsi* have been mentioned in various religious texts in Hinduism. Some selected verses are mentioned here. In Skanda Purana, it is mentioned that by worshiping *Tulsi* one can attain the same result which can be attained by worshipping *Saligrama Sila* on *Shravana dwadasi*. In Garuda Purana it is mentioned that by worshipping *Tulsi* then one will attain the same result which one can attain by observing fast on Janmashtami. Garuda Purana mentions that by worshipping Tulasi one gets the same result which one gets by bathing in Prayaga or leaving his body in Varanasi after death. There is a mention in *Agastya Samhita* that by worshipping *Tulsi* properly one fulfills the desires of the men and women in four varnas and ashramas. It is believed that in the *Kali Yuga*, if someone plants *Tulsi*, glorifies her, remembers her, irrigates her, gives her in charity, offers her to *Krishna*'s lotus feet and after offering eats the remnants of the sacred leaves then all his sins are burned. As per Narada Purana

a person's fortune increases everyday if one begins worshipping *Tulsi* daily. In Hari Bhakti Sudhodaya, it is mentioned that that Lord *Vishnu* always stays in *Tulsi* Forest hoping that some devotee will come and offer him an unbroken *Tulsi* leaf. Narada Purana further glorifying the worship of *Tulsi* mentions that "If one chants the names of Ganga then Ganga destroys all his sins. But when someone chants the holy names of Lord Hari then *Tulsi* Devi grants him the gift of devotional service." In Garuda Purana it is mentioned that "O great Garuda, those who plant Tulasi Devi attains liberation for sure." *Tulsi* is considered so sacred that her single branch is equal to a hundred *pipal* trees or thousand mango trees (discoverursupersoul.com, 2023).

It is believed that the presence of *Tulsi* plant near the house brings much cheer and health to the family. Keeps the heart and mind fresh. It bestows the energy of love and devotion on devotees. Lord *Sri Krishna*, the incarnation of *Narayan* or Lord *Vishnu* is very much pleased with *Tulsi* garland or a worship by *Tulsi* leaves. No other sacred flowers or plant parts equal to this sacred leaf. It is also believed that by pouring water on *Tulsi* plant, eliminates all the sin including brahmahatya (killing of human being). Drinking water-soaked sacred *Tulsi* leaves reduces stress. Scientific studies have established that *Tulsi* plant has significant medical properties. The *Tulsi* leaves extract brings down the fever. Chewing *Tulsi* leaves along with ginger and honey gives relief from cough, cold and flu. Gargling with the *Tulsi* leaves boiled water soothes the sore throat. It is also believed that the *Tulsi* extract with honey can expel kidney stones. *Tulsi* also reduces blood cholesterol. Chewing *Tulsi* leave daily reduces the chance of mouth infections. The paste of *Tulsi* roots is used against insect bites. Drops of *Krishna Tulsi* leaf extract in eyes soothes the sour eyes. Anyway, growing *Tulsi* plant at home is very helpful be as an indoor or outdoor plant. *Tulsi* plant acts as a purifying agent in house and its surroundings. The devotees of Lord *Krishna* always prepare a garden of this sacred plant in their courtyard (www.hinduismnet.com). In Jagannath temple, Puri a special sevayat (group engaged for offering service) are assigned the duty of supplying *Tulsi* from generation to generation from ancient times. That is reason why in *Tulsi* Puran, it is written as

"Yenmoole Sarvatheerthaani
Yenmadhye Sarvadevatha
Yadagre Sarva Vedaascha
Thulaseem-tham Namamyaham"

I bow down to the *Tulsi*, at whose base are all the holy places do exit, at whose middle all gods and goddess reside and at whose top, reside all *vedas*.

TULSI PLANT, ITS RELIGIOUS TIES AND THE ORIGIN OF DRIP IRRIGATION IN ODISHA

In Hindu religion, four salvation centers *(Mokhya dhams)* are considered as most sacred with respect to pilgrimage. Lord Vishnu is believed to visit these centers daily in his *pradhakhina*. When a devotee visits any one of the four centers, he or she gets the darshan of his or her Lord and achieves salvation or *mokhya*. These centers are Badrikadham in the north, Dwarakadham in the west, Jagannathdham in the east and Rameswaram dham in the south. Mythologically it is believed that Lord *Vishnu* gets up at Badrikadham and takes bath at river Alakananda, then he adorns dress or besha in Dwarakadham, he dines at Jagannath dham, Puri and sleeps at Rameswaram. That is the reason why daily *Chhapan Bhog* (56 prasad offerings) items are cooked in temple kitchen of Lord Jagannath temple and offered at Puri which is unique in the entire world. Even today the practices of *Arnaprasad* (cooked food including cereals) offered in various temples and its distribution to devotees, within and outside Odisha are in practice in almost all Jagannath temples. *Adi Shankaracharya* saw Lord Vishnu in these four places and hence established four maths for further spread of Hinduism (Mishra, 2011). Lord *Jagannath* is considered as one of the incarnations of Lord *Vishnu*. As Odisha was a famous site for Vaisnavas from ancient times, growing *Tulsi* plant for offering to Lord *Jagannath* was also intimately associated with Odia culture. In Odisha, each household grows at least one *Tulsi* plant in a raised earthen platform called *Tulsi Chaura* and worships it during both in the morning and evening times. In day time water and Prasad are offered, people eat a

few leaves of *Tulsi* as prasad. During evening times, Odia ladies after taking bath wearing fresh clothes, offer *Sanjabatti* (evening lamp) near the *Tulsi Chaura*. *Tulsi* plant is considered so sacred that its leaves are never plucked during night hours and during day time it is plucked only after chanting the following mantra.

"Maata Tulasi govinde hudrayanandakarini
Narayanasya pujartham chhinna masta jagat haste
Tulasi mruta namasi sadatwam keshabapriye
Keshabarthe bichhinani mama dosam na bidyate."

O *Tulsi*! You are always in the heart of Lord *Vishnu*. I am snatching your leaves for offering to Lord *Vishnu*, please excuse me. *Tulsi* is an arable plant and cannot withstand water logging. Odisha is coming under high rainfall area having annual precipitation of 1500 mm, so people grow this sacred plant on *Chaura*, a raised platform with soil, so that there will be good drainage and it wouldn't be subjected to water stagnation. People offer water daily to this plant and eat its leaves. It's a unique system in the entire Odisha. Because it is planted in raised platform, during summer months there is water deficit to this plant due to adequate drainage because of its stiff hydraulic gradient. So, people might have thought of prolonging water release to this plant which might have led to invention of drip irrigation. Due to its special geo-location being on the coast of Bay of Bengal, its environmental temperature rises sharply from early part of March and reaches up to 44.6°C (the highest temperature attended in any individual year) by 31st March, which is also highest in the country. During this period, a part of north India remains cooler and in south India, temperature rises little higher over north India. Higher air temperature might have accelerated the evapotranspiration of water from the *Tulsi chaura*, as a result, people might have thought to provide water to *Tulsi* plant during this hot summer. When they might have poured water from the pot to the base of *Tulsi* plant at *Chaura*, water might have drained quickly, subjecting the plants to desiccation due to its strong hydraulic gradient. This situation might have forced the Odia people to think of a process or method of watering *Tulsi* plant in which water will be trickled to plant at its base for a long period during summer season. In other words, they might have thought to develop a technique of water application where the release of water can be prolonged with a low rate of discharge so that water wastage can be reduced. This might have led to *Theki Basa* (Hanging up an earthen pot at the base of *Tulsi* plant with tine hole plugged with kusha grass) in Tulasi *chaura* starting from 1st of *Baisakh* month (14th day of the month April) i.e., *Mahabisuba Sankranti or Pana Sankranti* (Praharaj, 1931). In *Theki* basa earthen pot called *theki* with an earthen cover or lid is purchased from market and a small hole is made in the bottom of the pot. The hole is sealed through insertion of Kush grass *(Desmostachya bipinnata)* tightly, so that water will release drop by drop (Sahoo, 2014). An inverted U-shaped support is made through bamboo pieces and this *Theki* or earthen pot is hung with the help of jute *Sika* (one balance support made up off jute rope (Fig. 2). During summer months, after morning worship of *Tulsi* plant, water is poured in this earthen pot which trickles at the base of *Tulsi* plant drop by drop. This practice is continued during summer months and removed after onset of monsoon in the beginning of the month *Asadha*. This is the first application of drip irrigation to plants in the world which dates to beginning of *Sanatan dharma* or Hindu religion (Mohanty, 1979). The method of trickling of water to *Shivalinga* from the hanged pot filled with water having a tine hole plugged with grasses is also invented in India. It is difficult to say which practice or technology earlier and which is later. Both *Halahal* (poison) and *Tulsi* plant were obtained from churning of ocean during *Satya Yug* as per mythological scriptures. But one thing is sure that trickling of water drops on *Shivalinga* drop by drop might be first step in prolong release of limited supply of water to the targeted location by reducing wastage, whereas drop by drop release of water at the base of *Tulsi* plant during the summer months by hanging pot filled with water having tine hole with grass plugging is the first experiment in drip irrigation for the plants or crops in the entire world. All other forms of primitive drip irrigation have been started much later than the former as this practice was followed from the beginning of Hindu religion.

Fig. 2(a). Worship of *Tulsi* Chaura in Sriganga

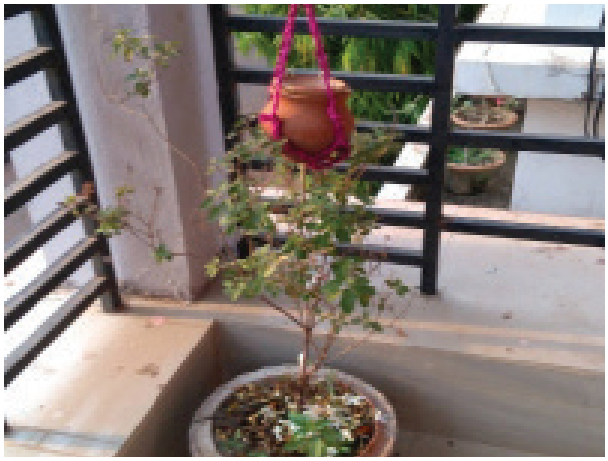

Fig. 2(b). Water trickle at the base of *Tulsi* plant in Maitrivihar

Fig. 2(c). Water trickle at the base of *Tulsi* plant in Patrapada

Fig. 2(d). Water trickle at the base of *Tulsi* plant in Bhubaneswar

Fig. 2(e). Lady devotee pouring water on mud pot to be trickled on *Tulsi* plant in Tulasipur area of Cuttack

Fig. 2(f). Shop selling holed earthen pots with hanging rope for water trickle over *Tulsi* plant at Bhubaneswar

Fig. 2(a-f) Photographs showing ancient drip irrigation technique in Odisha

CONCLUSION

Considering the above facts, it can be inferred that the first concept of water use efficiency by reducing its wastage was developed from India, which was trickling of water droplets on *Shivling* from a perforated pot with grass sealing. Similarly, the first experimentation and its application of drip irrigation to plants was made in Odisha state of India where water droplets are allowed to trickle down to *Tulsi* plant for its survival during summer months from a perforated mud pot hanged above the plant by providing artificial support. Both these religious practices are ancient to Hindu religion and are under practiced even today.

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Poacher's snare as threat to Indian wildlife: A review

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ABSTRACT

A snare is one of the easiest but most destructive hunting methods. In Asia, snares are the most widely used method of hunting because they are cheap to produce and easy to set in large numbers. Wildlife SOS, in collaboration with the Karnataka Forest Department, has attended a total of 42 wildlife rescue calls of sloth bears and leopards entrapped in such illegal snares or traps during the period 2009 to 2019. The snare traps entangled around the hind quarter, leads to internal organ damage and a slow death even after the rescue. Leopards and bears may also suffer serious dental problems from biting the snares, which results in medical problems and the inability to return the rescued animal to the wild. Intensive awareness programs in and around the protected areas regarding this barbaric and primitive trapping tools would help in reducing or avoiding such incidents. Human-animal conflict has been present ever since the fragmentation of forests and increase of biotic pressures have brought wild animals closer to humans in a fierce competition for survival. The present review deals with incidences of sloth bear and leopard mortality or injury due to snare documented across various districts of Karnataka and few other states, this review provides vital information about snares' threat to Indian wildlife. Based on content analysis of newspapers and news portals, we identified 446 incidents of wild animals caught in snare traps from January 2018 to October 2022. Most snare incidents involved wild boars, snakes, nilgai, Indian leopards, jackals and royal Bengal tigers. This review indicates large number of carnivore death as compared to both herbivore and omnivores. We therefore propose a shift in management focus, from current reactive practices to proactive measures that ensure safety of wildlife.

Key words: Animal welfare, electrocution, extinction, poaching, wildlife trade

INTRODUCTION

Globally poaching and illegal wildlife trade is driving many of the world's valuable species into extinction. Elephants, rhinos, and tigers are among the many exotic species that are poached for ivory, horn, and skin in order to make them targets for illegal trade (Kaul et al., 2004; Spillane, 2015). Other animals like wild pigs and deer are hunted as bush meat for protein (Warchol, 2004). Due to the development and emergence of several anti-poaching camps as well as the increased protection inside the protected areas, poachers' motives and hunting techniques have changed. Researchers believe that poachers vary; they may have varied motivations and motives, use different techniques, and use equipment of diverse types (Pires et al., 2016). One of the simple yet deadly methods used by poachers is to set up a snare and the practice of laying snares dates to the early 80s. It is no secret that hunters and poachers use rope, wire, or brake cables for making these simple, low-tech, noose-like traps, which they set in forests in order to capture animals. Snaring is one of the effortless but most effective hunting techniques followed in Asia (Belecky et al., 2020) and other parts of the world. It is becoming increasingly common to use wire snares in Asia due to their ease of construction from readily available materials like bicycle and motorcycle cable wires. Poachers set snares targeted for specific animals. There are tiny, thin, single strand wire snares used

to catch small animals like hares at lower levels on the trails, while larger, thicker snares used to trap bigger animals like wild pigs at higher levels.

Snares are essentially long pieces of wire connected at their ends with a loop and attached to stationary objects, such as trees or logs. Using a loop of wire, the snare is suspended from a branch or small tree, catching animals by their necks as they walk through the forest. The snare grips tightly and captures the animal as it continues to move forward. Snare traps are one of the most popular types of traps, not only because they are so easy to use, but also because they are so easy to make. In technical terms, they are wire or cable nooses that are anchored somewhere. It is impossible for the animal to escape the trap once it runs over it, as the noose tightens around the animal's body, neck or limb and it is unable to escape the trap as a result even though it may be simple and effective, it is not at all humane. According to the report by Mongabay on Snare traps decline, but still pose a threat to Leuser's Sumatran rhinos they explained that "Snares are typically made of steel or nylon wire and are easy to build. In addition, they are indiscriminate in what they capture, resulting in non-target species as well as females and juveniles being caught. While most of the trapped animals end up in local wildlife markets or are sold directly to restaurants as bush meat, the high-value species are typically traded in major cities or exported to foreign markets."

Throughout Southeast Asia, snaring is one of the most common types of hunting used to capture animals for human consumption and to stock wildlife farms in order to capture wildlife for human consumption (Becker et al., 2013; Gray et al., 2018). The ungulates are a very common species that is caught in Cambodia, Lao PDR, and Viet Nam, and there is evidence that they are a species that is traded more frequently in Asian countries (Cantlay et al., 2017). A study of wildlife seizures in Cambodia from 2005 to 2017 found that 46% of all wildlife meat seizures (61%) that likely came from snared animals (ungulates, carnivores, lagomorphs) occurred in markets (which were referred to as snared animals), whereas 48% (32% of biomass) occurred in restaurants and resorts. According to the WWF latest analysis report on Snaring crisis, it is concluded that, "There are an estimated 12.3 million snare on the ground in protected areas of Cambodia, Lao PDR and Vietnam." Similarly, according to the statement by Richard Thomas from TRAFFIC's, explained that, over 30,000 snares were removed in Cambodia in 2016 alone; it is likely that many more remain undiscovered. "As snares are a very dangerous device simply because they kill at random, which means all manner of wildlife is at risk. Snares are also very commonly used by poachers to steal tigers from Asia's forests due to their tendency to kill at random. In order to curb this crisis, there is an urgent need for the countries in the Tiger range to intensify their enforcement efforts."

The snaring technique is not only a common method in Asian countries, but all over Africa as well. As a result of the rising global demand for bushmeat in Africa, there is an increase in the silent capturing and poaching of wild animals with the use of snare traps. Since snaring has become a popular method throughout Africa because of the availability of the materials needed (e.g., fence wire, telecommunications or electrical cabling and nylon rope) at an affordable price, this method has become very widespread (Mowat et al., 1994; Obanda et al., 2008). It is widespread throughout Africa for bushmeat to be harvested using snares which is mainly done within the protected areas of forest and savanna as well as in communal or private lands. (Hitchcock, 2000; Poulsen et al., 2009; Lindsey et al., 2013; van Velden et al., 2018; van Velden et al., 2020).

Table 1. Sloth bear incidents with human hazards and distance from forest fringes

Type of hazard	Number of incidents	Average distance to forest edge (m)	Range of distances to forest edge (m)	Number of incidents in a forest area	Notes
Snares	18	2,117 (n=10)	240 - 8,825	3	5 Locations unknown

Table 2. Sloth bear incidents by gender with human hazards

Type of hazard	Number of incidents	Females (including with cubs)	Males
Snares	18	11	7

MATERIALS AND METHODS

Apart from the Table 1 and 2 data, the rest of the data collection was made through the secondary sources. The relevant information and data were collected by reviewing various website and research articles for content that explains snare wire and traps. In addition, the last five years (January 2018 - October 2022) wildlife injury and death data are collected and analyzed using daily newspapers like the Hindu, the Indian Express, as well as articles written by organizations such as WWF, Asia, etc. There is a strong presence of media in India, even in rural areas, and news about wild species is mostly covered by the media, making it a reliable source of information about large mammal conflicts. The study relied completely on information sourced from newspaper media reports, open-source government websites and remotely acquired data. Animal care and use committee approval was not required.

RESULTS AND DISCUSSION

Snares

Of the 18 sloth bears caught in snares that Wildlife SOS attempted to rescue (Table 1 and 2), twelve (67%) were eventually released back to the forest, and in all cases except one, back to the forest they were trapped nearby. Four bears (22%) died in the snare or from the wounds they received while being caught in the snare, and two bears (11%) were put into lifetime care at the Wildlife SOS Bannerghatta Bear Rescue Centre, due to the fact that their injuries were too substantial to release them back to the wild. Eleven of the bears were female, and seven of the bears were male. Half of the bears (n=9) were estimated at 2 years old or younger, the half (n=9) were estimated at 5 years or older.

Ten of the eighteen snares (56%) were found in agricultural areas, three (17%) were found in forest or scrublands and five (28%) did not have a location documented. The average distance of those found in agricultural fields was over 2,000 meters from forest edges. Two of the three snares found in forest or scrublands were less than 300 meters from agricultural fields while one was over 800 meters from agricultural areas.

There was a spike in the number of bears caught in snares between the months of September and December. This is during the harvest time when animals enter the agricultural areas to raid the crops. Six of the bears (33%) caught in snares were caught outside of the harvesting season. However, three of these were caught in the scrub areas, not the agricultural areas, and two of the them were caught in undocumented locations. Only one bear was caught in the agricultural areas outside of the harvesting time.

Snare traps in India

The snare traps are made from materials that can readily be found, including clutch wires, fencing wires, and other materials that can be found around the house. Considering the fact that they are light and easy to carry, they can be used to capture animals without them being aware that they are being caught. Using wire snares and electrocution are the most predominant ways to kill animals. It is generally the local village communities who set up these traps to be able to catch wild boars, small herbivores, etc. that wander around in the area. It is quite common to set up snares along game trails and near watering holes (Gubbi et al., 2021) where there is a greater chance of getting caught by the trap. Wildlife killing with snares is illegal in India, but snares remain a popular method for catching wildlife Indian wildlife populations are rarely studied empirically for the effects of snares (Madhusudan and Karanth, 2002; Gurung et al., 2008; Gubbi and Linkie. 2012).

Fig. 1. Snare trap/ cable removed by Karnataka forest department

Cases of snaring in India

According to records, over the course of the last decade, India has witnessed twenty-four tigers and one hundred and fourteen leopards becoming entangled in wire snares. Uttarakhand, Karnataka, and Madhya Pradesh are some of the states that have been high on the radar of snares. Nevertheless, a database compiled by Wildlife Protection Society of India (WPSI), a conservation organization fighting poaching and escalating wildlife trade, shows 24 tiger fatalities and 110 leopard deaths in the country in 2010-2018, including five tiger, 14 leopards and 30 other wild animals alone in Maharashtra state. It has also been reported that the greatest number of big cats, 26 leopards and three tigers, have been killed in Uttarakhand and at least 13 leopards have been injured. In Madhya Pradesh, five tigers have been killed by wire snares and one has been injured, the highest number of tigers killed in a state in the last decade.

Table 3. List of animals died by snare traps from 2018-22 in India

No.	Animal	Type	2018	2019	2020	2021	2022	Total
1	Royal Bengal Tiger	Carnivore	6	4	4	3	5	22
2	Indian leopard	Carnivore	11	7	7	2	5	32
3	Asiatic lion	Carnivore	0	0	1	1	0	2
4	Fishing cat	Carnivore	3	3	3	5	6	20
5	Wild boar	Omnivore	26	16	19	25	32	118
6	Snow leopard	Carnivore	0	0	1	0	2	3
7	Clouded leopard	Carnivore	0	0	0	0	1	1
8	Sloth bear	Omnivore	1	2	2	3	0	8
9	Indian gaur	Herbivore	0	0	0	2	0	2
10	Asian elephant	Herbivore	6	2	1	1	2	12
11	Lion tail macaque	Omnivore	0	0	1	2	3	6
12	Indian rhino	Herbivore	0	0	0	0	1	1
13	Wild water buffalo	Herbivore	0	1	0	2	0	3
14	Nilgai	Herbivore	13	5	7	9	11	45
15	Bengal fox	Carnivore	3	6	7	0	1	17
16	Striped hyena	Carnivore	1	0	0	0	0	1
17	Spotted deer	Herbivore	1	0	0	1	3	5
18	Swamp deer	Herbivore	0	0	1	0	0	1
19	Kashmir stag	Herbivore	1	0	0	0	0	1

20	Sambar deer	Herbivore	2	0	0	1	1	4
21	Indian pangolin	Carnivore	0	0	0	0	3	3
22	Hanuman langur	Omnivore	7	8	1	0	0	16
23	Indian cobra and others	Carnivore	0	12	11	24	11	58
24	Pygmy hog	Carnivore	0	1	0	0	0	1
25	Ganges shark	Carnivore	0	0	1	0	0	1
26	Red crowned roof turtle	Herbivore	3	0	1	1	1	6
27	Himalayan wolf	Carnivore	1	0	0	0	0	1
28	Nilgiri tahr	Herbivore	0	0	1	0	0	1
29	Jackals	Carnivore	7	2	13	1	3	26
30	Musk deer	Herbivore	1	0	0	0	0	1
31	Jungle cat	Carnivore	0	7	1	1	0	9
32	Wild dog	Carnivore	1	0	0	0	1	2
33	Tibetan fox	Carnivore	1	0	0	0	1	2
34	Red fox	Carnivore	1	0	0	0	0	1
35	Marbled cat	Carnivore	1	2	0	0	0	3
36	Large indian civet	Carnivore	0	0	0	1	0	1
37	Small indian civet	Carnivore	0	0	0	3	0	3
38	Asian palm civet	Omnivore	0	0	0	1	0	1
39	Himalayan black bear	Carnivore	0	0	0	1	1	2
40	Yellow throated marten	Carnivore	1	1	1	0	0	3
41	Gangetic dolphin	Carnivore	1	0	0	0	0	1
	Total cases		99	79	84	90	94	446

It is common for people living in close proximity to forests or protected areas to place snare traps either as a means of hunting bushmeat or as a means of defending crops against crop damaging animals. In India, 446 wild animals were strangled in snare traps in between 2018 and 2022 i.e., on an average 89-90 animals per year. During 2018, 99 deaths are reported, followed by 79 in 2019, 84 in 2020, 90 in 2021, and 94 in 2022 (Table 2). These deaths included 22 cases of Bengal tigers, 32 cases of Indian leopards, 12 cases of Asian elephants (mainly electrocuted snare), and 118 cases of wild boars. While snare traps are often set for small animals, it appears that large animals are most likely to fall victim to them. In spite of the fact that snare traps are often used to capture small mammals such as Indian hares, and other animals such as wild boars, the majority of the victims were non-targeted species i.e., Bengal tiger, Indian leopard and etc. The occurrence of carnivore cases is 49% (215) while omnivore cases are 33% (149) and herbivore cases are 18% (82); (Fig. 2). The following graphs are an overview of the death of wild animals from snaring from the years 2018 to 2022.

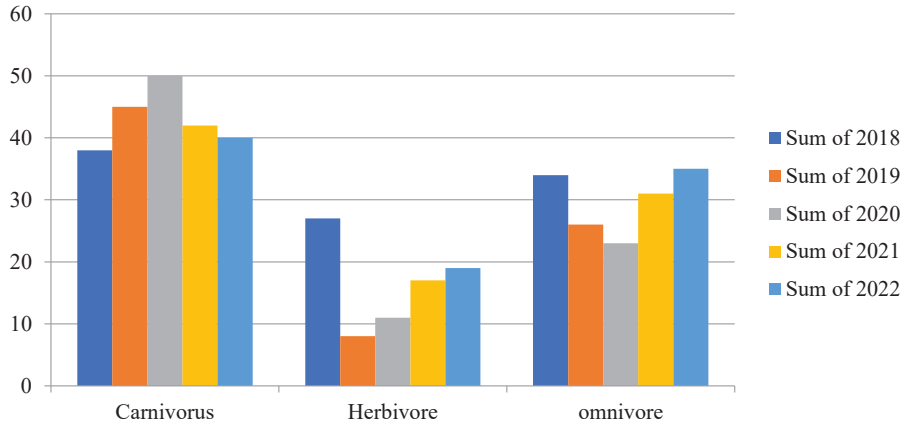

Fig. 2. Snaring victims in India in the past five years, grouped according to their diet

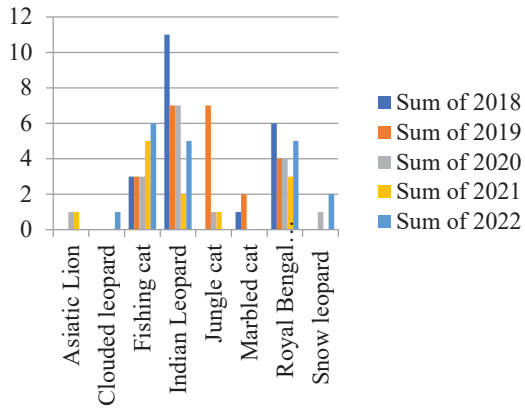

Fig. 3. Felidae

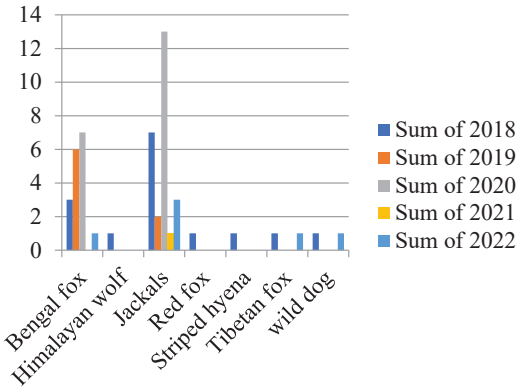

Fig. 4. Canidae

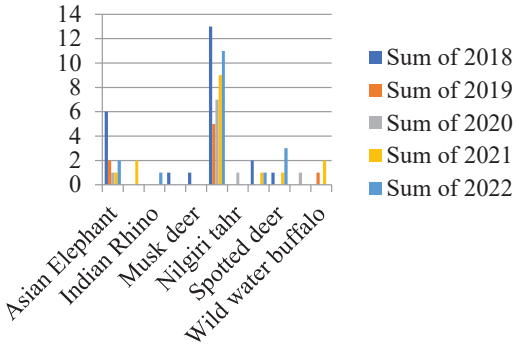

Fig. 5. Herbivores

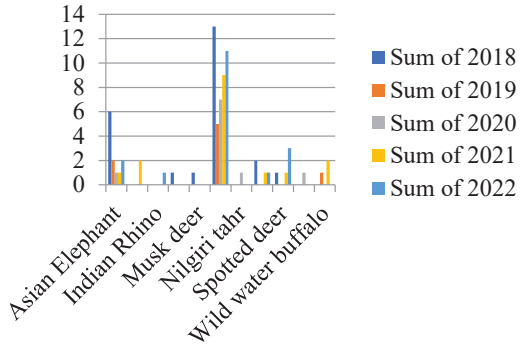

Fig. 6. Aquatic and snake

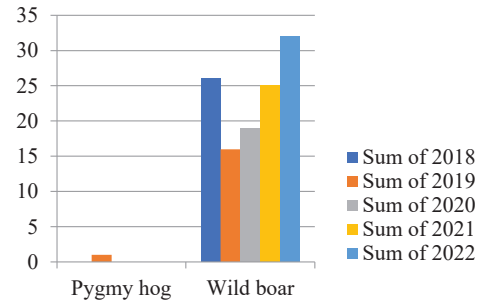

Fig. 7. Suidae

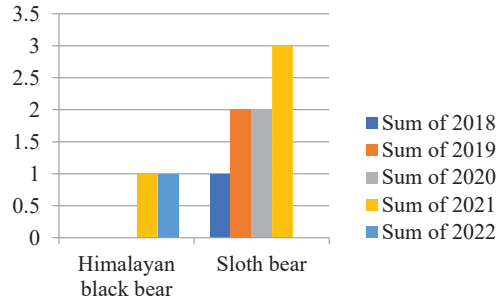

Fig. 8. Ursidae

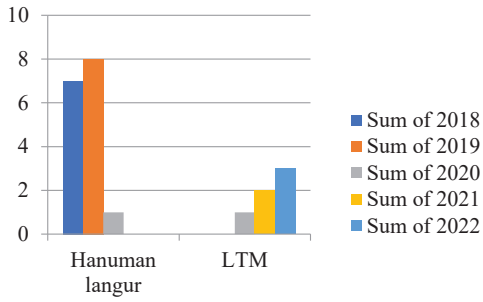

Fig. 9. Primates

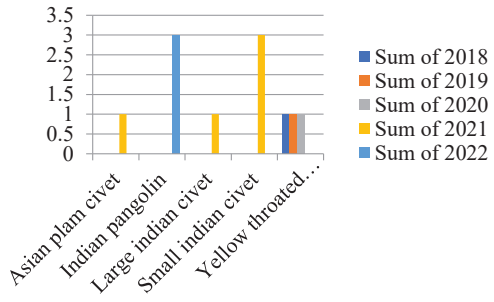

Fig. 10. Other small mammals

Indian wildlife continues to suffer from snare setting, despite a wide range of preventative measures implemented over the years. A number of reasons exist for the prevalence of snare-based poaching in India, including the low costs involved and the low risk of being caught and prosecuted if caught. A cable snare is directly responsible for providing food for the household and indirectly for producing income through the sale of bushmeat, which is sold to the public. Gubbi et al. (2021) explained that the number of snaring incidents were extremely high during the monsoon season which is the peak cropping season when farmers tend to put extra effort into protecting their crops and their livestock, including setting snares to stop herbivores from raiding their crops. There may be a reason for the high number of leopards that get caught in snares during monsoon season.

Finding in this study indicate high number of carnivore death as compared to both herbivore and omnivore animals. Although the traps are mainly set for small mammals like wild pigs, hare's, mongoose, mouse deer, civets and squirrels etc. (Fig. 15) but the prime victims were the large mammals like Bengal tiger (22 cases), Indian leopard (32 cases), nilgai (45 cases), sloth bear (8 cases), Wild boar (118 cases) and sometime even elephants etc. Apart from that, aquatic species like Gangetic dolphin (1 case), Ganges shark (1 case), red crowned roof turtles (6 cases) and snake species like Indian cobra, etc. (58 cases), have also been victims of the snare traps in India.

When it comes to hunting for human consumption and the stocking of wildlife farms in Southeast Asia, snaring is among the most prevalent methods of hunting for the purpose of capturing animals for human consumption (Harrison et al., 2016; Gray et al., 2018). In Southeast Asia, Cambodia, Lao PDR, and Viet Nam are among the nations most affected by the snaring crisis, with a greater number of snares than anywhere else in the region or in the world (Belecky and Gray, 2020). According to the data collected WWF 2020 Southeast Asia snaring crisis report, it is estimated that between 2005 and 2019 rangers from 11 protected areas in five Southeast Asian countries (Cambodia, Indonesia, Lao People's

Democratic Republic, Malaysia, and Viet Nam) removed 371,856 snares (approximately 53,000 snares a year) from 11 protected areas (Belecky and Gray, 2020). In Vietnam there is between 60% and 80% of the wildlife meat consumed in urban areas that is eaten in restaurants (Nguyen, 2003; Drury, 2011). The most commonly consumed species, which represents almost 75% of all wildlife meat consumption, is wild pig a species that is heavily hunted with snares in mainland Southeast Asia (WWF Vietnam, 2017). Similarly, a study of wildlife seizures collected in Cambodia between 2005 and 2017 revealed that 46% (representing 61% of the seized biomass) of wildlife meat that was likely to have been snared occurred in markets, whereas 48% (42% of biomass) were seized at restaurants and resorts (Heinrich et al., 2020).

Furthermore, a study conducted in Bayanga region of Central African Republic on cable snare hunting, stated that in the Bayanga hunting range, which includes Dzanga-Ndoki National Park, there are on average 4.2 cable snares per square kilometer, with an estimated 9000 total captures per year, or nine captures per square kilometer, which puts the total number of captures around 9 per square kilometer (Noss, 1998). There are, however, studies in South Africa that show that 30-60% of rural households living in communal tenure regions consume bushmeat as a matter of course (Grey-Ross et al., 2010; Martins and Shackleton, 2019).

Snares are cost-efficient, easy to carry and, unlike firearms, easy for poachers to conceal and transport throughout the world. Even though snares are simple in design, they frequently cause severe discomfort and pain to animals in controlled experiments following animal welfare guidelines. This is especially true in remote locations where hunters leave traps unattended for weeks or even months (Mowat et al., 1994; Powell, 2005; Gese et al., 2019). In spite of its indiscriminate nature, snaring has the potential to be very detrimental (i.e., non-target mortalities) (Fig. 14) and extremely wasteful if it is carried out in an irregular manner (Obanda et al., 2008; Lindsey et al., 2011).

Animals captured in such conditions will usually experience prolonged suffering before death. Some animals may be able to escape from the ensnaring trap, either by self-mutilation (such as chewing away at ensnared limbs to free themselves) or by self-harm Noss (1998). In order to survive, these crippled individuals will have to deal with a great deal of hardship. It is well documented that animals suffering from such injuries are likely to have smaller home ranges, to suffer from malnutrition, and to occupy degraded habitats, as they have difficulty defending their territories against healthy animals (Sunquist, 1981; Othman et al., 2019). Animals who suffer from physical ailments are more likely to engage in conflict as their behavior is altered (Becker et al., 2013). For example, it has been reported that physical impairments are the most common factor associated with human-killing tigers in Nepal (Gurung et al., 2008). It has also been reported that elephants wounded in snares pose serious dangers to rural villages, thus escalating conflict with the species that is already prone to antagonistic encounters with humans (Obanda et al., 2008; Becker et al., 2013; Abdullah et al., 2019; Othman et al., 2019).

An animal species with a dominant hunting instinct could be subjected to physical impairments including dental issues, tooth loss, and misaligned canine teeth, which could influence the genetic selection process in terms of breeding. An individual with incomplete or complete ocular or auditory deformities, as well as locomotion defects can alter the individual's behavior, hunting skills, and can even lead to the possibility of a human-wildlife conflict. In terms of physiological impact, cervical bone fractures or defects, dislocated joints, fractured limbs and physiological damages to vital organs are among the most common and leads to mortality such as kidney, spleen, liver, heart. These injuries occur as a result of an excess compression and pressure of the snare which is dependent upon the position of the snare within the animal.

Documented evidence of various types of illegally installed poacher's snare, species involved and injuries encountered by various Indian wildlife photographs are presented in Fig.11 to Fig. 15.

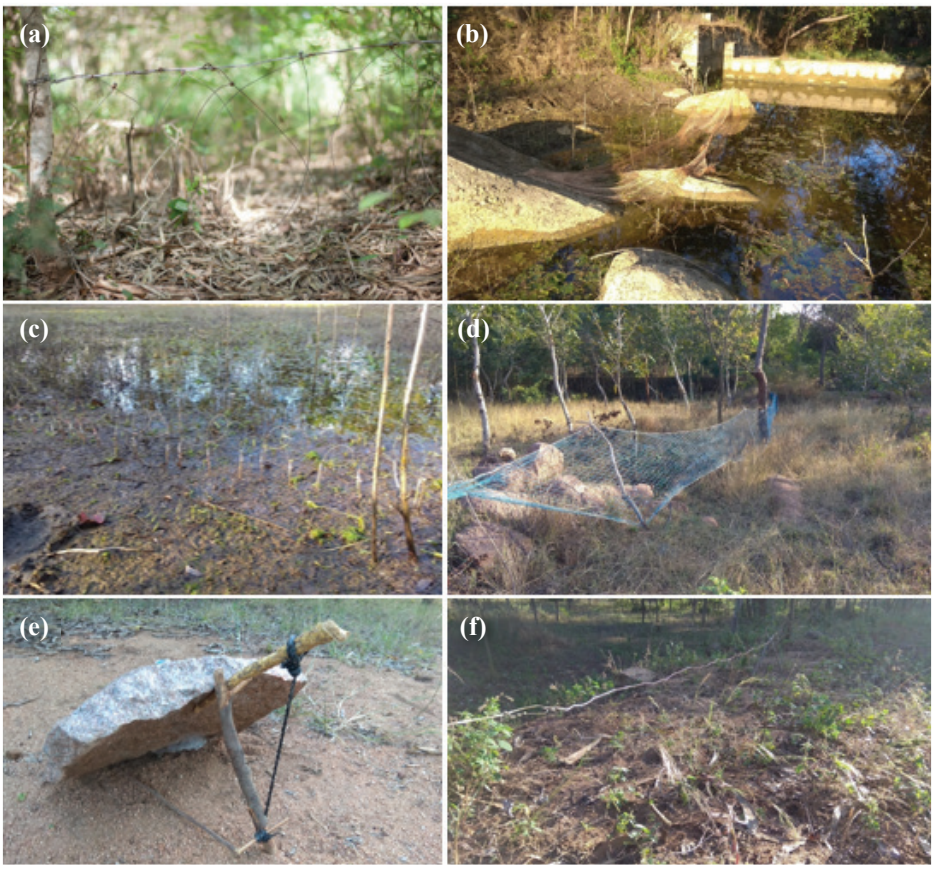

Fig. 11 (a, b, c, d, e, f). Different types of snares; Loop snares mainly used for capturing small animals like Indian hare etc.; net snare traps mainly used for capturing wild boar, spotted deer etc. and stone traps used for small mammals

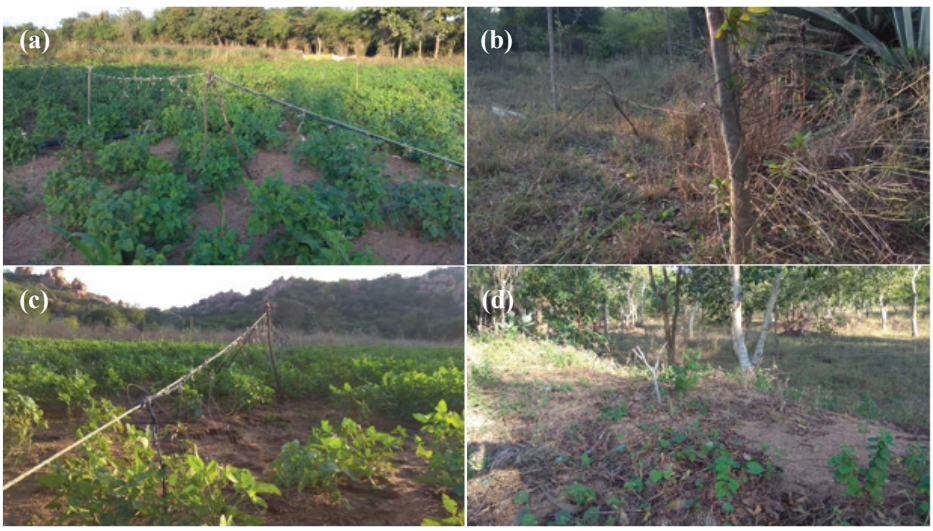

Fig. 12 (a, b, c, d). Wire snares and loops snares placed by people in agricultural fields

Fig. 13 (a, b, c, d, e). Different types of confiscated snares: Net, nylon wire, metal wire and clutch wire

Fig. 14 (a, b, c, d, e, f). Victims of some non-targetted species

Fig. 15 (a, b, c, d). Some of the most targeted species

Fig. 16 (a, b, c, d). Sloth bear (a and c) struggling after getting trapped in a barbed wire and leopard (b and d) trapped in a snare trap rescued by Wildlife SOS

RECOMMENDATIONS

A comprehensive review of this article over a wide range of wildlife snaring studies conducted in different countries and the Indian subcontinent led the author to make the following recommendations. Snare patrols should be conducted regularly in and around protected forests to pick up snares that have been set up. As part of its efforts to combat the incidence of snares, the Forest Department should seek help from a wide range of stakeholders and agencies. Poachers who use snares for illegal purposes must be punished and convicted with more severe legal consequences. Besides that, awareness sessions should be conducted continuously all the time, especially when hunting season is believed to be in full swing. Furthermore, educating the people living around forests' fringes that it is a punishable offense under the Indian Wildlife Protection Act 1972.

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Effect of straw mulch and irrigation frequency on yield and yield components of mung bean [*Vigna radiata* (L.) Wilczek]

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ABSTRACT

A field experiment was conducted at agricultural research farm of Agriculture faculty of Ghazni University in 2022. It was laid out in Factorial Randomized Complete Block Design with three levels of irrigation frequency as, irrigation interval at 5 (I05), 10 (I10) and 15 (I15) days; with straw mulch (SM) and non-straw mulch (NM) in three replications. The highest amount of grain yield (1102 kg ha^{-1}) was obtained from 10-day irrigation interval with straw mulch (SMI10) followed by SMI15 (1064 kg ha^{-1}) and SMI05 (1081 kg ha^{-1}) treatments, respectively. While NMI15 treatment significantly ($P<0.05$) reduced number of leaves (18 leaves per plant), leaf area (22 cm^2), number of pods (15 pods per plant), 1000-seed weight (48 g), and grain yield (531 kg ha^{-1}) than other treatments. In SMI10, SMI05, and SMI15 treatments, the crop water stress coefficient (K_s) values were recorded as 0.93, 0.91, and 0.90, respectively. In conclusion, the highest mung bean performance was achieved under SMI10 and SMI15 treatments, and for achieving suitable yield of mung bean, 15-day irrigation interval with straw mulch is recommended.

Key words: Irrigation frequency, mulch, mung bean, yield, yield components

INTRODUCTION

Mung bean (*Vigna radiata* L.) is a short-season grain legume crop that grows in summer predominantly indry conditions throughout tropical and subtropical (Robertson et al., 2004). This crop is rich in protein (25%) with high quality lysine (460 mg g^{-1}) and tryptophan (60 mg g^{-1}). Its seed has significant amount of ascorbic acid when sprouted, as well as riboflavin (0.21 mg 100 g^{-1}) and minerals (Praveena et al., 2018) specifically K, Fe, P and Ca with higher digestibility (Mahato et al., 2015). Due to its ability to fix atmospheric nitrogen in the soil (Mahunta et al., 2018), mung bean plays a major role in crop rotation (Sadiq et al., 2018). In case of drought stress, Allahmoradi et al. (2011) reported the adverse effects of drought stress during vegetative growth than in reproductive stage. There for in areas with limited water availability and high soil temperature, straw mulch may be beneficial for growth and yield of mung bean (Chaudhary et al., 1985). The contribution of crops residue uses as mulch on conservation of soil fertility for long time reported by Martin and Belfield (2007). The use of an appropriate Ks method having the potential to improve irrigation time table in better management of stress and ensuring optimum yield under limited irrigation water supply (Kullberg et al., 2017). Therefore, the aim of this experiment was the evaluation of mulch and irrigation frequency on yield and yield components of mung bean for efficient use of irrigation water under irrigated conditions with limited water supply.

MATERIALS AND METHODS

Experimental site

This experiment wa scarried out at agricultural research farm of the Ghazni University (lat. 33° 31' 58" N, long. 68° 28' 52" E, altitude 2204 m above MSL). Ghazni province is in the area with cold and snowy winter (October to March the fourth quarter of the Persian year) in which the most parts of precipitations occur and hot summer (Hemat et al., 2017).

Experimental design

This experimental design was laid out in Factorial Randomized Complete Block Design with three irrigation frequency and with and without straw mulch which consist of six treatments viz. NMI05, NMI10, NMI15, SMI05, SMI10, and SMI15 (Table 1) in three replications

Table 1. Description of the treatments

No.	Treatments description	Group abbreviation
1	Irrigation after 5 days interval (I05) with no-straw mulch (NM)	NMI05
2	Irrigation after 10 days interval (I10) with no-straw mulch (NM)	NMI10
3	Irrigation after 15 days interval (I15) with no-straw mulch (NM)	NMI15
4	Irrigation after 5 days interval (I05) with straw mulch (SM)	SMI05
5	Irrigation after 10 days interval (I10) with straw mulch (SM)	SMI10
6	Irrigation after 15 days interval (I15) with straw mulch (SM)	SMI15

Data collection and parameters

Growth and yield attributes

The plant growth parameters were directly measured from the row length of each treatment and recorded. A representative sample of 10 plants was used to represent the treatment and replication for determination of numbers of pods per plant, seeds per pod, 1000-seed weight and yield, and individual leaf are a measured as below:

$$LA = LL \times LW \times A \quad (1)$$

Where, LL and LW are leaf length and leaf width and A is constant (A=0.75), respectively, and total leaf area per plant was calculated by summation of individual leaf areas per plant.The leaf area index (LAI) was calculated from multiplying of leaf area per plant with number of plants per unit of land (Greaves and Wang, 2017) based on bellow equation:

$$LAI = LA/Plant \times No.\ Plant/m^2 \quad (2)$$

Crop water stress coefficient (K_s)

Crop water stress coefficient (K_s) shows the amount of water which received by plant during the growing season and calculated from relative yield reduction to maximum yield of crop. It ranges from 0-1 where the number 1 shows when crop received 100% of water and lower than 1 indicates the degree of stress. It is calculated by the following equation (Wahaj et al., 2007):

$$K_s = 1\text{-}1/K_y\ [1\text{-}Y_a/Y_m] \quad (3)$$

Where, K_s is crop water stress coefficient, Y_a= actual yield (kg ha^{-1}), Y_m= the maximum yield (kg ha^{-1}).

The SPSS software was used for analyzing of variance and Duncan test was used for determination of the significant differences between each treatment.

RESULTS AND DISCUSSION

Growth parameters

According to the research results, all the growth parameters, viz. plant height, number of leaves per plant, leaf area, leaf area index, and number of branchesper plant increased with increasein irrigation frequency under no mulch treatments (Table 2). The highest value of plant height (34 cm) was observed in SMI05 and with SMI10 (30 cm) and SMI15 (28 cm) but there was no significant *($p<0.05$)* difference between all the treatments. Also, no significant difference was found in leaf area index and number of branches per plant among the treatments. The treatment of SMI10 had higher number of leaves per plant (36) than the remaining treatments except NMI10 and NMI15 which were significant. In case of leaf area, there were statistically no significant differences among all the treatments except NMI15 which had significantly the lowest leaf area (22 cm^2) than other treatments.

Table 2. Effects of irrigation frequencies and straw mulch on growth attributing characteristics of mung bean

Treatments	Plant height (cm)	Leaves per plant	Leaf area (cm^2)	Leaf area index	Branches per plant
NMI05	26	29^{cd}	31^{ab}	1.15	5
NMI10	26	26^{de}	28^{bc}	0.90	5
NMI15	20	18^{f}	22^{d}	0.52	5
SMI05	34	35^{ab}	34^{a}	1.48	6
SMI10	30	36^{a}	31^{ab}	1.40	6
SMI15	28	32^{bc}	31^{ab}	1.26	6
LSD	NS	*	*	NS	NS
CV%	16	22	13	13	11

*Represent the significant difference (P<0.05), NS = no significant difference, LSD=least significant deviation, CV=coefficient of variation.

The result of this experiment is in conformity with the finding of Patel et al. (2020) who opioned that the plant growth depends on photosynthetic net produces, nutrient and water absorption from the soil, hence the proper and timely use of irrigation frequencies with reduction in water losses from plant rhizosphere causes cell turgidity resulting in higher meristematic activity, photosynthetic rate, and improves morphological parameters and ultimately enhance plant development (Yadav et al., 2021).

Yield and yield components of mung bean

The yield components of mung bean in response to irrigation frequency varied from the highest to the lowest, where as no significant reduction were found among the treatments under straw mulch. Significantly the highest values in number of pods per plant (23, 22, and 22) and 1000-seed weight (59, 58, and 60 g) were recorded from SMI15, SMI10 and SMI05, respectively, compared to the remaining treatments (Table 3). Itwas in conformity with Haqqani and Pandey (1994) finding who reported the suffering of mung bean due to water stress resulted in reduction of seed yield, number of seeds per pod and 1000-seed weight. The similar result was reported by Bunkar et al. (2013) who recorded significantly higher values in all growth and yield parameters as well as yields under weed and straw mulches.

Table 3. Effects of irrigation frequencies and straw mulch on yield and yield components of mung bean

Treatments	Pods per plant	Seeds per pod	1000-seed weight (g)	Yield (kg ha^{-1})
NMI05	20^{bc}	8	56^{cd}	972^{cd}
NMI10	20^{bc}	8	54^{de}	784^{e}
NMI15	15^{d}	8	48^{f}	531^{f}
SMI05	22^{ab}	9	60^{a}	1081^{ab}
SMI10	22^{ab}	8	58^{bc}	1102^{a}
SMI15	23^{a}	9	59^{ab}	1064^{bc}
LSD	*	NS	*	*
CV%	13	6	8	24

*Represent the significant difference (P<0.05), NS = no significant difference, LSD=least significant deviation, CV=coefficient of variation.

The yield of mung bean due to irrigation frequency and straw mulch differed from NMI05 to SMI15. The grain yield of mung bean (1102 kg ha^{-1}) was recorded from the SMI10 treatment, that was not significantly different than SMI05, SMI15, and NMI05 treatments, except NMI10 and NMI15 treatments that was significantly different (Table 3). The similar findings reported by Swain et al. (2007) who stated that the greater up take of nutrients and moisture by plant under straw mulching by conserving moisture and mulching benefits yield by improving soil physical conditions including improved stability in the top soil (de Silva and Cook, 2003).

Crop water stress coefficient (K_s)

On the basis of the research results, the performances of mung bean to various irrigation frequency and straw mulch from NMI05 to SMI15 were dissimilar. The treatments (SMI10, SMI05, SMI15 and NMI05) whose K_s values were recorded 0.93, 0.91, 0.90, and 0.83, respectively, indicating very less waterstress. However, the treatments of NMI10 and NMI15 whose K_s values (0.70 and 0.51, respectively) had decreased significantly ($P<0.05$) indicating the sensitivity of the mung bean to water stress (Fig. 1).

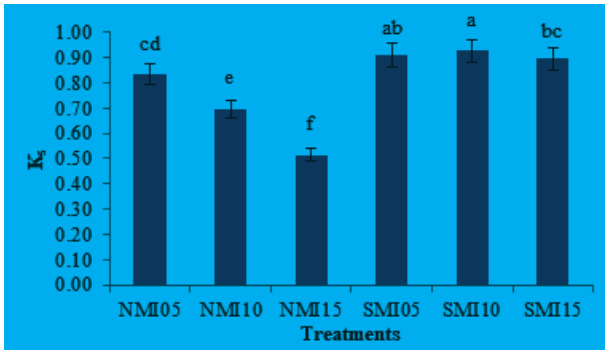
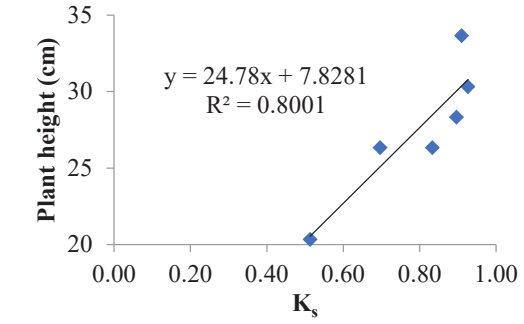
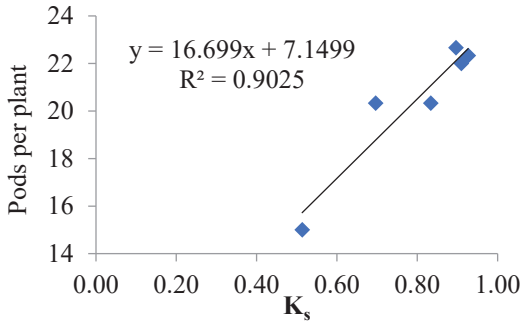
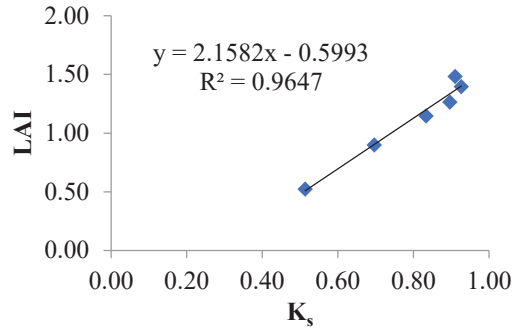


Fig. 1. Crop stress coefficient under various irrigation frequencies and straw mulch

Furthermore, the relationships of K_s with growth, yield and yield components are presented in Fig. 2. The significant correlations ($R^2 = 0.9$) showed among growth, yield, and yield components with K_s. The growth, yield, and yield components values were decreased whenever the mung bean exposed to water shortage. The essentiality of maximizing water use efficiency through crop water stresse valuation was advocated by Kokkotos et al. (2020) and use of appropriate K_s method to manage limited irrigation water supply for ensuring optimum yield are already expressed and confirm the result of our study in case of K_s. Like wise, our result is similar with findings of Terán-Chaves et al. (2022) who concluded that various water levels affect ryegrass by water depletion in the soil, resulting in reduction of biomass production by 76% at stress levels between 50% and 90% of total available water.







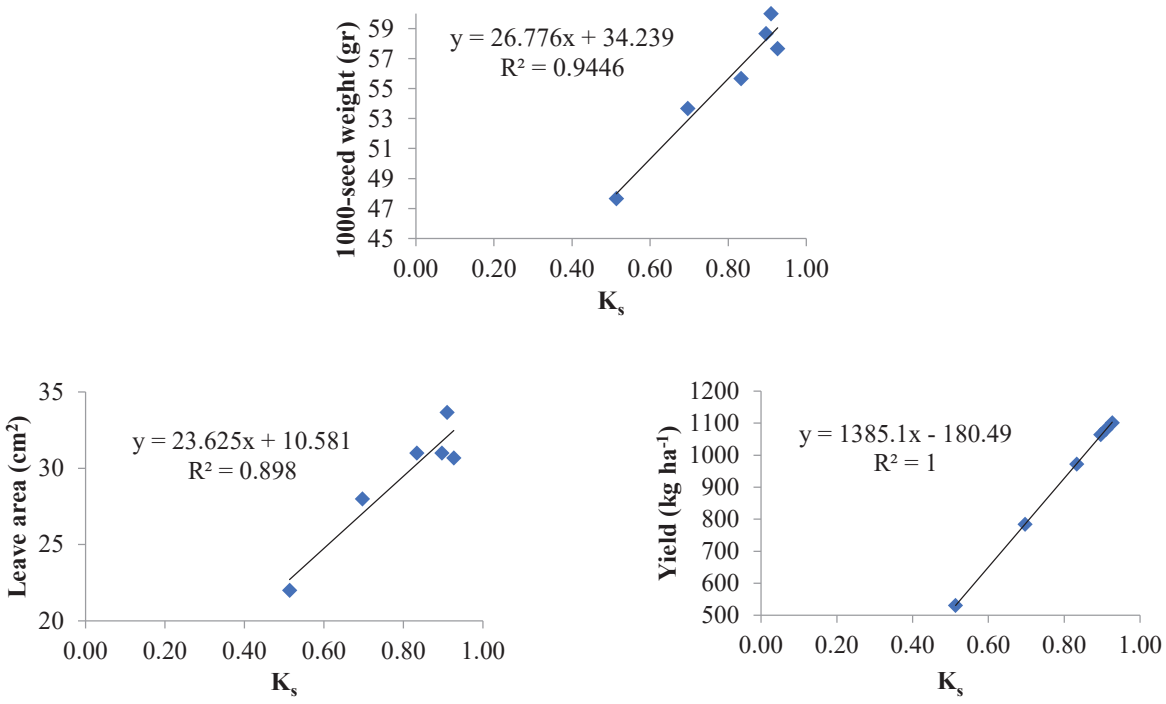


Fig. 2. Relationship of crop water stress coefficient with yield and yield components of mung bean

CONCLUSION

Mung bean crop showed dissimilar performances to various irrigation frequencies and mulch. Although its response was better in 5-day and 10-day irrigation frequency with or without mulch cover, in addition SMI15 had satisfactory performance with respect to water in adequacy and scarcity. Therefore, mulching can be a better option for water saving and is recommended to be used between rows of cultivated crops.

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Effect of nitrogen and sulfur applications on growth, chlorophyll content and yield of soybean [*Glycine max* (L.) Merr.]

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ABSTRACT

The current investigation reports the effect of basal and splitting application of nitrogen and sulfur on growth, chlorophyll content and yield at R_2 and R_5 growth stages of soybean in *kharif season* during year 2018. A field experiment was conducted at the Research Farm, Indian Institute of Soybean Research, Indore, India under different 14 treatment combinations involving different doses of N (12.5, 25 and 50 kg ha^{-1}) and S (12.5, 25 and 50 kg ha^{-1}) as basal and split application at R_2 growth stage of crop. The experiment was laid out in randomized block design with three replications. The results revealed that the basal and split application of nitrogen and sulfur induces the improvement in plant height, dry matter accumulation, chlorophyll content, leaf area, no. of branches per plant, pod per plant, seed index and grain yield over control. Average highest plant height, dry matter accumulation, chlorophyll content, leaf area, no. of branches per plant, pod per plant, seed index and grains yield were recorded with the application of N25+25, S25+25 at R_2 and R_5 growth stages. Thus, it can be concluded that split application N and S (N25+25, S25+25) can be applied to achieve better growth and yield in soybean crop.

Key words: Chlorophyll content, growth, nitrogen, soybean, sulfur, yield

INTRODUCTION

Soybean is one of the most important oil seed crops of India and the world. Majority of soybean area in India is in central part and is grown on vertisols and associated soils and by and large the crop is grown under rain fed situations. There is a considerable potential to bride the yield gap between the real and potential yield by adopting of appropriate improved resource management approaches. Nitrogen and sulfur are essential nutrients as they are involved in many biochemical processes and their deficiency not only affects crop productivity but also the quality of produce for human and animal consumption (Salvagiotti et al., 2008; Arata et al., 2017; Krishnan and Jez, 2018). Soybean has a large nutrient requirement throughout the growing season and has an especially high N requirement due to its seed protein content that averages about 40% based on seed dry weight (Bellaloui et al., 2015). Crop growth, grain yield will be increased when N fertilizer is added (Aulakh and Malhi, 2004). Nitrogen is a crucial plant nutrient, as it is an important component of chlorophyll. Nitrogen application time may be an important factor when considering N fertilizer application to soybean. Applying N at later stages may increase yield by supplying N at a vital time when N supply may be limited. Barker and Sawyer (2005) concluded that fertilizing soybean with N at the beginning of R3 increased yield over the control. Kinugasa et al. (2012) also reported that N fertilization after flowering significantly increased soybean seed number per plant. Over the last two decades, sulfur (S) deficiency has been recognized as a constraint on crop production all over the world

(Schonhof et al., 2007; Mascagni et al., 2008). The levels of the sulfur-containing amino acids cysteine and methionine in soybean seed proteins are inadequate for optimal growth and development of monogastric animals and the role of sulfur in plant metabolism, human and animal diet assumes significance (Krishnan and Jez, 2018). Fertilization with S enhances the effect of N and intervenes in soil processes that improve N-use efficiency by the crop. This improvement has been shown to be due to greater N recovery, without changes in internal efficiency (Salvagiotti et al., 2008). Plants must be supplied adequately with nutrients during the critical growth period for the normal maintenance of their physiological and biochemical processes. For this reason, obviously the concentration of plant nutrients in the soil solution must be maintained at a satisfactorily level for optimum plant growth. Therefore, the present study was undertaken to develop an optimum N and S management approach for soybean.

MATERIALS AND METHODS

Field experiment

A field experiment was conducted at the Research Farm, Indian Institute of Soybean Research, Indore, India (22° 8' N latitude; 75° 4' E longitude) during *kharif* 2018 (rainy season) to evaluate the effect of N and S application on growth and yield of soybean. The experimental soil (vertisols) belonged to Sarol soil series (Fine, iso-hyperthermic, montmorillonitic, Typic Haplusterts) with soybean-wheat as the predominant cropping pattern. The characteristics of the soil at zero time are as follows: pH 8.2, Organic carbon 4.6 g kg^{-1}, clay content 56.2%. The experiment consisted of 14 treatments involving different doses of N (12.5, 25 and 50 kg ha^{-1}) and S (25 and 50 kg ha^{-1}). Nitrogen and sulphur were applied as basal and split application at R_2 stage of soybean in various combinations. The experiment was laid out in randomized block design with three replications. The size of each plot was 6 m long and 3.6 m wide. All other recommended agronomic practices were followed to harmonize with prevalent practices. At R_2 and R_5 growth stage of soybean growth stage, three replicates were utilized for growth and yield parameters. Plant samples (5 plants) were collected randomly to assess plant height, branches per plant, pods per plant, dry matter accumulation. For dry matter accumulation plant samples were oven dried at 70° C till constant weight. Leaves from each plant were taken and fed into the automatic leaf area meter (CI-203, portable leaf area analyzer (CID, USA) to measure leaf area. The total chlorophyll content was determined by dimethyl sulfoxide (DMSO) method (Hiscox and Israelstam, 1979). For the extraction of chlorophyll 50 mg well-cleaned fresh leaf was chopped and transferred to a test tube containing 10 ml of DMSO. The contents were incubated at 65°C for 3 h and volume was made up to 10 ml with DMSO. The contents were allowed to settle down and the absorbance of supernatant was recorded at 645 and 663 nm. Finally, the total chlorophyll content was calculated by using the following formula:

Total Chlorophyll (mg g^{-1} leaf fresh weight) = [20.2 x (A645 + 8.02 + A663) V]/(1000 x W)

Where, A, V and W were absorbance, final volume, and weight of sample, respectively.

Statistical analysis

The data was analyzed by using SAS statistical software (ver.9.2; SAS Institute., Cary, NC). One-way analysis of variance (ANOVA) was carried out with the ANOVA procedure in SAS enterprise guide 4.2 and the Fisher least significant differences and Duncan multiple range test were used to separate the treatment means.

RESULTS AND DISCUSSION

Plant height

Data in Table 1 shows the effect of nitrogen and sulfur on plant height and dry matter accumulation. Plant height significantly increased with basal and split application of N and S as compared to control as well as alone and combined treatments of N and S at R_2 and R_5 growth stages of crop. The application of N25; N50 and split application of N and S significantly increased the plant height at R_2 and R_5 stages of crop growth. The highest plant height was recorded with N25+25,

S25+25 which was statistically similar with N25; N50 at R_2 growth stage and significantly different from control and other treatment combination. At R5 stage the highest plant height recoded with N25+25, S12.5+12.5 which was at par with N25; N50; N25+25; N12.5+12.5, S25+25; N25+25, S25+25 and N25+S50 while the smallest plants were recorded in control in both stages. The almost same results were also reported by (Sharma et al., 2014; Khalili et al., 2021).

Table 1. Effect of different levels and methods of nitrogen and sulfur applications on dry matter and plant height at different stages of soybean crop growth

Treatment	Plant height (cm)		Dry matter accumulation (g plant^{-1})	
	R_2	R_5	R_2	R_5
Control	46±3.51^{h}	66±9.29^{e}	8.80±0.40^{f}	17.39±2.18^{f}
N25	64±3.60^{abc}	81±3.60^{abc}	11.87±1.02^{abcd}	24.84±2.66^{abcd}
N50	65±3.51^{ab}	80±8.66^{abc}	12.93±0.83^{ab}	26.74±3.46^{abc}
N25+25	58±2.88^{cde}	83±2.64^{ab}	12.47±0.41^{ab}	27.36±2.47^{a}
N12.5+12.5	50±5.29^{gh}	72±2.51^{cde}	9.53±0.41^{f}	18.44±1.27^{ef}
S25	53±3.05^{efg}	74±5.68^{bcde}	9.47±2.53^{f}	19.72±1.55^{ef}
S50	54±4.58^{efg}	78±3.00^{abcd}	10.00±0.87^{def}	21.62±1.76^{de}
S12.5+12.5	46±1.52^{h}	69±3.60^{de}	8.80±0.20^{f}	17.83±1.13^{f}
S25+25	56±2.64^{def}	72±1.52^{cde}	10.33±2.46^{def}	23.62±1.18^{cd}
N25+25, S12.5+12.5	61±3.78^{bcd}	86±3.60^{a}	12.27±0.46^{abc}	28.02±1.26^{a}
N12.5+12.5, S12.5+12.5	51±3.05^{fgh}	72±9.00^{cde}	10.40±0.34^{cdef}	20.18±1.38^{ef}
N12.5+12.5, S25+25	57±4.16^{def}	79±2.88^{abc}	9.67±1.70^{ef}	23.93±1.83^{bcd}
N25+25, S25+25	69±3.00^{a}	82±9.60^{ab}	11.53±1.30^{bcde}	27.53±1.95^{a}
N25+S50	63±2.08^{bc}	77±4.58^{abcd}	13.73±0.41^{a}	27.11±2.75^{ab}
LSD (p=0.05)	10.35	17.13	3.41	6.08

Data are mean values of three replicates± SD; means with different letters in the same column differ significantly at P=0.05 according to Fisher LSD

Dry matter accumulation

Alone N and combined with S fertilization significantly increased the dry matter accumulation of crop. At R_2 growth stage, significantly the highest dry matter accumulation was recorded with the application of N25+S50 which was not significantly different from N25; N50; N25+25 and significantly different from other treatments. At R_5 stage, the highest dry matter accumulation was produced by N25+25, S12.5+12.5 which was statistically same with N25+S50; N25; N50; N25+25 and N25+25, S25+25 and significantly different from other treatment combinations. At R_5 growth stage, combined split application of N and S (N25+25, S12.5+12.5; N25+25, S25+25) and alone split application N (N25+25) treatments were recorded significantly higher dry matter accumulation of crop over other treatments and control.

Chlorophyll content

There were no significant changes in chlorophyll content with the application of N and S at R_2 and R_5 stages of soybean, however, these treatments were significantly increased as compared to control (Table 2). The basal application of N25; N50; S50 and split application of N and S (N25+25, S25+25;

N25+25, S12.5+12.5; N25+S50) were recorded significantly higher chlorophyll content as compared to other treatments and control at R_2 growth stage. At R_5 growth stage, basal application of (N25; N50) and splitting of N and S (N25+25, S25+25; N25+S50; N25+25 with S12.5+12.5) recorded significantly higher chlorophyll content than other treatments and control. Leaf area surface was significantly increased by split application of N and S over control. Significantly higher chlorophyll content at different crop growth stages statement is endorsed by (Chavan et al., 2008; Sharma et al., 2022).

Table 2. Effect of different levels and methods of nitrogen and sulfur applications on total chlorophyll content and leaf area surface at different stages of soybean crop growth

Treatment	Total chlorophyll content (mg g^{-1} leaf fresh weight)		Leaf area surface (cm^2)	
	R_2	R_5	R_2	R_5
Control	28.99±2.33^{b}	45.43±4.74^{d}	955±174^{d}	1511±284^{e}
N25	45.07±9.63^{a}	53.63±3.43^{abcd}	1770±315^{abc}	2361±259^{abcd}
N50	43.26±4.38^{ab}	58.12±5.25^{ab}	1904±98^{ab}	2636±238^{ab}
N25+25	42.11±6.76^{ab}	55.38±2.00^{abcd}	1849±51^{ab}	2461±321^{abc}
N12.5+12.5	39.42±2.44^{ab}	49.57±1.86^{abcd}	1156±156^{cd}	2087±124^{abcde}
S25	39.76±6.48^{ab}	47.93±0.55^{cd}	977±42^{d}	1669±102^{ed}
S50	45.00±4.29^{a}	52.00±2.95^{abcd}	1107±114^{d}	1944±428^{bcde}
S12.5+12.5	36.81±2.30^{ab}	47.75±1.22^{cd}	942±218^{d}	1503±54^{e}
S25+25	40.79±0.82^{ab}	48.83±1.29^{bcd}	1186±69.47^{cd}	1977±295^{abcde}
N25+25, S12.5+12.5	44.16±3.85^{ab}	57.16±4.91^{abc}	2035±229^{a}	2400±23^{abc}
N12.5+12.5, S12.5+12.5	33.03±5.52^{ab}	46.53±5.01^{d}	1185±129^{cd}	1911±88^{cde}
N12.5+12.5, S25+25	38.92±2.32^{ab}	46.05±1.95^{d}	1286±299^{bcd}	1624±273^{e}
N25+25, S25+25	45.77±7.42^{a}	59.52±2.01^{a}	2190±505^{a}	2675±330^{a}
N25+S50	44.39±4.57^{a}	58.05±4.33^{ab}	2024±92^{a}	2444±196^{abc}
LSD (p=0.05)	15.19	10.01	647	715

Data are mean values of three replicates± SD; means with different letters in the same column differ significantly at P=0.05 according to Fisher LSD

Leaf area

Application of (N25+25, S25+25) produced the maximum leaf surface area at R2 and R5 stages of crop over control which did not significantly various with basal application of N (N25; N50) and split application of N and S (N25+25; N12.5+12.5; N25+25, S12.5+12.5; N25+S50) and statistically differ from other treatments combinations together with control. These results are recommended by significantly higher leaf area at different stages of the crop by many scientists (Chavan et al., 2008; Sharma et al., 2022).

Branches per plant

The number of branches per plant was improved by alone nitrogen and combined with sulfur as basal and splitting fertilization (Table 3). The highest number of branches per plant were found with combined split application of N and S (N25+25, S25+25) which was statistically similar with (N50; N25+25; N25+25, S12.5+12.5; N12.5+12.5, S25+25; N25+S50) and various with other treatments.

Yield attributes

The data on no. of pods per plant revealed that highest pods yield was obtained with application of N25+S50, S25+25 which were at par with application of N25+S50 and N25+25 and it is significantly different from other treatments during the year of investigation (Table 3). These findings are in accordance with the results of Khalili et al. (2021). The seed index was significantly higher with the application of basal application of N50, and split application of N and S (N25+25; N25+25, S25+25; N25+25, S12.5+12.5; N25+S50) as compared to control and other fertilized treatment in the study and lowest seed index was found with the control. However, Khalili et al. (2021) reported that N rate and time of application did not influence the seed index of soybean.

Table 3. Effect of different levels and methods of nitrogen and sulfur applications on no. of branch per plant, pods per plant, seed index and grains yield at harvest

Treatment	No. of branch per plant	No. of pods per plant	Seed index (g)	Grains yield (kg ha^{-1})
Control	5±1.00^{d}	93±3.60^{g}	10.79±0.44^{g}	1918±57^{g}
N25	6±1.15^{bcd}	123±2.08^{bcde}	12.46±0.09^{abc}	2517±182^{bcde}
N50	7±1.00^{abc}	126±20.03^{bcd}	12.75±0.51^{a}	2724±345^{ab}
N25+25	7±0.57^{ab}	131±14.52^{abc}	12.34±0.99^{abcd}	2556±28^{bcd}
N12.5+12.5	7±0.00^{abc}	112±3.21^{def}	11.43±0.14^{efg}	2127±232^{fg}
S25	6±0.57^{cd}	109±16.28^{ef}	11.77±0.11^{bcdef}	2168±155^{efg}
S50	6±0.00^{bcd}	118±8.88^{bcdef}	11.87±0.68^{bcde}	2199±121^{defg}
S12.5+12.5	6±1.15^{cd}	92±6.42^{g}	11.55±0.29^{defg}	2056±102^{g}
S25+25	6±0.57^{bcd}	107±5.68^{efg}	11.91±0.91^{bcde}	2278±21^{def}
N25+25, S12.5+12.5	7±0.57^{ab}	127±8.73^{bcd}	12.54±0.34^{ab}	2684±302^{abc}
N12.5+12.5, S12.5+12.5	6±0.57^{cd}	104±3.05^{fg}	11.70±0.29^{cdef}	2156±157^{fg}
N12.5+12.5, S25+25	7±1.00^{abc}	117±7.54^{cdef}	11.02±0.18^{fg}	2347±182^{cdef}
N25+25, S25+25	8±0.57^{a}	145±4.35^{a}	12.43±0.45^{abc}	3011±397^{a}
N25+S50	7±1.15^{ab}	134±9.84^{ab}	12.20±0.26^{abcde}	2875±282^{ab}
LSD (p=0.05)	1.34	16.22	0.82	359

Data are mean values of three replicates ± SD; means with different letters in the same column differ significantly at P=0.05 according to Fisher LSD

Grain yield

Data presented in Table 3 indicated that the yield of grains in soybean was significantly affected by basal and split application of N and S alone and both by combined treatments. The highest seed yield was obtained with the application of N25+25, S25+25 and statistically similar with N25+25, S12.5+12.5; N25+S50; N50 and significantly different from other basal and splitting of N and S as well as control. However, the lowest grains yield was produced by the control. The above results are in conformity with those of Khalili et al. (2016), Mamatha et al. (2018) and Khalili et al. (2021) who reported that the application of N and S recorded significantly higher grain yield as compared to control in maize and soybean.

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Malva sylvestris L. (Malvaceae): A new distributional species record for Odisha and Eastern India

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ABSTRACT

The members of genus *Malva* L. (Malvaceae) are widely grown as ornamental plants due to their attractive appearance and few are edible as leafy vegetables and medicinal usage too. During the exploration for germplasm collection of minor leafy vegetable in parts of Odisha, the occurrence of an interesting plant species of mallow viz. *Malva sylvestris*, found as a weed and used as leafy vegetable by locals was explored from western part of Odisha. On critical assessment of its distribution, its natural occurrence was found to be a new genus record for the flora of Odisha and new species record for Eastern India. A detailed taxonomic description on morphology along with field photographs and economic uses are provided for ease of identification and sustainable utilization.

Key words: Eastern India, germplasm collection, mallow, *Malva sylvestris*, new species record, Odisha

INTRODUCTION

The genus *Malva*, commonly known as *"*mallow*"* of family Malvaceae is primarily native to temperate, subtropical, and tropical regions of Asia, Africa, and Europe. The genus comprises about 40 species (Mabberly, 1997), however, it is represented by 29 accepted species names and further 2 taxa of infra-specific rank (The Plant List, 2013). The genus includes both annual and perennial herbs and under-shrubs. Several species are widely grown as ornamental plants due to their showy appearance and few are edible as leafy vegetables. Bodo tribes of Northeast India and local people of Kashmir domesticate few species of *Malva* viz. *Malva parviflora, M. sylvestris* and *M. verticillata* as traditional cuisine or vegetable dish. Leaves and flowers of few species have been used as traditional medicine for treatment of coughs, sore throats, and gastro-intestinal tract.

Malva sylvestris L. known as "common mallow or tall mallow", alien to Indian flora, is native to the regions of Western Europe, Northern Africa, North-West Asia, and Iran. Over the time, it has been introduced to and naturalized in parts of Eastern Australia, Canada, Mexico, and United states as an invasive species (Hinsley, 2014). It is a tall herb with showy bright mauve purple colour flowers with dark decorative stripes, spreads itself and grows in wastelands, crop fields, fallow lands etc. It is often domesticated as an ornamental plant in gardens for its attractive flowers. However, this species has a limited distribution in India and was recorded earlier in parts of north, west and central India and not reported previously in Eastern India. However, the occurrence of *Malva sylvestris* in central table land phyto-geographical zone leads to a new genus record of *Malva* for the flora of Odisha and new species record for Eastern India.

MATERIALS AND METHODS

During the course of plant exploration and germplasm collection of minor leafy vegetable in parts of Odisha during 2014 in collaboration with ICAR-Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, the occurrence of an

interesting plant species of Malvaceae found as a weed in the border of a fallow field was recorded from Bargarh district of Odisha. The plant specimens bearing both vegetative and flowering parts were collected from the natural habitat and the voucher specimens were deposited in the herbarium of ICAR-National Bureau of Plant Genetic Resources (NBPGR), Base Centre, Cuttack, Odisha along with one set at the National Herbarium of cultivated plants (NHCP), ICAR-NBPGR, New Delhi. The herbarium specimens were studied and collected specimens were compared with the images of the authentic herbarium type specimens deposited at the Royal Botanical Garden, Kew (K000914119) to confirm the identity of the plant. The seed germplasm bearing accession number IC-610775 and collection number RCM/PKS/110 were conserved in the National Gene Bank, ICAR-NBPGR, New Delhi for long term storage. The images of vegetative, flowering, and fruiting parts of the plant were presented for reference and ease of identification (Fig. 1).

Fig. 1 (a)

Fig. 1 (b)

Fig. 1 (c)

Fig. 1 (d)

Fig. 1 (e)

Fig. 1 (f)

Fig. 1 (g)

Fig. 1 (h)

Fig. 1 (i)

Fig. 1. *Malva sylvestris*: a. Natural occurrence at village Dahita in Bargarh district, Odisha, b. Regenerated population in fallow lands, c. Twig with leaves and flowers, d. Flower, e. Dried fruits, f. Herbarium specimen preserved at Base Centre, Cuttack, g. Image of the herbarium type specimen (K000914119) at Royal Botanical Garden, Kew, h. Local woman shows the use of plant, i. Woman collects leaves to prepare curry

RESULTS AND DISCUSSION

After thorough examination of vegetative and floral characters of live plants along with study on herbarium specimens and perusal of literature, the species was identified as *Malva sylvestris*, a species reported so far from some states of India (Master, 1874; Cooke, 1901; Gamble, 1915; Duthie, 1976; Nair and Henry, 1983; Chowdhery and Wadhwa, 1984; Saldanha, 1984; Sharma et al., 1984; Roy et al., 1992; Verma et al., 1993; Singh and Karthikeyan, 2000). It was observed that the species was found as an escape and as a weed in crop fields and adjoining fallow lands near village Dahita in Padampur block of Bargarh district in Odisha. On verification of major published Indian literature, it was found that it has not been reported till date from Eastern India including Odisha and Andhra Pradesh (Prain, 1903; Haines, 1921; Mooney, 1950; Deb, 1981; Guha Bakshi, 1984; Sanyal, 1994; Saxena and Brahmam, 1994; Venkata Raju and Pullaiah, 1995; Pullaiah and Chennaiah, 1997; Singh et al., 2001). Therefore, the present collection counts an addition of genus *Malva* to the flora of Odisha and forms a new distributional plant record for Eastern India. A detailed taxonomic description on morphology along with field photographs and herbarium images (Fig. 1) and economic uses of the plant are provided for easy identification and sustainable utilization.

Taxonomic description

Malva sylvestris L. Sp. Pl. 689. 1753; Mast.: In. Hook. F. Fl. Brit. India 1: 320; 1874; Gamble, Fl, Madras 1: 88 (63). 1915; Paul: In Sharma et al. (eds) Fl. India 3: 357, f. 99. 1993. *M. sylvestris var. maurtiana* (L.) Boiss. Fl. Orient. 1:819. 1867; Cooke, Fl. Bombay 1: 96. 1901.

Annual or perennial erect herbs or undershrub up to 90 cm height. Stem stout, terete, glabrous. Leaves up to 15 cm long including petiole; lamina 4-6 × 5-8 cm, roundish in outline, sub-orbicular, shallowly 3-5 lobed, base truncate or broadly cordate, apex obtuse, margin crenate, glabrous, minutely hairy on nerves beneath; petiole slender, up to 9 cm long. Flowers 5-10, in each axillary fascicles; pedicels up to 2 cm long. Epicalyx segments 3, small, free, ovate to oblong, 3-5 × 2.0 mm, shorter than calyx. Calyx lobes 4.0-6.0 × 3.0 mm, cupular or rotate, broadly triangular, ovate-lanceolate to oblong, connate at base, ciliate along margins, glabrescent, accrescent in fruit. Corolla rotate or infundibuliform, much longer than calyx, 3-4 cm across; petals 5, bright purple with dark pink to violet ornamented stripes, 1.5-2.5 × 1.5-2.0 cm, obovate, emarginate, clawed, bearded at base. Staminal tube ca. 3 mm long, divided at the top into antheriferous filaments, column antheriferous at apex, shorter than petals. Carpels and styles 9-12, ovary many-celled, ovule solitary in each cell, styles as many as cells, filiform; stigmas linear; cocci forming a round depressed fruit, muticous, separating when ripe from each other and from the axis, indehiscent, 1-seeded. Schizocarp discoid with a depressed centre, ca.6 mm across. Mericarps 10-14, 1.5-2.0 mm across, reniform, reticulate at back, 2-keeled, nearly glabrous, flattened, indehiscent. Seeds 1.5-2.0 mm across, sub-reniform, brownish black.

Flowering and fruiting: November – February.

Germplasm collected and conserved and specimens examined

India, Odisha state, Bargarh district, Padampur block, Gram Panchayat and nearby village: Dahita, 20° 54' N latitude and 83° 04' E longitude, elevation 234 m from mean sea level, R.C. Misra HS number 22943 (Herbarium of NHCP, ICAR-NBPGR, New Delhi); date of collection: 20.01.2014; seed germplasm accession number IC 610775 (collection number RCM/PKS/110); source: disturbed, weed in a crop field/fallow land; sampling method: selective, frequency: rare; (Local name: *Gangatiria Nalita, Gangatiria saga*). Images of herbarium type specimens of *M. sylvestris* bearing barcode number K000914119 of Royal Botanic Garden, Kew, London.

Economic uses

Despite its use as ornamental plant, the local inhabitants use the leaves as leafy vegetable. They collect and cut the tender leaves into pieces, boil and or fry with tomato and brinjal and prepare curry. It is said to be of very tasty and they consume it with

day meal. Tender leaves, shoots, flowers, and fruits are consumed in salads, soups, or boiled vegetables.

Methanolic extracts from the leaves of *M. sylvestris* exhibited antibacterial activity against *Staphylococcus aureus, Enterococcus faecalis*, *Streptococcus agalactiae*, and *Erwinia carotovora* and the flowers showed activity against *Escherichia coli* (Razavi et al., 2011). Fluid extracts of *M. sylvestris* leaves and flowers are used to treat inflammatory diseases of mucous membranes, cystitis, and diarrhea (Farina et al., 1995). The results of studies on the antimicrobial properties of *M. sylvestris* indicate that the plant also has antibacterial and antiviral activity against many human pathogens (Benso et al., 2015). This amazing plant has antimicrobial, hepato-protective, anti-inflammatory and antioxidant properties and is considered as one of the most promising medicinal species. The traditional use of this species in treating many diseases such as cold, cough, bronchitis, digestive problems, eczema and cut wounds and preparing pharmaceutical compounds highlights the drugs used to produce antibiotics and other therapeutic agents (Mousavi et al., 2021).

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Sensory evaluation and microbial analysis of gulkand under ambient storage

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ABSTRACT

An experiment was carried out at Post Harvest Lab, Department of Horticulture, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur (Rajasthan) to analyze sensory evaluation, hunter colour *(L* values)*, non-enzymatic browningand microbial count of bourbon rose gulkand product at ambient storage. All sensory characters i.e., taste, flavour, texture, and overall acceptability showed decreasing trend, whereas fungal growth, bacterial growth and non-enzymatic browning showed increased trends at ambient storage duration. The C_9-Bourbon rose petals + sugar candy (1.0:2.0 ww^{-1}) was found to be the best among all ingredient combinations with regard to non-enzymatic browning (0.70), lightness *(L*)* value (20.10) and no microbial and fungus growth, as well as attainment highest organoleptic score for taste, flavour, texture and overall acceptability as compared to C_{11}-Check I market product from Rajsamund (Khamnore), C_{12}-Check II market product from Chittorgarh (Ghodakheda) and C_1-Bourbon rose petals + Sugar (1.0:1.25 ww^{-1}- Check III) at 0 to 120 days ambient storage.

Key words: Bourbon rose, lightness, microbial count, non-enzymatic browning, sensory score

INTRODUCTION

Floriculture is the largest sector in terms of profit-making. Since flower crops are called "High profit, low volume crops,", flower supply and value addition play an important role in determining market value. As flower crops are highly perishable, proper post harvest techniques and value addition are required to increase their value. *Rosa hybrida* contains the diploid chromosome number 2n=14 belonging to the family Rosacea. Gulkand has several benefits on human health, viz. heat problems like tiredness, scratching, pains, and reduction of burning sensations in soles and palms, reduce acidity and stomach heat, which prevents ulcers and intestines swelling as per National Institute of Ayurvedic Medicine of India. It acts as a tonic which decreases stress and strengthens the heart and central nervous system of human beings. It increases vision and decreases redness and swelling of the eyes. It treats ulcers in the mouth and makes teeth and gums powerful. It is a good rejuvenator that corrects skin problems like pimples and blemishes. It repairs sperm abnormalities in males, such as less number or weak sperm. Protects from sunstroke and prevents bleeding of the nose during the summer season. More over, this is a gentle laxative, it reinforces the body's 7 Dhatus (fundamental elements or tissues such as plasma, blood, muscles, fat, sperm, bone, and bone marrow), as par suggested by Rudrawar and Singar (2017). Gulkand contains a variety of biochemical substances, including alkaloids, flavonoids, glycosides, tannins, triterpenoids, and saponins which is a valuable source of therapeutic and preventative agents for diseases (Sindhura et al., 2013). The only disadvantage of gulkand is that diabetics should avoid it. It has a lot of sugar in it, which might cause your blood sugar to rise (Sao and Sharma, 2021)

Bourbon rose belongs to *R. bourboniana* group derived from a natural cross between *Rosa chinensis* and *Rosa damascena.* It is a vigorous shrub with erect shoots, flowers double, deep rosy red, scented, about 7.5 cm in diameter, borne singly or in small clusters. It originated from Bourbon Isle de of Reunion (then known as Bourbon) in 1817. At present, cultivated in Ajmer, Nagaur, Chittorgarh, Banswara and Udaipur districts of Rajasthan. Commercial rose products such as rose water, rose oil, gulkand, pankhuri and gulroghan are available both in international and national markets. Rose hips are an excellent source of vitamin C. Rose water is also one of the most important rose products due to its body-cooling properties, widely used as an ingredient in medicines, perfumes, eye lotions, eye drops and confectionery products. If the sugar and sugar candy levels are optimum set TSS (65°B) or above, will be helpful to draw the moisture or water through osmosis. When a higher concentration of sugar enters the petal cell, the water activity is stopped. Hence, micro-organisms do not grow and ultimately, colony-forming units of fungi, bacteria and actinomycetes decrease. Eventually, the shelf life of gulkand increases.

MATERIALS AND METHODS

The experiment was conducted at Post Harvest Lab, Department of Horticulture, Maharana Pratap University of Agriculture and Technology, Udaipur from October 2020 to March 2021, which is located at 24°35' Latitude, 73°42' Longitude and an elevation of 559.65 meters above the mean sea level. The daily high and minimum temperature in Udaipur during the experimental period of October 2020 to March 2021 was 22.5°C to 33.4°C and 3.8°C to 21.5°C, accordingly, while the high and minimum relative humidity varied from 54.4 per cent to 90.6 per cent and 18.3 per cent to 57.2 per cent, respectively. Sunlight hours vary from 3.4 to 9.6 hours per minute, and annual rainfall varies from 0.0 to 12.6 mm according to the Meteorological Observations, Deptt. of Agronomy, RCA, Udaipur. Bourbon rose flowers were procured from Mr. Parixit Singh a farmer's field at village Barodiya, District of Banswara, Rajasthan. Ingredients like sugar and sugar candy were purchased from the local market.

The experiment was laid out in a completely randomized design with 12 treatments combination replicated thrice, viz. C_1-Bourbon rose petals + Sugar (1.0:1.25 w w^{-1}- Check III), C_2- Bourbon rose petals + Sugar (1.0:1.50 w w^{-1}), C_3- Bourbon rose petals + Sugar (1.0:1.75 w w^{-1}), C_4-Bourbon rose petals + Sugar (1.0:2.00 w w^{-1}), C_5- Bourbon rose petals + Sugar (1.0:2.25 w w^{-1}), C_6-Bourbon rose petals + Sugar candy (1.0:1.25 w w^{-1}), C^7-Bourbon rose petals + Sugar candy (1.0:1.50 w w^{-1}), C8-Bourbon rose petals + Sugar candy (1.0:1.75 w w^{-1}), C_9-Bourbon rose petals + Sugar candy (1.0:2.00 w w^{-1}), C_{10}-Bourbon rose petals + Sugar candy (1.0:2.25 ww^{-1}), C_{11}-Check I market product from Rajsamund (Khamnore) and C_{12}-Check II market product from Chittorgarh (Ghodakheda).

Fresh rose flowers of Rosa bourboniana were chosen, washed and pre-cooled overnight. While next morning rotted, off-coloured rose petals, pollen, anther, stigma, epicalyx, and pedicel were separated and removed. For the preparation of gulkand, selected healthy rose petals were used. In a big mouth glass jar, weighed amounts of petals, i.e., 1 kg and varied levels of sugar or sugar candy, after weighing by electronic balance were mixed with the help of a blender as per treatment. The mouth of the gulkand fill-up jar was covered and bound with a white muslin cloth, and then kept in sunlight for one month to the impregnation process. The prepared gulkand was packed in food-grade plastic containers (500 g) and leaving 2-3 cm head space. The jars were then air-tightly sealed with lids. They were then labelled as per treatment details and kept at ambient temperature for 0, 30-, 60-, 90-, and 120-days storage for observations. The number of bacteria and fungus were counted using a medium of Potato Dextrose Agar (PDA) for fungus and Luria Bertani Agar (LBA) for bacteria. The non-enzymatic browning (NEB) in gulkand was calculated by measuring the optical density (OD) of methanol extracts of a sample at 440 nm by spectrophotometer. The Hedonic Rating Test of gulkand was assessed organoleptically by a jury of five people (Amerine et al., 1965). The data were analysed using a completely randomized design (Fisher, 1950).

RESULTS AND DISCUSSION

Fungal growth (cfu × 10^4 g^{-1})

The data in Table 1 regarding fungal growth shows an inclined trend in bourbon rose gulkand with the advancement of ambient storage duration and declined trends of the improved quantity of sugar and sugar candy. Initially, no colonies were observed up to 60 days of ambient storage in all treatment combinations. However, the maximum fungal growth was detected (2.00 cfu × 10^4 g^{-1}) in lower-level combinations of sugar and sugar candy at C_1, C_2, C_3, C_6, C_7 and C_8, while no fungal growth was detected at C_4-Bourbon rose petals + sugar (1.0:2.00 w w^{-1}), C_5-Bourbon rose petals + sugar (1.0:2.25 w w^{-1}), C_9-Bourbon rose petals + sugar candy (1.0:2.00 w w^{-1}) and C_{10}-Bourbon rose petals + sugar candy (1.0:2.25 w w^{-1}) as compared to best market product C_{11}-check I from Rajsamund (Khamnore) and C_{12}-check II from Chittorgarh (Ghodakheda) at 0 to 120 days ambient storage period. The decreasing number of fungal growths in the improved level of sugar and sugar candy might be due to the fact that when a higher concentration of sugar enters to bourbon rose petal cell, the free water comes out from the petal cell and hence not available to fungal and microorganism growth. Hence, micro-organisms do not grow and ultimately, the colony-forming units of fungi, bacteria and actinomycetes lower down at the higher level of sugar and sugar candy of bourbon rose gulkand.

Krishna et al. (2020) also observed a very negligible growth of fungal units upto 120 days of storage in mango jam and mentioned that the added sugar exerted an osmophillic load in the jam. Present findings are in conformity with the findings of Bafna and Manimehalai (2013) in kokum fruit jam, Jat (2018) and Jat et al. (2018) in rose petal jam.

Bacterial growth (cfu × 10^6 g^{-1})

It is apparent from the mean data in Table 1 that bacterial growth was increased significantly in bourbon rose gulkand with the advancement of ambient storage duration and reduced with the addition of a greater level of sugar and sugar candy. Initially, no bacterial colonies were observed up to 30 days of ambient storage in all treatment combinations. However, The highest bacterial growth was detected (5.00 cfu × 10^6 g^{-1}) with a lower level of sugar and sugar candy at C_6-Bourbon rose petals + sugar candy (1.0:1.25 w w^{-1}) and (4.00 cfu × 10^6 w w^{-1}) at C_1-Bourbon rose petals + sugar (1.0:1.25 w w^{-1}) at 120 days of ambient storage time, while no fungal growth was detected with a higher level combination under C_4-Bourbon rose petals + sugar (1.0:2.00 w w^{-1}), C_5-Bourbon rose petals + sugar (1.0:2.25 w w^{-1}), C_9-Bourbon rose petals + sugar candy (1.0:2.00 w w^{-1}) and C_{10}-Bourbon rose petals + sugar candy (1.0:2.25 w w^{-1}) as compared to best market product C_{11}-check I from Rajsamund (Khamnore) and C_{12}-check II from Chittorgarh (Ghodakheda) at 0 to 120 days ambient storage period.

The reduced bacterial growth seen at later stages of ambient storage might be attributed to an increase in the sugar content and titratable acidity of the gulkand, as sugar, sugar candy and increased acid have preservative action that inhibits microbial development (Jat et al., 2018). The present findings are less or more in agreement with the results of Bafna and Manimehalai (2013) in kokum fruit jam, Krishna et al. (2020) in mango jam and Rana et al. (2021) in mixed fruit jam.

Non-enzymatic browning (NEB)

The mean data about non-enzymatic browning of bourbon rose gulkand in Table 2 reveals that there was a gradually increasing trend observed upon advancement of storage duration from 0 to 120 days and improve quantity of sugar and sugar candy. The highest non-enzymatic browning (0.711) was found at C_5-Bourbon rose petals + sugar (1.0:2.25 w w^{-1}) and the lowest NEB (0.657) at C_6-Bourbon rose petals + sugar candy (1.0:1.25 w w^{-1}) at 120 days of ambient storage. While the best market products C_{11}-check I from Rajsamund (Khamnore) and C_{12}-check II from Chittorgarh (Ghodakheda) were statistically found to be equivalent to best desirable combination trends at C_9-Bourbon rose petals + sugar candy (1.0:2.00 w w^{-1}) and C_4-Bourbon rose petals + sugar (1.0:2.00 w w^{-1}) respectively. Present findings are consistent with those of Burdurlu and Karadeniz (2003) in apple juice and Kumar and Dean (2017) in wood apple jelly.

Table 1. Effect of sugar and sugar candy levels on fungal and bacterial growth of bourbon rose gulkand at ambient storage

Treatments (w w^{-1}) Ambient storage (days)	Fungal growth (cfu × 10^4 g^{-1})						Bacterial growth (cfu × 10^6 g^{-1})					
	0	30	60	90	120	Mean	0	30	60	90	120	Mean
C1- Bourbon rose petals + Sugar (1.0:1.25 Check III)	0.00	0.00	0.00	2.00	2.00	0.80	0.00	0.00	2.00	3.00	4.00	1.80
	(0.71)	(0.71)	(0.71)	(1.58)	(1.58)	(1.06)	(0.71)	(0.71)	(1.58)	(1.87)	(2.12)	(1.40)
C2- Bourbon rose petals + Sugar (1.0:1.50)	0.00	0.00	0.00	1.00	2.00	0.60	0.00	0.00	1.00)	2.00	3.00	1.20
	(0.71)	(0.71)	(0.71)	(1.22)	(1.58)	(0.99)	(0.71)	(0.71)	(1.22)	(1.58)	(1.87)	(1.22)
C3- Bourbon rose petals + Sugar (1.0:1.75)	0.00	0.00	0.00	1.00	2.00	0.60	0.00	0.00	0.00	1.00	1.00	0.40
	(0.71)	(0.71)	(0.71)	(1.22)	(1.58)	(0.99)	(0.71)	(0.71)	(0.71)	(1.22)	(1.22)	(0.91)
C4- Bourbon rose petals + Sugar (1.0:2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)
C5- Bourbon rose petals + Sugar (1.0:2.25)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)
C6- Bourbon rose petals + Sugar candy (1.0:1.25)	0.00	0.00	0.00	2.00	2.00	0.80	0.00	0.00	2.00	2.00	5.00	1.80
	(0.71)	(0.71)	(0.71)	(1.58)	(1.58)	(1.06)	(0.71)	(0.71)	(1.58)	(1.58)	(2.35)	(1.39)
C7- Bourbon rose petals + Sugar candy (1.0:1.50)	0.00	0.00	0.00	1.00	2.00	0.60	0.00	0.00	1.00	1.00	3.00	1.00
	(0.71)	(0.71)	(0.71)	(1.22)	(1.58)	(0.99)	(0.71)	(0.71)	(1.22)	(1.22)	(1.87)	(1.15)
C8- Bourbon rose petals + Sugar candy (1.0:1.75)	0.00	0.00	0.00	1.00	1.00	0.40	0.00	0.00	0.00	0.00	1.00	0.20
	(0.71)	(0.71)	(0.71)	(1.22)	(1.22)	(0.91)	(0.71)	(0.71)	(0.71)	(0.71)	(1.22)	(0.81)
C9- Bourbon rose petals + Sugar candy (1.0:2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)
C10- Bourbon rose petals + Sugar candy (1.0:2.25)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)
C11- Check I market product from Rajsamund (Khamnore)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)
C12- Check II market product from Chittorgarh (Ghodakheda)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)
SEm±	0.001	0.001	0.001	0.003	0.003	-	0.001	0.001	0.003	0.003	0.003	-
C.D. (P=0.01)	NS	NS	NS	0.012	0.010	-	NS	NS	0.010	0.011	0.013	-

Note: values in parenthesis are transformed by √x+0.5

Table 2. Effect of sugar and sugar candy levels on NEB and colour L* value of Bourbon rose gulkand at ambient storage

Ambient storage (days) Treatments (w w^{-1})	Non-enzymatic browning						Colour L* value					
	0	30	60	90	120	Mean	0	30	60	90	120	Mean
C1- Bourbon rose petals + Sugar (1.0:1.25 Check III)	0.594	0.610	0.634	0.656	0.671	0.63	20.24	19.45	17.57	16.15	15.14	17.71
C2- Bourbon rose petals + Sugar (1.0:1.50)	0.599	0.619	0.641	0.663	0.675	0.64	21.01	20.18	19.20	18.16	17.09	19.13
C3- Bourbon rose petals + Sugar (1.0:1.75)	0.611	0.628	0.648	0.672	0.686	0.65	22.49	21.18	20.73	20.12	18.20	20.54
C4- Bourbon rose petals + Sugar (1.0:2.00)	0.623	0.644	0.661	0.686	0.698	0.66	23.20	22.60	21.73	20.61	19.10	21.45
C5- Bourbon rose petals + Sugar (1.0:2.25)	0.631	0.650	0.672	0.695	0.711	0.67	23.33	22.71	21.86	20.72	19.32	21.59
C6- Bourbon rose petals + Sugar candy (1.0:1.25)	0.580	0.598	0.616	0.641	0.657	0.62	20.66	19.85	18.03	16.77	15.43	18.15
C7- Bourbon rose petals + Sugar candy (1.0:1.50)	0.591	0.613	0.630	0.654	0.670	0.63	22.15	21.40	20.26	18.46	17.90	20.03
C8- Bourbon rose petals + Sugar candy (1.0:1.75)	0.604	0.626	0.643	0.665	0.682	0.64	22.75	21.78	21.14	20.34	19.29	21.06
C9- Bourbon rose petals + Sugar candy (1.0:2.00)	0.616	0.635	0.653	0.681	0.695	0.66	24.55	23.24	22.16	21.58	20.10	22.33
C10- Bourbon rose petals + Sugar candy (1.0:2.25)	0.625	0.640	0.670	0.686	0.692	0.66	24.65	23.27	22.21	21.71	20.21	22.41
C11- Check I market product from Rajsamund (Khamnore)	0.617	0.638	0.656	0.685	0.697	0.66	24.50	23.21	22.13	21.54	20.06	22.29
C12- Check II market product from Chittorgarh (Ghodakheda)	0.627	0.642	0.664	0.688	0.705	0.67	23.16	22.57	21.68	20.57	19.00	21.40
SEm±	0.007	0.007	0.001	0.006	0.008	-	0.23	0.26	0.23	0.21	0.20	-
C.D. (P=0.01)	0.028	0.029	0.007	0.027	0.033	-	0.92	1.02	0.90	0.83	0.80	-

A similar increasing trend was observed by Shafaly et al. (2019) in bael-mango jam during ambient storage and they stated that it might be owing to the effect of acidity, which accelerated the hydrolytic process, resulting in browning. Acids also accelerate the Maillard reaction and caramelization, resulting in more browning in the jam. The acceptability and freshness of the final product of papaya are jam reduced due to changing the colour by non-enzymatic browning (Pinandoyo and Siddiqui, 2020).

Hunter Colour *(L*)*

The data about the *L** value of bourbon rose gulkand in (Table 2) indicates that the colour coordinates for lightness *(L*)* of bourbon rose gulkand decreased gradually as the storage time increased from 0 to 120 days in all treatments. Present findings indicate that light colour decreased and dark colour increased at the advancement of the ambient storage period of bourbon rose gulkand. Colour changes might be caused by the Millard reaction, ascorbic acid breaking down, enzymatic browning and polymerization of colour pigments (carotenoids and anthocyanins) with some other phenolic compounds (Shah et al., 2015). Almost similar results were reported by Burdurlu and Karadeniz (2003) in apple juice and Jat (2018) in rose petal jam.

Organoleptic evaluation

The data in Table 3 & Table 4 revealed that the taste, flavour, and texture of bourbon rose gulkand were significantly affected by the different ratios of bourbon rose petals, sugar, and sugar candy levels. The taste, flavour and texture score decreased as the ambient storage time increased from 0 to 120 days. The highest mean score of 0 to 120 days for taste (8.25), flavor (7.92) and texture (7.88) were observed in the desirable treatment combination at C_9-Bourbon rose petals + sugar candy (1.0:2.00 w w^{-1}) and the

Table 3. Effect of sugar and sugar candy levels on taste and flavour of bourbon rose gulkand at ambient storage

Ambient storage (days)	Taste score						Flavour score					
Treatments (w w^{-1})	0	30	60	90	120	Mean	0	30	60	90	120	Mean
C1- Bourbon rose petals + Sugar (1.0:1.25 Check III)	6.50	6.41	6.26	6.10	5.81	6.22	7.51	7.34	6.13	5.89	5.65	6.50
C2- Bourbon rose petals + Sugar (1.0:1.50)	7.75	7.61	7.50	6.41	6.15	7.08	7.52	7.35	7.15	6.92	6.63	7.11
C3- Bourbon rose petals + Sugar (1.0:1.75)	8.05	7.91	7.79	7.65	7.48	7.78	8.61	8.44	8.23t	7.99	7.40	8.13
C4- Bourbon rose petals + Sugar (1.0:2.00)	8.63	8.48	8.35	8.21	8.06	8.35	8.86	8.58	8.37	8.18	7.82	8.36
C5- Bourbon rose petals + Sugar (1.0:2.25)	8.24	8.09	7.93	7.81	7.72	7.96	7.64	7.47	7.24	7.09	6.10	7.11
C6- Bourbon rose petals + Sugar candy (1.0:1.25)	6.20	6.05	5.70	5.35	5.10	5.68	6.38	6.21	6.00	5.76	5.47	5.96
C7- Bourbon rose petals + Sugar candy (1.0:1.50)	7.43	7.25	7.12	5.55	5.30	6.53	6.45	6.28	6.07	5.83	5.62	6.05
C8- Bourbon rose petals + Sugar candy (1.0:1.75)	7.91	7.85	7.74	7.56	7.13	7.64	7.73	7.56	7.35	7.12	5.90	7.13
C9- Bourbon rose petals + Sugar candy (1.0:2.00)	8.78	8.62	8.51	8.39	8.25	8.51	8.91	8.78	8.53	8.26	7.92	8.48
C10- Bourbon rose petals + Sugar candy (1.0:2.25)	8.16	8.05	7.91	7.77	7.50	7.88	7.62	7.45	7.25	7.01	6.72	7.21
C11- Check I market product from Rajsamund (Khamnore)	8.70	8.50	8.39	8.30	8.21	8.42	8.89	8.75	8.50	8.25	7.88	8.45
C12- Check II market product from Chittorgarh (Ghodakheda)	8.59	8.45	8.33	8.19	8.10	8.33	8.84	8.56	8.35	8.16	7.80	8.34
SEm±	0.12	0.08	0.09	0.09	0.10	-	0.10	0.09	0.08	0.09	0.09	-
C.D. (P=0.01)	0.48	0.31	0.34	0.37	0.39	-	0.39	0.36	0.31	0.35	0.34	-

lowest score were observed at T6–Bourbon rose petals + sugar candy (1.0:1.25 w w^{-1}) at 0 to 120 days of the storage period, which were better over C1, statistically at par with best market product C_{11}-Check I from Rajsamund (Khamnore) and C_{12}-Check II from Chittorgarh (Gohakheda) respectively. It can be concluded that the biochemical changes during storage i.e., fluctuations in acids, pH, sugar/acid ratio and the storage duration, had a significant impact on the organoleptic characteristics of the bourbon rose gulkand. Jat et al. (2018) indicates that the loss of flavour value is caused by the loss of highly volatile aromatic compounds, which are extremely susceptible to high temperature storage and some enzymatic degradation of phenols and oxidative changes in sugars, which cause flavour loss during storage. Similarly, a decreasing trend was reported by Patel et al. (2015) in banana and pineapple blended jam, Shah et al. (2015) in apple and olive fruit blended jam, Rahman et al. (2018) in guava jam and Khan et al. (2020) in fig fruit jam blended with apple.

Overall acceptability

It is apparent from the data in Table 4 that the overall acceptability of bourbon rose gulkand, which is a combined effect of taste, flavour and texture was significantly affected by the different ratios of ingredients. The overall acceptability score decreased as the storage time increased from 0 to 120 days at ambient storage period. The maximum score for overall acceptability (8.02) was found in C_9-Bourbon rose petals + sugar candy (1.0:2.00 w w^{-1}) and the lowest score (5.26) was observed in T_6 –Bourbon rose petals + sugar candy (1.0:1.25 w w^{-1}) at 120 days of ambient storage period. The C_9-Bourbon rose petals + sugar candy (1.0:2.00 w w^{-1}) and C_4-Bourbon rose petals + sugar (1.0:2.00 w w^{-1}) respectively were better

Table 4. Effect of sugar and sugar candy levels on texture and acceptability of bourbon rose gulkand at ambient storage

Ambient storage (days) Treatments (w w^{-1})	Texture score						Overall acceptability score					
	0	30	60	90	120	Mean	0	30	60	90	120	Mean
C1- Bourbon rose petals + Sugar (1.0:1.25 Check III)	7.62	7.50	7.36	7.13	6.87	7.30	7.21	7.08	6.58	6.37	6.11	6.67
C2- Bourbon rose petals + Sugar (1.0:1.50)	7.88	7.71	7.59	7.36	7.12	7.53	7.72	7.56	7.41	6.90	6.63	7.24
C3- Bourbon rose petals + Sugar (1.0:1.75)	8.25	8.14	8.00	7.74	7.48	7.92	8.34	8.18	8.02	7.82	7.47	7.96
C4- Bourbon rose petals + Sugar (1.0:2.00)	8.65	8.47	8.35	8.13	7.70	8.26	8.71	8.51	8.36	8.17	7.86	8.32
C5- Bourbon rose petals + Sugar (1.0:2.25)	8.19	8.03	7.86	7.63	7.37	7.82	8.02	7.86	7.68	7.51	7.06	7.63
C6- Bourbon rose petals + Sugar candy (1.0:1.25)	6.29	6.13	5.99	5.76	5.20	5.87	6.29	6.13	5.90	5.62	5.26	5.84
C7- Bourbon rose petals + Sugar candy (1.0:1.50)	6.55	6.34	6.23	6.00	5.26	6.08	6.81	6.62	6.47	5.79	5.39	6.22
C8- Bourbon rose petals + Sugar candy (1.0:1.75)	8.36	8.18	8.04	7.81	7.53	7.98	7.96	7.85	7.70	7.47	6.84	7.56
C9- Bourbon rose petals + Sugar candy (1.0:2.00)	8.79	8.55	8.41	8.20	7.88	8.37	8.83	8.64	8.48	8.28	8.02	8.45
C10- Bourbon rose petals + Sugar candy (1.0:2.25)	8.03	7.94	7.80	7.57	7.31	7.73	7.94	7.81	7.65	7.45	7.18	7.61
C11- Check I market product from Rajsamund (Khamnore)	8.74	8.53	8.40	8.18	7.86	8.34	8.78	8.59	8.43	8.24	7.98	8.41
C12- Check II market product from Chittorgarh (Ghodakheda)	8.61	8.45	8.32	8.10	7.68	8.23	8.68	8.49	8.33	8.15	7.86	8.30
SEm±	0.08	0.10	0.09	0.09	0.08	-	0.09	0.09	0.08	0.08	0.08	-
C.D. (P=0.01)	0.34	0.39	0.35	0.34	0.33	-	0.35	0.37	0.31	0.31	0.32	

over C1, statistically at par with best market product C_{11}- Check I from Rajsamund (Khamnore) and C_{12}-Check II from Chittorgarh (Gohakheda) for overall acceptability. A similar decline in overall acceptability was observed by Shah et al. (2015) in apple + olive fruit blended jam, Ullah et al. (2018) in carrot + apple blended jam, Jat et al. (2018) in rose petal jam, Rahman et al. (2018) in guava jam, Pinandoyo and Siddiqui (2020) in papaya jam and Khan et al. (2020) in fig fruit jam blended with apple.

CONCLUSION

It is concluded that C_9-Bourbon rose petals + sugar candy (1.0:2.00 w w^{-1}) was found to be statistically at par with regard to non-enzymatic browning (0.70) lightness *(L*)* value (20.10), *a** value (7.22), yellowness *(b*)* value (3.06) and no microbial and fungus growth, as well as attainment of the highest organoleptic score as compared to best market product C_{11}-Check I from Rajsamund (Khamnore), C_{12}-Check II from Chittorgarh (Ghodakheda) and C1-Check.

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Dump management in South Kaliapani chromite mines, Jajpur, Odisha, India

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ABSTRACT

The South Kaliapani Chromite Mines of Odisha Mining Corporation (OMC Ltd.) Limited is in ultramafic complex in Sukinda valley of Jajpur District. The mine is connected through the nearest rail head at Jajpur- Keonjhar Road railway station on Howrah Chennai line of South-Eastern Railway. Huge quantity of overburden waste or mine spoil is generated in Chromite mines due to high ore to overbudern ratio having the maximum output of 14.55 Metric Tons of waste generated against 1.0 Metric Tons of ore. The waste having diverse chemical and mineralogical characteristics, is challenged with designing stable waste dumps up to 120 m high dumps with stable configuration. This article elucidates study of slope stability and results of implementation of control measures in designing and maintaining a safe slope of the overburden thereof in South Kaliapani Chromite Mines of OMC Ltd. Authors also recommend the management of active dumps, control measures at active dumps to avoid water accumulation, stabilization of inactive dumps through plantations and overall better dump monitoring.

Key words: Chrome ore, dump, overburden, slope stability

INTRODUCTION

Odisha has a rich source of chromite minerals. Chromite, or iron chromium oxide ($FeCr_2O_4$) is the mineral source of chromium. In its purest form, chromite comprises chromium (Cr_2O_3) at 68% and iron oxide (FeO) at 32%. High purity chromite deposits are rare, due to the natural replacement of chromium and ferrous iron by other elements. After extraction of minerals, the residual remains in dumps are liable to pass on through rainwater to lower lands, if not properly managed may affect human health, plants, and animals. Panda and Patra (2004) studied the chromium phytotoxicity and its bioavailability in rice seedlings in both water and soil culture experiments using chelating agents, organic acids, and some mineral irons. The rapid industrialization in India has currently increased the consumption of natural resources with consequent generation of wastes and pollutants. This has serious consequences on the human health and the environment (Mohanty et al., 2010).

A combination of poor mining methods, waste storage and disposal systems, as well as the day-to-day activities associated with tribute and contract chromite mining are primarily responsible for environmental problems in the mines (Maponga and Ruzive, 2002). Contaminated soil and water pose a major environmental and human health problem. Due to open cast mining process lots of over burden are being generated and leaching from this over burden adds the hexavalent chromium to ground water regime. Ground water in the valley is encountered at a shallow depth in semi-confined aquifer (Mishra and Sahu, 2013). Therefore, it is essential to stabilize the dumps and make necessary surface run-off arrangements to avoid leaching. The surface runoff thus collected is treated in Effluent Treatment Plant before final discharge.

MATERIALS AND METHODS

South Kaliapani Chromite Mine of M/s Odisha Mining Corporation Limited (OMC) is in Jajpur District of Odisha. South Kaliapani Chromite Mine in Sukinda ultramafic complex is in Sukinda Tehsil of Jajpur district in Orissa. The nearest railway station is Baghupal on Padapahar Jn. (on SE-Railway's Rajkharsawan- Barajamda Jn. B.G. Branch Line) – Banspani – Keonjhar – Jakhapura Jn. (on the Howrah - Kharagpur - Cuttack – Visakhapatnam B.G. Main Line) which is at an aerial distance of ~21.5 km east of the mine. The leasehold area is linked with Daitari-Paradeep Express Highway. State Capital at Bhubaneswar and district head quarter at Jajpur are located at road distances of ~150 km (~80 km aerial distance) and ~98 km (~60 km aerial distance), respectively from South Kaliapani leasehold area. It is located between latitudes 21° 00' 49.64076"N and 21° 03' 25.10625" N and longitudes 85° 46' 25.96764" E & 85° 48' 28.53433"E.

The location of the South Kaliapani chromite mine is shown in Fig. 1. The location of the ML area on Google Earth has been shown in Fig. 2. The chromite mining process is largely related to the generation of a huge quantity of wastes and rocks in the form of overburden (OB; Dhakate et al., 2008). The CSIR-Central Institute of Mining and Fuel Research (CIMFR) has rendered its services for assessment of safe slope design. The analysis of strength properties along with engineering judgement was used in the process of analyzing and evaluating the stability of OB Dump with different geometrical configurations.

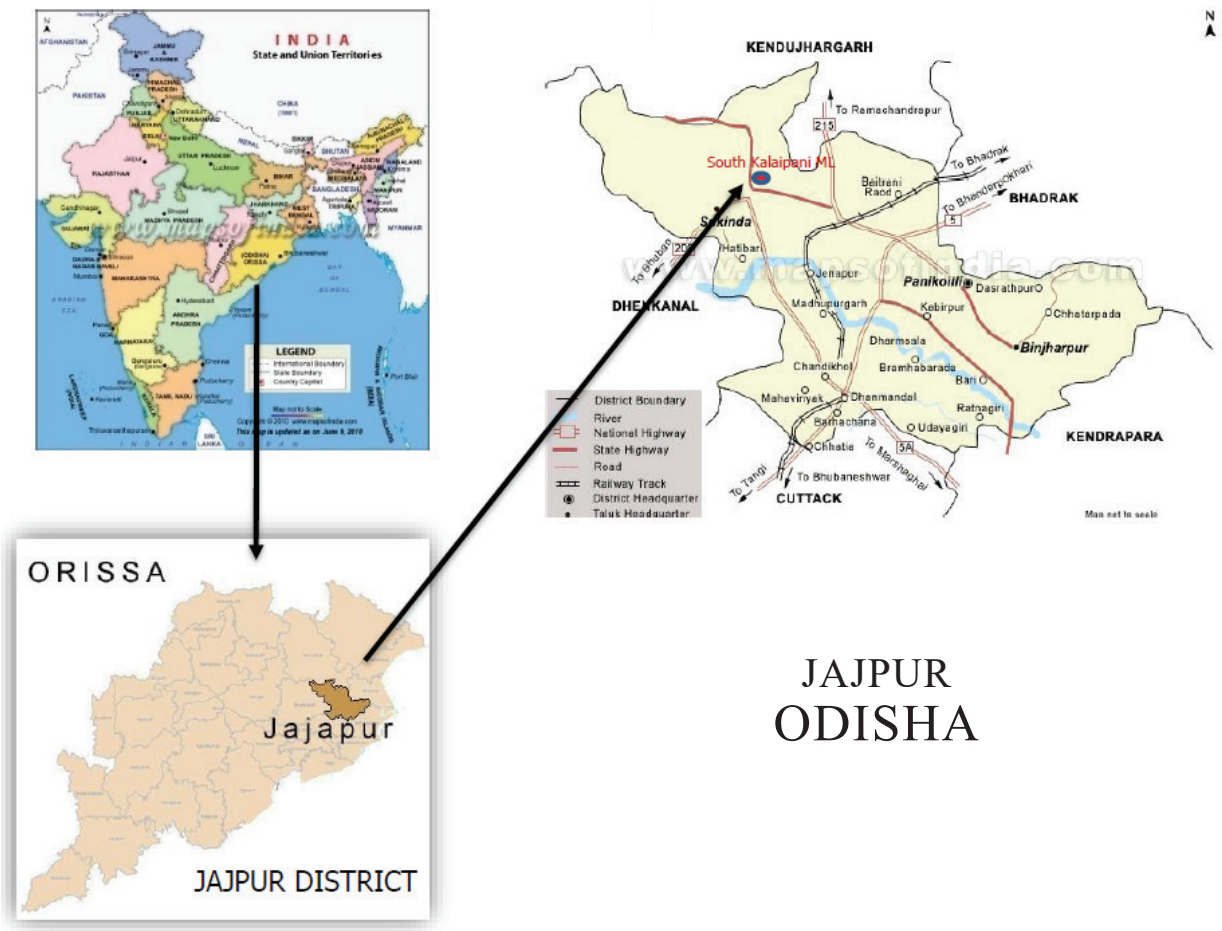

Fig. 1. Location map of south Kalipani chromite mines

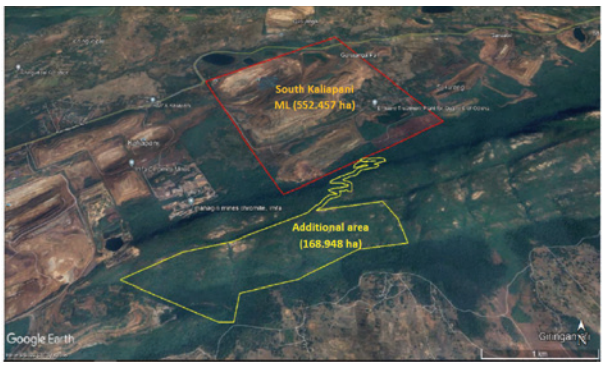

Fig. 2. Location map of south Kalipani chromite mines

The slope stability depends upon the slope geometry and the strength of the properties of slope materials. Engineered properties of materials of OB dump influences the analysis of slope stability. The limit equilibrium analysis using GALENA software was used along with the analysis of the geo-mechanical properties in the dump soil in the rock mechanics laboratory of CIMFR was used for analysis of slope stability (CSIR-CIMFR Report, 2021).

RESULTS AND DISCUSSION

Slope stability analysis

Huge piles of overburden or waste dump are stockpiled near mining sites having high levels of toxic substances which create dust clouds with fumes of many other toxic gases leading to air and water pollution need to be stabilized before monsoon season.

The static external dump has been stabilized through afforested or re-grassed to check wash offs. Waste dump is guarded with retaining walls at their toes along the lower contours. Following the retaining wall, a garland drain is developed for carrying water to the natural drainage system. Settling pits have also been constructed in the drains to arrest solid particles. The retaining walls are of 1.5 m height and 1.5 m thick at the base. The existing waste dumps are stabilized with bio-degradable coir geo-textile. It facilitates new vegetation by absorbing water and preventing topsoil from drying out. Grass-seedling or plantation is done after blanketing the coir matting on the dump slope. This provides support to the dump soil allowing natural vegetation to become established.

Garland drains are dug around 1 m beneath the adjoining contour level at the lower peripheral areas of the dump. The width of the drains is around 1.5 m. A series of settling pits is provided to arrest the wash-off solid particles. The OB dumps areas area compacted, and afforestation is carried out on the terraces as well as along the slopes after spreading a layer of topsoil over it before rehabilitation. Topsoil being generated during mining is used stored in an earmarked area and used for plantation purposes only. Biologically reclaimed part of existing external OB dump along with retaining wall and garland drain in South Kaliapani ML and settling pit at toe of existing external OB dump along with retaining wall in South Kaliapani ML is shown in Fig. 3 and Fig. 4 below (EIA Report, 2021).

Fig. 3. Biologically reclaimed part of existing external OB dump along with retaining wall and garland drain

Fig. 4. Settling pit at toe of existing external OB dump with retaining wall

Management of active dumps

The slopes of dumps are maintained at an overall angle of less than 28 degrees with individual lifts at less than 37 degrees. As the dumps attain final position, the slope is terraced, and proper vegetation is laid which will cause binding of the soil preventing any slope failure. Retaining wall is built across dumps except at few places leaving access to the dumps which will have weep holes for passage of storm water to join garland drains study conducted by CIMFR vide their report dated January 2021, stipulates the following measures:

Dumping of spoil or OB is so done that spoil- banks are benched in accordance with the recommendations of scientific study carried out by an agency having expertise in this regard and in accordance with the stipulations of the permission granted by Directorate General of Mines Safety (DGMS). The width of such benches shall not be less than the height of the bench, and the general slope of the spoil bank shall not exceed one vertical to 1.5 horizontal. The height of spoil bank or OB dump shall also not exceed the height as recommended by the scientific study.

During dumping of OB and maintenance of the dump or its benches, it is ensured that no water is allowed to accumulate at the dump-top or any of the benches already formed. Natural gravitational drainage is provided by sloping the dump-top and the intermediate benches, as well as the haul road leading to dump- top, and collector drains to collect and drain out the rainwater and prevent its seepage into the dump shall further be provided. It is ensured that the collector drains are kept maintained free of debris and loose material that may slide in as shown in Fig. 5(a & b).

Fig. 5(a). GI sheets with drainage arrangements

Fig. 5(b). Pipeline arrangement with vat at the slope of the dump.

Control measures at active dumps to avoid water accumulation

The top as well as benches of all active dumps are regularly inspected at intervals fixed by the Manager, more particularly during rainy season and post-monsoon, by a person not below the rank of Mine Foreman and any deformation or formation of tensile cracks are brought to the notice of the Manager forthwith. If any cracks are observed, they are filled with sandy material immediately to check the entry of water in the dump mass. A record of such inspections is duly maintained in a bound paged book. The dump-design and dump-slope profile parameters of dumps exceeding 60 meters in height are monitored by suitable survey instruments to ensure their accurate and up-to-date geometry. Frequency of such measurement is fixed by the manager and may be increased during monsoon and post-monsoon periods when dump-slopes are more likely to fail because of rain, which may lower cohesion of the dump-material mass.

A record of such measurement is maintained in the bound paged book kept for the purpose, which is signed by the surveyor entrusted with such responsibility and is counter-signed by the Manager. Persons engaged in inspection of the dumps and their benches, and those engaged in monitoring of dump-geometry are imparted job-specific training before being deployed for the purpose. OB or spoil removal from the mine and its dumping in OB dump-yards is placed under overall charge of an Assistant Manager, who shall ensure during dumping of spoil or OB that dump-design and dump-slope parameters are kept maintained in accordance with this SOP.

Dumping of OB or spoil in OB dumps and regulation of movement of tipper-trucks on dumping platform in each of the working shifts is carried out under personal supervision of a competent person (Mine Foreman or Mining Mate). Before commencement of dumping operations and at regular intervals during his shift, the competent person shall inspect the dumping platforms or dump-tops, where from dumping of OB or spoil is carried on, for any signs of sinking and or formation of tensile cracks. If signs of any formation of tensile cracks or sinking of dumping surface are observed, dumping operations in the said area is stopped immediately and brought to the notice of the Assistant Manager or Manager forthwith. Dumping in the said area shall resume only after the matter has been investigated, corrective actions have been taken and formal approval has been accorded by the Manager. The competent person shall regulate the movement of dumpers or tipper-trucks in an orderly manner so that there is no crowding at the dumping platform and shall also ensure that dumpers or tipper-trucks dump OB or spoil at the designated places and in the manner prescribed. Only top dumping of OB or spoil is done to prevent dumpers or tipper-trucks rolling back and down the dump-slope. Dumped OB or spoil material is regularly pushed down the dump slope by dozer.

Where edge dumping of spoil or OB becomes unavoidable, a berm or embankment is provided at the edge of the dumping platform whose width at top shall not be less than 1 meter and its height is not less than the diameter of the tyres of the largest dumper or tipper-truck engaged in dumping or stacking. Movement of the dumpers or tipper-trucks is regulated by the competent person standing on an elevated traffic island to prevent the risk of him being run-over by the tipper-trucks. He shall not stand near the dump-edge or in the operational area of dumpers or tipper-trucks engaged in dumping of OB or spoil. Suitable code of signals by means of coloured flags or batons are framed and implemented for the purpose as shown in Fig. 6.

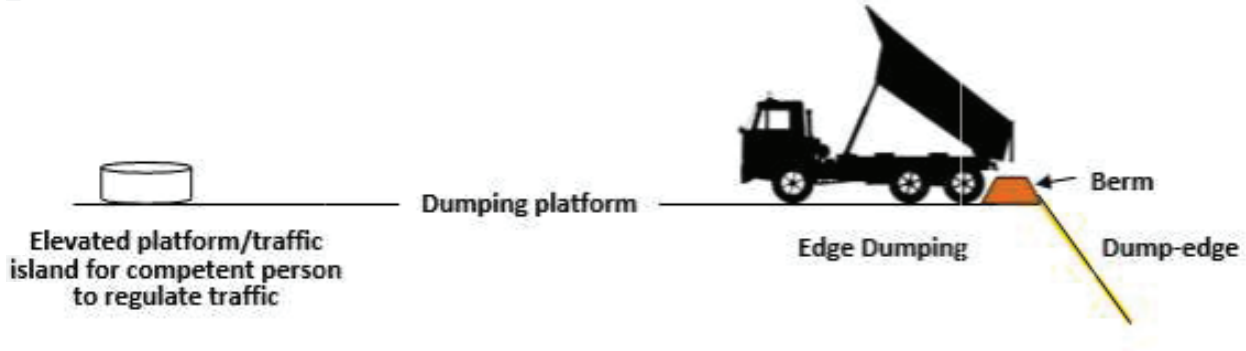

Fig. 6. Safe dumper movement schematics

All ore, including low-grade ore, is stacked at places belonging to the mine and duly approved by the manager in writing. The sites chosen for stacking of different categories of ore, including low-grade ore is such that ore is not stacked beneath or close to power, transmission, or telephone lines, also ensuring that the toe of the ore- stock piles do not get extended within 100 m of any public works, public road, power or transmission or telephone lines and permanent structures not belonging to Mine. A suitable fence is erected between such public works or road or building or structure not belonging to Mine and the toe of the stockpiles to prevent unauthorized persons from approaching it. The slope of stockpiles is the natural angle of repose of the ore being stacked and should not exceed 37 degrees from horizontal. The toe of the stockpiles shall not be retained by artificial means as to increase their slope more than natural angle of

repose of the ore being stacked. Stacking of ore including low-grade ore is done in a manner that stockpile slopes are benched in accordance with the stipulations, if any, of permission granted by DGMS or at intervals not exceeding 20 m in height. The width of such benches shall not be less than the height of the bench. Stockpiles slopes may as well be benched at lesser intervals in the interest of safety if space requirements so permit. The height of stockpiles shall not exceed 40 meters.

Stabilization through plantation

The species for plantation is selected based on soil quality, place of plantation, chances of survival, commercial value (timber value, ornamental value, etc.), etc. It is to be noted that only indigenous species is planted. Exotic species like Subabool (*Leucaena leucocephala*), Eucalyptus and Australian Acacia (*Acacia auriculiformis*) will not be planted. Also, Teak (*Tectona grandis*) will not be planted as it is not native to the region. The species for green belt or vegetation covered development is selected in consultation with the State Forest Department. Mixed plantations are done keeping optimum spacing between the saplings. However, the species suitable for planting in the area as recommended by Central Pollution Control Board (CPCB) in their publication "Guidelines for Developing Green belts" (CPCB, 2000).

Once dumping or back filling has been completed, a path is cleared to the designated area so that the basic inputs (water, manure, and seedlings) can be carried up to the site. Next, a layer of topsoil must be spread over the area and roughly leveled. Grass seeds or seedlings are planted on the soil layer to stabilize the soil. Plants selected for plantation in and around the waste dumps should have pollution hardy nature, fast growth rate, glabrous or pendulous leaves, and large crown volume to surface area of fluttering leaves.

Trenches of 45 cm × 45 cm are dug on the flat top of the dumps and the excavated material is used to form a bund on the dip side of the trenches to retain maximum water in the trenches during rains. Suitable benches are made on the waste dumps and a size of 60 cm × 60 cm pits are dug on the benches at 23 m intervals. The pits are filled with a mixture of topsoil, organic manure, and phosphoric fertilizers. Saplings are planted in these pits once monsoon has commenced to ensure the maximum survival of the saplings. Initially hardy pioneers' species, *Ficus benghalensis, Ficus religiosa* and *Acacia auriculiformis* are planted to help build up the soil. Subsequently, *Acacia auriculiformis* is cut down (as this species is an invasive species) replaced by species such as *Azadirachta indica*, *Annona squamosa*, *Pongamia pinnata*, *Ziziphus mauritiana*, *Ficus* spp. etc.

Plantation on the slope of the dumps will commence as soon as the first bench is ready. The terrace on the slopes is sloped in ward. 60 cm × 60 cm pits are dug at 1.5 m intervals and filled with a mixture of topsoil and organic manure. There are open masonry drains on the terraces. These will receive water from the higher terraces and convey it to the next lower terrace. Before the commencement of the monsoon the slopes and terraces are covered with a layer of soil (held with suitable mechanical soil binder) and sprinkled with water. Just before the commencement of the monsoon seeds of grasses and small shrubs are sprinkled on the soil covering of the dump slopes or seedlings of such plants are planted on the slopes.

Besides, it is also proposed to stabilize the existing dead waste dump with bio-degradable coir geo-textile. It facilitates new vegetation by absorbing water and preventing topsoil from drying out. Grass-seeding or plantation is done after blanketing the coir matting on the dump slope. This will provide support to the dump soil allowing natural vegetation to become established (Singh, 2010).

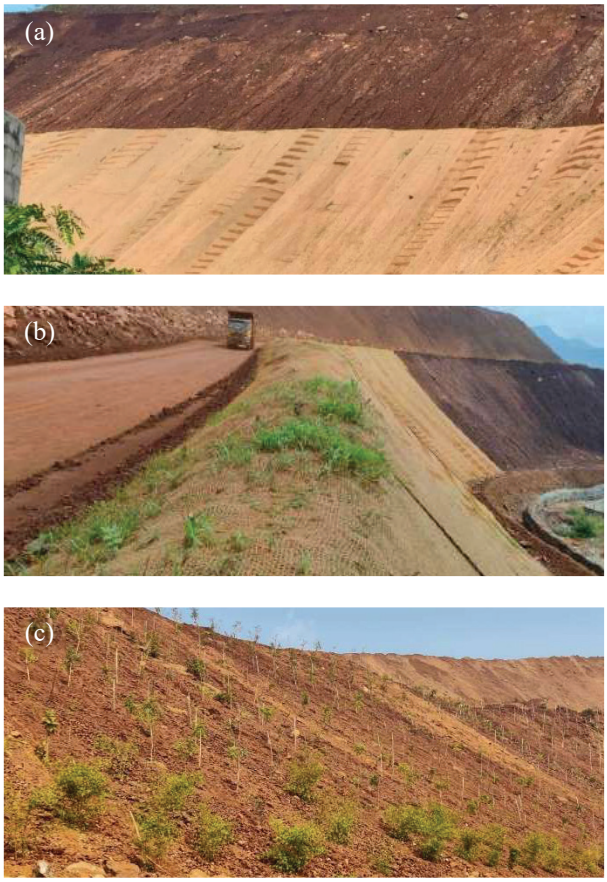

Fig. 7 (a,b,c). Photos of plantation with coir matting under taken at stabilized parts in the active dumps

Dump monitoring

The following dump monitoring practices are followed: The operations connected with stacking of ore in each stockpile are placed under the charge of a competent person in each of the working shifts who shall personally supervise such operations. Re-handling operation for dispatch of ore from the mine (if carried out simultaneously along with stacking of ore) shall also be placed under the charge of a separate competent person. Operations connected with stacking of ore received from mine and re-handling of ore from stockpile for dispatch are kept segregated, above or below each other and shall not be done simultaneously from the same place or vertically.

Before commencement of dumping of ore in stockpiles or re-handling of ore there-from, and at regular intervals during the shift, the dumping and loading platforms or surfaces is inspected by the competent person for signs of sinking and or formation of tensile cracks. If signs of any formation of tensile cracks are observed, dumping of ore or re-handling operations is stopped immediately and the same is brought to the notice of the manager forth with. Dumping of ore or re-handling shall resume only after the matter has been investigated, corrective actions have been taken and formal approval is accorded by the Manager. The competent person (s) shall regulate movement of dumpers or tipper-trucks engaged in stacking of ore or of buyers' trucks engaged in dispatch or ore from the mine in an orderly manner so that there is no crowding during stacking of ore or re-handling of stockpile.

Movement of the dumpers or tipper-trucks or buyers' trucks is regulated by the competent person standing on an elevated traffic island to prevent risk of him being run- over by the tipper-trucks or buyers' trucks. Suitable code of signals by means of coloured flags or batons are framed and implemented for the purpose. Only top-dumping of ore is done to prevent dumpers or tipper-trucks rolling back and down the stock-pile slope. Dumped ore shall either be levelled or pushed down the stock-pile slope by dozer at regular intervals. Stockpiles exceeding digging height of the loading equipment deployed for re-handling is re-handled top-down wards by forming artificial benches on the stack-slopes. The width of such benches is adequate as to permit safe operation of the equipment deployed for loading and transport of ore.

CONCLUSION

In chrome ore mines, dumping of spoil or OB shall be so done that spoil-banks are benched in accordance with the recommendations of scientific study carried out by an agency having expertise in this regard and in accordance with the stipulations of the permission granted by DGMS. The mining process creates over burden and waste rock while extraction of mineral content from the excavated reserve (typically the strip ratio of over burden to actual mineral reserve is very high in chrome mining).

As a result, huge piles of over burden or waste dump is collected near mining sites and contains significant levels of toxic substances and creates dust clouds with fumes of other toxic gases there by leading to air pollution and water pollution as a result of run off during monsoon season. Hence, the waste dump needs to be stabilized with procedures as described above. The slope monitoring allows failures to be predicted and safe working conditions. The review of monitoring results, visual inspection and regular briefing of field people help to detect the onset of failure. The slope monitoring of dumps is being done by the mine management with the help of total station surveying equipment. To date no large-scale failure is reported.

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e-planet **21** (1): 56-59 (June 2023)

Distribution of honey badger *(Mellivora capensis)* in Similipal Tiger Reserve, Odisha, India

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ABSTRACT

The distribution of Ratel or Honey Badger, *Mellivora capensis*, is poorly known within the Asian portion of its global range. Targeted camera-trapping produced the first known records of this species from Similipal Tiger Reserve (STR) in Odisha, India. During the exercise the tiger reserve was divided into different block. In the I block total 126 cameras were fixed within the four ranges. Similarly in the Block II total 187 cameras was fixed in seven ranges. In the Block III total 214 and Block IV 131 camera were fixed within the six ranges and four ranges respectively. Total nineteen ratels were captured from three divisions. Out of the three divisions, the highest number of ratels were captured in Kaptipada range of Baripada division (N =13) followed by Pithabata range (N=03), Chahala range (N=01), Nawana North (N=01) of Similipal core division and Manada range of Rairangpur division (N=01) only one ratel was captured. It shows that the maximum photos were captured in Buffer division of the Similipal Tiger Reserve.

Key words: Camera trap, distribution, honey badger, Similipal Tiger Reserve

INTRODUCTION

The Ratel, *Mellivora capensis*, is widely distributed throughout Africa, the Middle East, and South Asia (Begg et al., 2008; Do Linh San et al., 2016), including most of India (Prater, 1980; Menon and Daniel, 2003). Although their status and distribution have been well-documented in parts of Africa and the Middle East (Krunkand Mills, 1983; Begg et al., 2003) there have been relatively few records from India, with most published records from Central India and the Western Ghats (Kumara and Singh, 2006; Gupta et al., 2012; Gubbi et al., 2014; Krishnan et al., 2016). It has a very wide habitat tolerance occurring from sea level to > 2500 m and from desert steeps to rain forests but prefers drier arid landscapes. The current note presents the first known camera-trap records of ratel from Similipal Tiger Reserve in Odisha.

Study area

Similipal Tiger Reserve is in the Mayurbhanj District of Odisha and spreads over 2750 km^2 of the Chotanagpur plateau (Fig. 1). The park is surrounded by high plateaus and hills, the highest peak being the twin peaks of Khairiburu and Meghashani (1515 above mean sea level). Twelve rivers cut across the reserve, all of which drain into the Bay of Bengal. These are Budhabalanga, Palpala, Bandan, Salandi, Khairi, Khadkei, Budhabalanga, West Deo and East Deo rivers. The reserve forms parts of the Eastern Ghats and the main habitat type is moist deciduous forest. Sal (*Shorea robusta*) trees are the most dominant tree species (Sahoo et al., 2016).

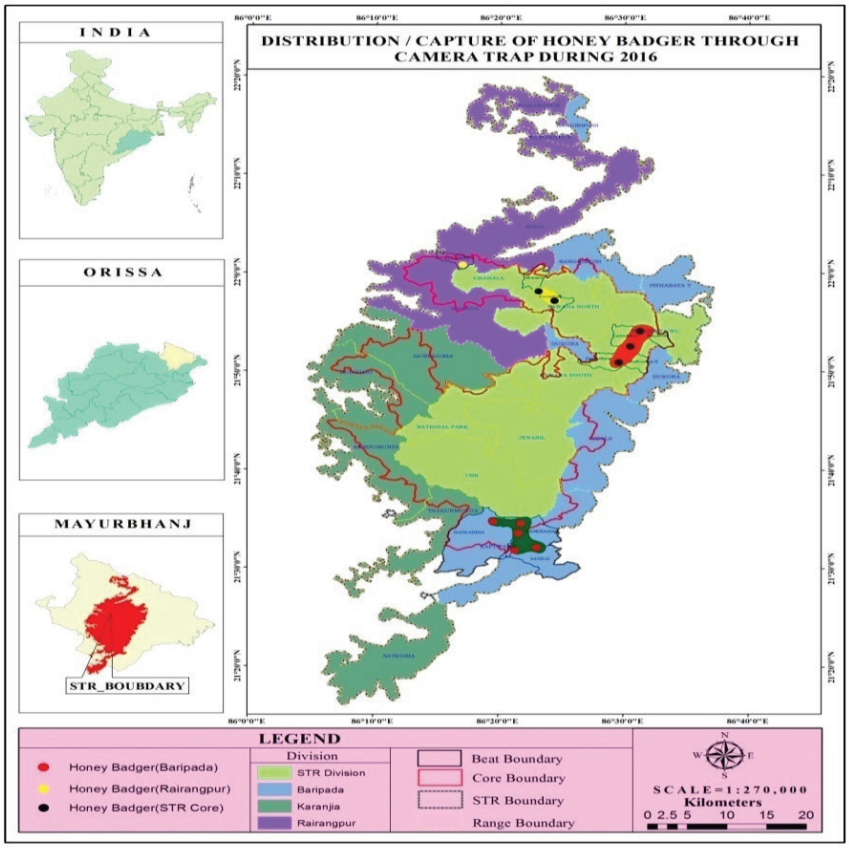

Fig. 1. Distribution pattern of honey badger during camera trapping

MATERIALS AND METHODS

In 2016 the Odisha Forest Department has undertaken camera trapped method in the reserve to study the status of tiger *(Panthera tigris)*. The camera traps were set in locations that would maximize tiger detections (e.g., by recent kills, Pug mark and other signs). The Camera-trapping exercise lasted from February 2016 to May 2016 for 120 days. Total area was divided into four blocks and the sampling period was 35-47 days. Camera trapping is a non-invasive technique for wildlife and landscapes monitoring. Along with the rapid assessment of modern ecological analysis and modeling tools, camera trapping is being a vital role in wildlife research at various levels. Mean while along with improvements in techniques decreasing cost and increasing application interest this practice is adopted by many researchers and wild life managers in the protected area. The camera traps were used to survey the nocturnal animals in the study area.

RESULTS AND DISCUSSION

During the camera trap exercise from February 2016 to May 2016 each block was sampled for 30 days. The cameras were active for 24h period that accounted for one sampling occasion. Each camera was assigned a unique identification number, date, time, and camera ID was recorded for every capture. The locations of each photo-capture of honey badger were recorded and mapped to understand their geographic distribution in the study area. Although primarily this exercise was taken up mainly for the purpose of the phase-IV Tiger monitoring in STR the authors could take privilege to study the distribution pattern of honey badger in the process. During the exercise the tiger reserve was divided into different blocks. In the I block total 126 cameras were fixed within the four ranges. Similarly in the Block II total 187 cameras were fixed in seven ranges. In the Block III total 214 and Block IV 131 camera were fixed within the

six ranges and four ranges respectively. A total of nineteen ratels were captured from three divisions (Table 1; Fig. 1, 2a, 2b). Out of the three divisions the highest number of ratels were captured in Kaptipada range of Baripada division (N=13) followed by Pithabata range (N=03), Chahala range (N=01), Nawana North (N=01) of Similipal Core division and Manada range of Rairangpur division (N=01) only one ratel was captured. It shows the maximum photos were captured in Buffer division of the Similipal Tiger Reserve (Table 1). The study revealed that the primary habitat of the ratel happened to be the types of deciduous forest (both dry and moist) and ever green forest. The study area varied from dry deciduous, moist deciduous, semi ever green to ever green forest. There is a need to develop management plans that look in to and beyond protected area. Present study can give an insight to develop requisite management plans for sustenance of the population of honey badgers. Further studies may be undertaken on their exact status, population density, ecology, connectivity, and threats in the said reserve to facilitate developing strategies for conservation of the species.

Fig. 2(a). Honey badger captured through camera trapping in Kaptipada range

Fig. 2(b). Honey badger captured through camera trapping in Manada range

Table 1. Location of camera-trapped images

Sl.	Division	Range	Longitude	Latitude	No. of photo capture
1	STR(core)	Pithabata	86°29'33.0"	21°50'56.7"	1
2	STR(core)	Pithabata	86°30'29.7"	21°52'33.0"	1
3	STR(core)	Pithabata	86°31'15.4"	21°54'05.5"	1
4	STR(core)	Chahala	86°23'06.4"	21°58'07.4"	1
5	STR(core)	Nawana (N)	86°24'22.3"	21°57'10.6"	1
6	Baripada	Kaptipada	86°21'45.0"	21°34'32.9"	2
7	Baripada	Kaptipada	86°21'33.4"	21°33'32.3"	1
8	Baripada	Kaptipada	86°21'33.0"	21°33'32.2"	1
9	Baripada	Kaptipada	86°23'01.9"	21°32'05.5"	2
10	Baripada	Kaptipada	86°21'17.0"	21°31'48.5"	4
11	Baripada	Kaptipada	86°19'31.6"	21°34'44.1"	3
12	Rairangpur	Manda	86° 23' 05.3"	21° 58' 03.1"	1

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Diversity and abundance of butterfly in Kalyani Lake park, West Bengal, India: A reconnaissance

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ABSTRACT

Butterfly species are one of the most important biodiversity indicators of nature. Study was done in Kalyani Lake Park area from June 2022 to December 2022 on status, abundance, and diversity of butterfly species. In recent times local butterfly species survives under threat and their count decreases. The objective of that study was to know about the abundance and diversity of butterfly species in the selected study area, to analyze what measures should be taken for conservation approach. From the present study, a total number of 1328 butterfly species individuals are found from 5 families belong to 44 genus, 58 species. Among them family Nymphalidae consists of 18 species followed by Lycaenidae (17 species), Papilionidae (5 species), Pieridae (8 species), Hesperiidae (10 species) were recorded. out of these 58 species, 3 species recognized as rare and vary rare type; these species of butterfly species are immediately needed to be conserved. By following a simple step everyone can contribute to butterfly species conservation; implantation of saplings, keep the park clean. This study focuses to identify the threats for butterfly species and to contribute in conservation approach.

Key words: Abundance, butterfly species, biodiversity, conservation, Kalyani Lake Park

INTRODUCTION

Butterflies are a large group of insect species belonging to the order Lepidoptera in phylum Arthropod (Robbins and Opler, 1997). There are more than 28,000 species of butterflies world wide, about 80 per cent of which are found in tropical regions. The subcontinent carries a diverse terrain, climate and vegetation that host about 1,504 species of butterfly species (Tiple, 2011; Nair et al., 2014). There are about 200,000 known species of Lepidoptera of which about 10% are butterfly species (Holloway et al., 1987; Qureshi, 2020). Butterfly species occupy an important position in the ecosystem, acting as pollinators, food has good source and aesthetic value (Klein et al., 2007; Syaripuddin et al., 2015; Day et al., 2017; Samal et al., 2021), enables monitoring of species diversity in a region on the potential functional role of the species. Tools to reduce human disturbance and pollution in urbanization, rural and managed areas and urban ecosystems can be used as species diversity monitors (Wilson, 1997; Mukherjee et al., 2015; Abdullahi et al., 2019; Iserhard et al., 2019).

Pollinators play an important role in the world's food supply and they have an important role in ecosystems (Losey and Vaughan, 2006; Lindstrom et al., 2018; Mukherjee and Mondal, 2020; Pradhan and Khaling, 2020). This taxon

is vulnerable due to their response to climatic conditions, land-use patterns, changing habitat and management intensity (Thomas, 2005; Rundlof et al., 2008; Zingg et al., 2018; Schwarz and Fartmann, 2021). They are important components of the food chain. Butterfly species play the role of pray of birds, bats, and other insectivorous animals. There may be minor changes in their habitat that cause immigration or local extinction (Blair, 1999; Kunte, 1997; Mennechez et al., 2003; Ghosh and Saha, 2016). They help in controlling the number of plants and insect population (Conrad et al., 2007; Kulkarni et al., 2021). Butterfly species and plants lives are exceptionally interlinked, which leads to different patterns in their distribution depending on the availability of their food plants (Feltwell, 1986; Silambarasan et al., 2016; Burghardt et al., 2009; Vina and Liu, 2017). Thus, conservation of butterfly species will improve our environment and enrich human life. Because it depends on plants, butterfly species diversity can reflect the overall flora diversity in a given area (Padhye et al., 2006; Dhadse, 2022).

Plant species that serve as rich nectar sources influence butterfly species occurrence (Tipple et al., 2006; Singh et al., 2020). Taxonomic and functional diversities of butterfly species can be increased by creating native vegetation outside the urban parks. In urban matrices, native vegetation's help to maintain the levels of functional butterfly species (Iserhard et al., 2019). A total of 58 butterfly species belonging to the five families of Papilionidae, Nymphalidae, Lycaenidae and Hesperiidae were identified in the present investigation. Butterfly species can be diverse protected by planting host-specific native plants to make sure that there will be at least the common species don't go on to the verge of devastation. The objective of the present study is quantification of butterfly species diversity, their status and abundance in and around the Kalyani Lake Park area.

MATERIALS AND METHODS

Study Area

The present study was conducted in Kalyani Lake Park, West Bengal, India from June 2022 to December 2022 to assess the diversity of butterfly species. Kalyani Lake Park is located in between 88°0.45' E longitudes and 22°0.98' N latitude of West Bengal, India. The vegetation of the area is very rich with a variety of flora species consisting of different types of woody plant, shrubs, herbs, palms, and climbers which are well present.

Fig. 1. Overview of the study area

Data collection

The field survey was conducted between July 2022 to December 2022. Butterfly species diversity at Kalyani Lake Park, West Bengal, India, was studied on the monsoon (June to September) and post monsoon (October and November) season. Butterfly species were carried out in the study area two days a week for a period of six months. Butterfly species were accessed in the study area from morning 10`am to afternoon 3 pm in the day time by direct observations during walking transects (Pollard, 1977; Pollard and Yates, 1993; Caldas and Robbins, 2003; Patil and Shende, 2014) of 200 m 500 m length with 2 m to 5 m on either side in the study area. Their identification was done during flight, feeding, basking, and mating activities using field guides (Kehimkar, 2013). Data were analyzed with the help of Microsoft Excel 2007 (Majumder et al., 2012; Trivedi et al., 2022) to understand butterfly species community structure in the study area.

RESULTS AND DISCUSSION

The butterfly fauna of Kalyani Lake Park area was studied to be fairly rich. A total of fifty-eight butterfly species representing 44 genera belonging to five different families were recorded (Fig. 2; Table 1 and 2). The present findings reveled that species diversity of Nymphalidae (31%) was the highest followed by Lycaenidae (29%), Hesperiidae (17%) and Pieridae (14%) while, Papilionidae (9%) has the lowest diversity. Nymphalidae was the most diverse family with 18 species consisting of 11 genera, followed by Lycaenidae (17 species, 13 genera), Hesperiidae (10 species, 10 genera), Pieridae (8 species, 7 genera), and Papilionidae (5 species, 3 generations). Among these species, 5 (9%) were not rare, 2 (3%) were very rare 1 (2%) were rare, 31 (53%) were commonly occurring and 19 (33%) were very common (Fig. 3). Among these 58 recorded species, Common four ring, Common crow, Gray pansy, Common grass yellow and Psyche were found in high frequencies in the Kalyani Lake Park (Fig. 5).

The study area has vegetation comprising shrubs, herbs, grasses, trees, and fruit plants. Following is a list of butterfly species along with their preferred food plants found in the study area (Table 3). The usefulness of Lepidoptera insects like butterfly species as an indicator of environmental conditions is a basis for study diversity of butterfly species at a spatiotemporal scale (Stefanescu et al., 2004). Butterfly species are indicators of a healthier ecosystem. They act as a pollinator, also serves as a prey for insect eating birds. Basically, butterfly species helps to maintain the food chain, in larger aspect the species richness (Tiple, 2012).

Table 1. Family-wise composition of butterfly community in Kalyani Lake Park

Family	Genus	Species	No. of individuals
Papilionidae	3	5	96
Nymphalidae	11	18	588
Pieridae	7	8	210
Lycaenidae	13	17	325
Hesperiidae	10	10	109
Total	44	58	1328

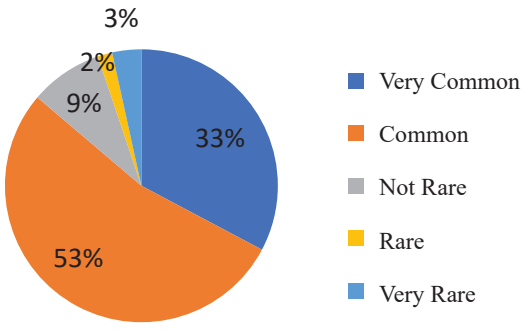

Fig. 2. Family-wise distribution of butterfly species at Kalyani Lake Park

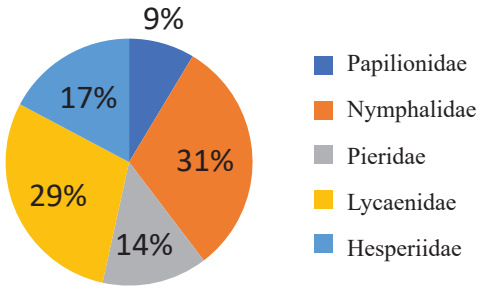

Fig. 3. Status abundance of butterfly species at Kalyani Lake Park

Table 2. Family-wise checklist of butterfly species observed in Kalyani Lake Park with its status

Sl.	Common name	Scientific name	Family	Status abundance
1	Common mormon	*Papilio polytes (Linnaeus)*	Papilionidae	C
2	Common jay	*Graphium doson (C. and R. Felder)*	Papilionidae	C
3	Tailed jay	*Graphium agamemnon (Linnaeus)*	Papilionidae	VC
4	Lime butterfly	*Papilio demoleus (Linnaeus)*	Papilionidae	C
5	Common rose	*Pachliopta aristolochiae (Fabricius)*	Papilionidae	C
6	Psyche	*Leptosia nina (Fabricius)*	Pieridae	C
7	Common grass yellow	*Eurema hecabe (Linnaeus)*	Pieridae	C
8	Eastern striped albatross	*Appias olferna (Swinhoe)*	Pieridae	C
9	Lemon emigrant	*Catopsilia pomona (Fabricius)*	Pieridae	C
10	Mottled emigrant	*Catopsilia pyranthe (Linnaeus)*	Pieridae	VC
11	Common gull	*Cepora nerissa (Fabricius)*	Pieridae	c
12	Common jezebel	*Delias eucharis*	Pieridae	C
13	Indian wanderer	*Pareronia hippia (Fabricius)*	Pieridae	C
14	Common quaker	*Neopithecops zalmora (Butler)*	Lycaenidae	C
15	Dark grass blue	*Zizeeria karsandra (Moore)*	Lycaenidae	C
16	Indian lime blue	*Chilades lajus (Stoll)*	Lycaenidae	VC
17	Plains cupid	*Chilades pandava(Horsfield)*	Lycaenidae	C
18	Common pierrot	*Castalius rosimon (Fabricius)*	Lycaenidae	C
19	Apefly	*Spalgis epius(Westwood)*	Lycaenidae	VR
20	Pale grass blue	*Psuedozizeeria maha*	Lycaenidae	C
21	Falcate oakblue	*Mahathala ameria (Hewitson)*	Lycaenidae	VR
22	Yamfly	*Loxura atymnus (Stoll)*	Lycaenidae	C
23	Common silverline	*Spindasis vulcanus (Fabricius)*	Lycaenidae	VC
24	Indigo flash	*Rapala varuna (Horsfield)*	Lycaenidae	R
25	Slate flash	*Rapala manea (Hewitson)*	Lycaenidae	C
26	Common lineblue	*Prosotas nora (C. Felder)*	Lycaenidae	VC
27	Pointed ciliate blue	*Anthene lycaenina (R. Felder)*	Lycaenidae	VC
28	Silverstreak blue	*Iraota timoleon (Stoll)*	Lycaenidae	C
29	Common ciliate blue	*Anthene emolus (Godart)*	Lycaenidae	C
30	Monkey puzzle	*Rathinda amor (Fabricius)*	Lycaenidae	NR
31	Chocolate pansy	*Junonia iphita (Cramer)*	Nymphalidae	NR
32	Grey pansy	*Junonia atlites (Linnaeus)*	Nymphalidae	VC
33	Peacock pansy	*Junonia almanac (Linnaeus)*	Nymphalidae	VC
34	Lemon pansy	*Junonia lemonias (Linnaeus)*	Nymphalidae	VC
35	Common palmfly	*Elymnias hypermnestra (Linnaeus)*	Nymphalidae	VC
36	Common four-ring	*Ypthima huebneri(Kirby)*	Nymphalidae	VC
37	Common five-ring	*Ypthima baldus (Fabricius)*	Nymphalidae	C
38	Common bushbrown	*Mycalesis perseus (Fabricius)*	Nymphalidae	VC

39	Plain tiger	*Danaus chrysippus (Linnaeus)*	Nymphalidae	VC
40	Striped tiger	*Danaus genutia (Cramer)*	Nymphalidae	VC
41	Blue tiger	*Tirumala limniace (Cramer)*	Nymphalidae	C
42	Common castor	*Ariadne merione (Cramer)*	Nymphalidae	C
43	Common crow	*Euploea core (Cramer)*	Nymphalidae	VC
44	Common evening brown	*Melanitis leda (Linnaeus)*	Nymphalidae	VC
45	Dark-branded bushbrown	*Mycalesis mineus (Linnaeus)*	Nymphalidae	C
46	Common sailer	*Neptis hylas (Linnaeus)*	Nymphalidae	C
47	Chestnut-streaked sailer	*Neptis jumbah (Moore)*	Nymphalidae	VC
48	Commander	*Moduza procris (Cramer)*	Nymphalidae	C
49	Chestnut bob	*Iambrix salsala (Moore)*	Hesperiidae	VC
50	Rice swift	*Borbo cinnara (Wallace)*	Hesperiidae	C
51	Straight swift	*Parnara guttatus (Bremer & Grey)*	Hesperiidae	C
52	Small branded swift	*Pelopidas mathias (Fabricius)*	Hesperiidae	C
53	Palm dart	*Telicota colon (Fabricius)*	Hesperiidae	NR
54	Common dartlet	*Oriens gola (Moore)*	Hesperiidae	VC
55	Suffused snow flat	*Tagiades gana (Moore)*	Hesperiidae	NR
56	Common redeye	*Matapa aria (Moore)*	Hesperiidae	C
57	Common bush hopper	*Ampittia dioscorides (Fabricius)*	Hesperiidae	NR
58	Indian palm bob	*Suastus gremius (Fabricius)*	Hesperiidae	C

** VC-Very Common (>100 sightings), C-Common (50-100 sightings), NR-Not Rare (15-30 sightings), R-Rare (5-10 sightings), VR-Very Rare (1-2 sighting).

Table 3. List of butterfly host plants of Kalyani Lake Park

Sl.	Butterfly species	Plant species name	Family
1	*Papilio polytes (Linnaeus)*	*Aegle marmelos*	Rutaceae
2	*Graphium doson (C. & R. Felder)*	*Polyalthia longifolia*	Annonaceae
3	*Graphium agamemnon (Linnaeus)*	*Huberantha cerasoides*	Annonaceae
4	*Papilio demoleus (Linnaeus)*	*Ixora coccinea*	Rutaceae
5	*Pachliopta aristolochiae (Fabricius)*	*Aristolochia indica*	Aristolochiaceae
6	*Leptosia nina (Fabricius)*	*Capparis* spp.	Capparaceae
7	*Eurema hecabe(Linnaeus)*	*Acacia* spp.	Fabaceae
8	*Appias olferna (Swinhoe)*	*Cleome rutidosperma*	Cleomaceae
9	*Catopsilia pomona (Fabricius)*	*Cassia* spp.	Fabaceae
10	*Catopsilia pyranthe (Linnaeus)*	*Cassia* spp.	Fabaceae
11	*Cepora nerissa (Fabricius)*	*Cleome viscosa*	Cleomaceae
12	*Delias eucharis (Drury)*	*Scurrula* spp.	Loranthaceae
13	*Pareronia hippia (Fabricius)*	*Capparis baducca*	Capparaceae
14	*Neopithecops zalmora (Butler)*	*Glycosmis pentaphylla*	Rutaceae
15	*Zizeeria karsandra (Moore)*	*Amaranthus* spp.	Amaranthaceae

16	*Chilades lajus (Stoll)*	*Glycosmis mauritiana*	Rutaceae
17	*Chilades pandava (Horsfield)*	*Acacia* spp.	Fabaceae
18	*Castalius rosimon (Fabricius)*	*Ziziphus* spp.	Rhamnaceae
19	*Spalgis epius (Westwood)*	*Mangifera indica*	Anacardiaceae
20	*Psuedozizeeria maha*	*Oxalis corniculata*	Oxalidaceae
21	*Mahathala ameria (Hewitson)*	*Terminalia* spp.	ombretaceae
22	*Loxura atymnus (Stoll)*	*Dioscorea* spp.	Dioscoreaceae
23	*Spindasis vulcanus (Fabricius)*	*Allophylus cobbe*	Sapindaceae
24	*Rapala varuna (Horsfield)*	*Zizyphus rugosa*	Rhamnaceae
25	*Rapala manea (Hewitson)*	*Camellia* spp.	Theaceae
26	*Prosotas nora (C. Felder)*	*Acacia* spp.	Fabaceae
27	*Anthene lycaenina (R. Felder)*	*Acacia* spp.	Fabaceae
28	*Iraota timoleon (Stoll)*	*Punica* spp.	Lythraceae
29	*Anthene emolus (Godart)*	*Mangifera indica*	Anacardiaceae
30	*Rathinda amor (Fabricius)*	*Ixora* spp.	Rutaceae
31	*Junonia iphita (Cramer)*	*Justicia neesii*	Acanthaceae-
32	*Junonia atlites (Linnaeus)*	*Sida rhombifolia*	Malvaceae
33	*Junonia almana (Linnaeus)*	*Sida rhombifolia*	Malvaceae
34	*Junonia lemonias (Linnaeus)*	*Sida rhombifolia*	Malvaceae
35	*Elymnias hypermnestra (Linnaeus)*	*Areca catechu*	Arecaceae
36	*Ypthima huebneri (Kirby)*	*Cynodon dactylon*	Poaceae
37	*Ypthima baldus (Fabricius)*	*Axonopus* spp.	Poaceae
38	*Mycalesis perseus (Fabricius)*	*Oplismenus compositus*	Poaceae
39	*Danaus chrysippus (Linnaeus)*	*Calotropis gigantea*	Apocynaceae
40	*Danaus genutia (Cramer)*	*Asclepias curasavica*	Apocynaceae
41	*Tirumala limniace (Cramer)*	*Asclepias* spp.	Apocynaceae
42	*Ariadne merione (Cramer)*	*Tragia involucrata*	Euphorbiaceae
43	*Euploea core (Cramer)*	*Ficus* spp.	Moraceae
44	*Melanitis leda (Linnaeus)*	*Brachiaria mutica*	Poaceae
45	*Mycalesis mineus (Linnaeus)*	*Setaria barbata*	Poaceae
46	*Neptis hylas (Linnaeus)*	*Canavalia* spp.	Fabaceae
47	*Neptis jumbah (Moore)*	*Ziziphus* spp.	Rhamnaceae
48	*Moduza procris (Cramer)*	*Mussaenda frondosa*	Rubiaceae
49	*Iambrix salsala (Moore)*	*Bambusa* spp.	Poaceae
50	*Borbo cinnara (Wallace)*	*Setaria pumila*	Poaceae
51	*Parnara guttatus (Bremer and Grey)*	*Axonopus* spp.	Poaceae
52	*Pelopidas mathias (Fabricius)*	*Axonopus* spp.	Poaceae
53	*Telicota colon (Fabricius)*	*Coccos nucifera*	Palmae
54	*Oriens gola (Moore)*	*Axonopus* spp.	Poaceae
55	*Tagiades gana (Moore)*	*Dioscorea* spp.	Dioscoreaceae
56	*Matapa aria (Moore)*	*Bambusa* spp.	Poaceae
57	*Ampittia dioscorides (Fabricius)*	*Leersia hexandra*	Poaceae
58	*Suastus gremius (Fabricius)*	*Calamus* spp.	Arecaceae

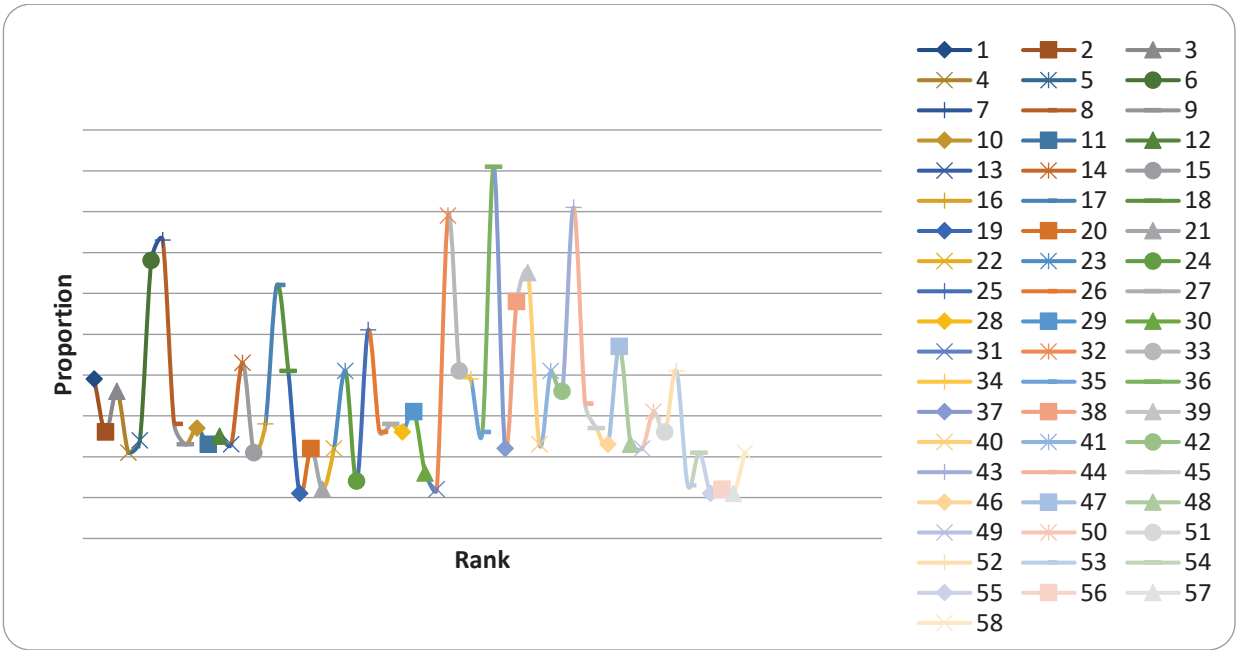

Fig. 4. Rank-abundance of butterfly species community in Kalyani Lake Park

Studies on butterfly species diversity gives us information about the species richness of that study area, we will learn about the vegetations of that landscape mainly about the adult nectar plants and host larval plants (Harrington and Stork, 1995; Tam and Bonebrake, 2016). The rich number of butterflies specially Nymphalids indicates floral diversity of this study area. The study area having large number of herbs, shrubs and trees seems to be a tropical climate plant species belonging to families such as Rutaceae, Annonaceae, Aristolochiaceae, Capparaceae, Fabaceae, Cleomaceae, Loranthaceae, Amaranthaceae, Rhamnaceae etc are found in the study area. Namely, the species are *Aegle marmelos, Polyalthia longifolia, Huberantha cerasoides, Ixora coccinea, Aristolochia indica, Capparis* spp., *Acacia* spp., *Cleome viscosa, Cassia* spp., *Cleome viscosa, Scurrula* spp., *Capparis baducca, Glycomis pentaphylla, Amaranthus* spp., *Glycosmis mauritiana, Acacia* spp., *Ziziphus* spp., *Mussaenda frondosa, Mangifera indica, Oxalis corniculata, Dioscorea* spp., *Allophylus cobbe, Zizyphus rugosa, Punica* spp., *Bambusa* spp., *Calotropis gigantea , Coccos nucifera, Ficus* spp., *Sida* spp., and *Lantana camara.* This kind of rich vegetation provides appropriate feeding and breeding place of butterfly species (Kaneria et al., 2013; Mohapatra et al., 2013; Dasgupta and Rao, 2014).

Along with seasonal and climatic changes butterfly species variety also varies (Thomas et al., 2004). March- April and October are the peak seasons for butterfly species abundance in India identified by Wynter-Blyth (1957). Butterfly abundance can be affected by Excess heat, humidity, rainfall etc. Our present study was done in the month of July to December, the monsoon and post monsoon (Tiple and Khurad, 2009).

From our present study highest number of butterfly species found from family Nymphalidae 18 species (31%) followed by Lycaenidae 17 species (29%), Hesperiidae 10 species (17%), Pieridae 8 species (14%), Papilionidae 5 species (9 %), (Table 1, Fig. 2). From the Nymphaladae family the butterfly species found in this study are *Junonia iphita, Junonia atlites, Junonia almana, Junonia lemonias, Elymnias hypermnestra, Ypthima huebneri, Ypthima baldus, Mycalesis perseus, Danaus chrysippus, Danaus genutia, Tirumala limniace, Ariadne merione, Euploea core, Melanitis leda, Mycalesis mineus, Neptis hylas, Moduza procris, Neptis jumbah;* from the family Lycaenidae. Butterfly species found in this recent study are *Neopithecops*

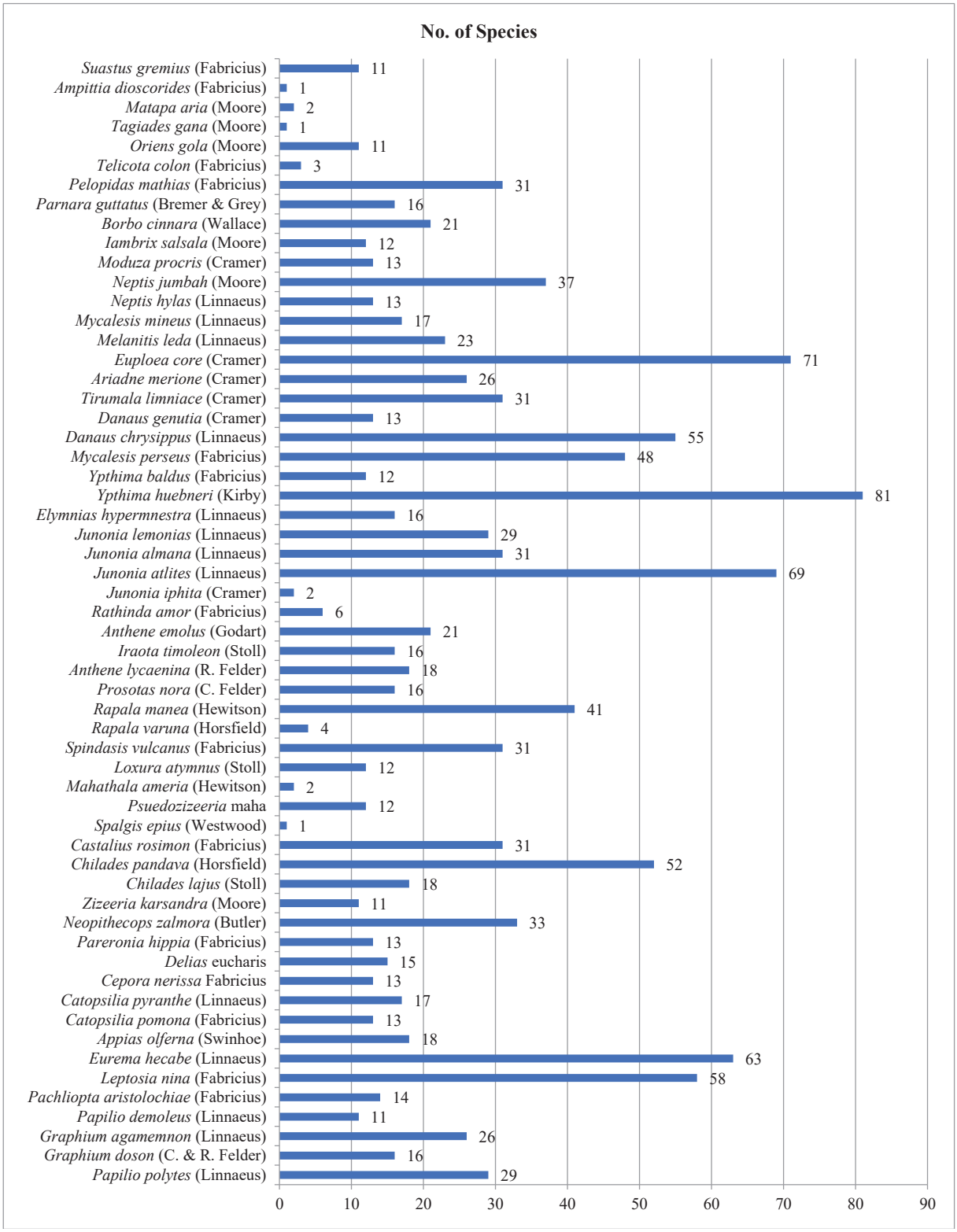

Fig. 5. Distribution of butterfly species at Kalyani Lake Park

zalmora, Zizeeria karsandra, Chilades lajus, Chilades pandava, Castalius rosimon, Spalgis epius, Psuedozizeeria maha, Mahathala ameria, Loxura atymnus, Spindasis vulcanus, Rapala varuna, Rapala manea, Prosotas nora, Anthene lycaenina, Iraota timoleon, Anthene emolus, Rathinda amor; from the family Papilionidae butterfly species that found in this recent study are *Papilio polytes, Graphium doson, Graphium agamemnon, Papilio demoleus, Pachliopta aristolochiae;* from the family Pieridae butterfly species that found in this recent study are *Leptosia nina, Eurema hecabe, Appias olferna, Catopsilia pomona, Catopsilia pyranthe, Delias eucharis, Cepora nerissa, Pareronia hippia;* from the family Hesperiidae butterfly species found in this recent study are *Iambrix salsala, Borbo cinnara, Parnara guttatus, Pelopidas mathias, Telicota colon, Oriens gola, Tagiades gana, Matapa aria, Ampittia dioscorides, Suastus gremius* (Table 2).

Status abundance of butterflies from this study recognized are very common 19 species (33%), common 31 species (53%), not rare 5 species (9%), rare 1 species (2%), very rare 2 species (3%). For the common, very common and not rare species the environment, food supply, breeding places of Study area is mostly friendly (Fig. 4). More than 48 species of butterfly recognized in dominant highest numbers in this study area. The most dominant butterfly species of this study area are *Ypthima huebneri, Euploea core, Junonia iphita, Eurema hecabe, Leptosia nina, Danaus chrysippus, Chilades pandava, Mycalesis perseus, Rapala manea etc.* (Fig. 5). From the present study, one butterfly species designated as rare species in this study area i.e., Indigo Flash *(Rapala varuna)* from the family Lycaenidae and two butterfly species are designated as Very rare; Apefly (*Spalgis epius*) from the family Lycaenidae, Falcate Oak blue *(Mahathala ameria)* from the family Lycaenidae.

CONCLUSION

The present findings of this study show us that Kalyani Lake Park is a resourceful habitat for butterfly species. Moreover, parks are one of the very appropriate place for butterfly species conservation. If proper management taken, a few steps to keep clean the park, implant some saplings routinely diversity of butterfly species may increase. This study also helps to understand the importance of butterfly species in nature and as well as the symbiotic relation between butterfly species and plants. Butterfly species are important to maintain the food web which is an essential component of ecosystem. It also acts as a bio indicator. Now a days, increased urbanization, improper garbage disposition, severe pollution, deforestations etc. seriously affect the butterfly populations. Now, at least to maintain the present levels of butterfly diversity, more saplings need to be planted, urban places be cleaned, parks hygiene and management may be taken care of to conserve a healthy ecosystem at this region.

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Morphological and molecular identification and classification of Passeriform birds in Kabul, Afghanistan

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ABSTRACT

It is thought that the Passeriformes order of bird has monophyletic origin. However, new studies show that many passerine families are not monophyletic in traditional classifications. A more complete understanding of Passeriform phylogeny is possible only by conducting extensive molecular studies. Therefore, this molecular study was conducted to evaluate the Passeriformes birds of Kabul Province, Afghanistan. Samplings were collected from ten stations during three stages during the year 2019 and 2021 in Kabul province, including DehSabz, Bagrami, Sarubi, KhakJabar, Shekar Dara, Gol Dara, Paghman, Char Asiyab, Andrabi Road and Kafroshi. Totally 190 samples were taken using a mist net out of which only 110 samples were taken to the laboratory. After the morphological studies, it was identified by the identification key that all the species belong to the Passeriformes order belonging to 13 families, 23 genera and 35 species. After performing morphometry, the samples were transferred to the laboratory for molecular studies and DNA extraction and *COX1* gene sequencing to identify the species in the tree drawn by Bayesian method which shows the position of genera and species within families and superfamilies. In this study, genus *Passer* with four species, genus *Motacilla* with one species, genus *Carduelis* with one species, genus *Emberiza* with six species and genus *Serinus* with one species were included in the super family *Passeroidea*. The genus *Corvus* with one species and the genus *Lanius* with one species were included in the *Corvida* clade. Two species of the genus *Luscinia* with one species of the genus *Muscicapa* and one species of the genus *Acridotheres* were included in the superfamily *Muscicapoidea*. Two species of the genus *Hirundo*, one species of the genus *Phylloscopus*, one species of the genus *Riparia*, one species of the genus *Sitta*, one species of the genus *Sylvia* and one species of the genus *Eremophila* were included in the super family *Sylvioidea*.

Key words: *COX1 gene*, Kabul, molecular classification, passeriformes

INTRODUCTION

Birds, with more than 10,000 species, constitute a diverse group of vertebrates in all aquatic and terrestrial ecosystems of the globe (Britannica ISLS, 2008). This category of vertebrates with their ability to fly, which makes them unique among other vertebrates, are scattered in various habitats of Afghanistan every year. Kabul province is one of the most important provinces in the country in terms of protection of seasonal migratory birds and their habitats. The presence of the Hindu Kush and the Pamir Mountain range in the east of the province indicate the extra ordinary habitat diversity for birds. Kabul province has an area of about 3128 square kilometers, equivalent to half of the country's total area (Nuristani, 1971). Kabul province has a dry climate with average temperatures between -17°C

and 40°C rainfall 100 to 150 millimeters per year. Afghanistan assumed to be one of the most enriched sources of natural resources including wildlife. Birds in turn is a main portion of the resources and need to be scientifically identified for successful management and improvement (breeding) of biodiversity programs. In ancient times the common way for identification and classification was the morphology while in recent years molecular studies were entered to the classification field, in which organisms are classified accurately and precisely (Mohanta et al., 2012). Forests, fruit trees, and seasonal crops provide well living habitat for birds in terms of feeding and nesting (Khan et al., 2021). Afghanistan particularly Kabul province by having seasonal crops and trees that pave the way for feeding and nesting of different migratory and non-migratory birds. To manage birds successfully, the classification is essential to be science-based.

Though some of the foreign researchers morphologically studied and listed 483 bird species of Afghanistan, but data on molecular studies on birds are unavailable. The goal of this research is to investigate and identify the birds of Kabul province based on morphology and DNA barcoding method by COX1 gene to place them in different clades. This research is done for the first time in the country in Kabul province.

MATERIALS AND METHODS

Place of study

Kabul province is located in the west of the Hindukush and the Pamir mountain range. This province has an area of about 3128 km^2, equivalent to half of the area of Kabul. Sampling was done from the Sanan in Kabul province and 7 cities, namely, DehSabz, Bagrami, Seroubi, KhakJabar, Shekardara, Gol Dara and Paghman. This sampling consisted of collecting feathers and muscles for molecular studies. Totally 190 samples were taken using a mist net out of which only 110 samples were taken to the laboratory for investigation. Sampling stations are shown in red on the map of Kabul province (Fig. 1).

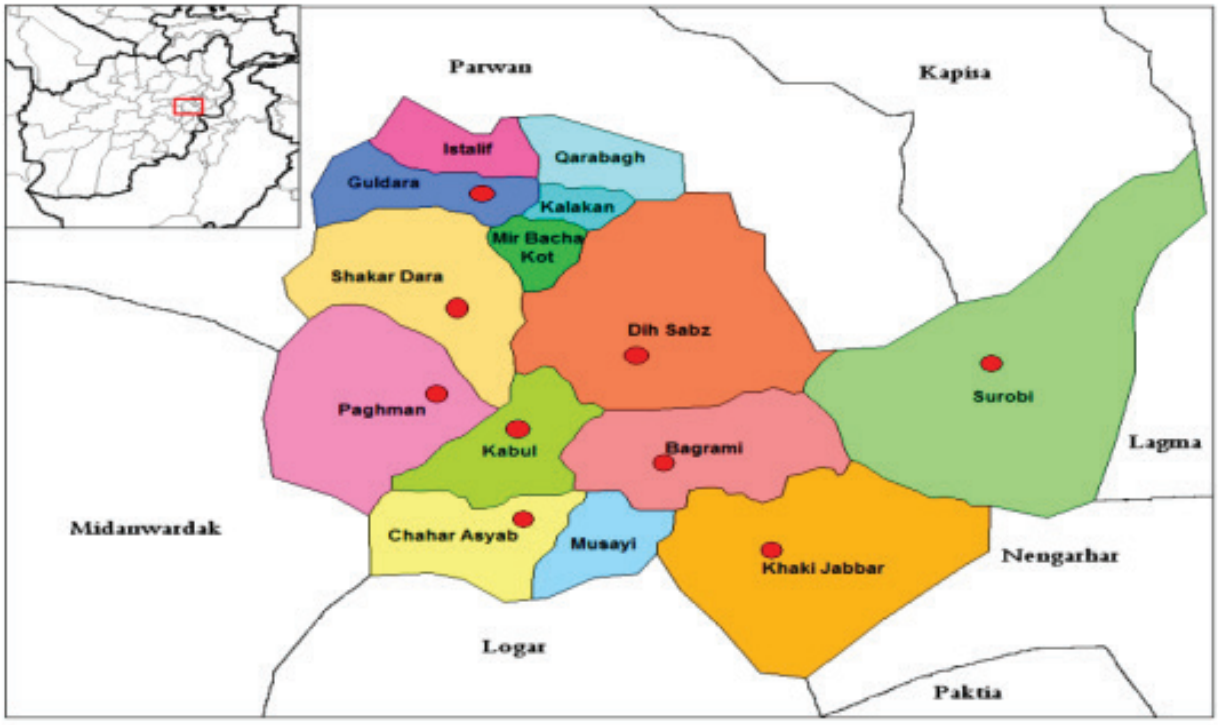

Fig. 1. Map of Kabul province, the central part of Kabul, the areas from which the sampling was done are shown with red marks

Table 1. Details of sampling areas and the number of samples in Kabul province

Sampling location	Number of samples	Sampling date	Longitude	Latitude	Altitude
Die-sabz	5	2019	34°38'54.06"N	69°14'54.44"E	1748 m
Die-sabz	6	2020	34°38'54.06"N	69°14'54.44"E	1748 m
Die-sabz	35	2021	34°38'54.06"N	69°14'54.44"E	1748 m
Istalif	22	2021	34°83'44.72"N	69°14'40.61"E	1748 m
Istalif	15	2021	34°83'44.72"N	69°14'40.61"E	1749 m
Bagrami	4	2020	34°28'63.32"N	69°16'31.61"E	1791 m
Bagrami	1	2021	34°28'63.32"N	69°16'31.61"E	1791 m
serobi	5	2021	34°35'18.09"N	69°44'48.94"E	1001 m
serobi	5	2021	34°35'19.42"N	69°44'50.53"E	1001 m
serobi	5	2021	34°35'18.09"N	69°44'48.94"E	1001 m
Khaki-jabar	3	2019	34°20'00.02"N	69°26'00.09 "E	
Char-asiab	5	2019	34°24'57.55"N	69°66'51.91"E	
Paghman	20	2021	34°35'05.24"N	69°58'41.62"E	2208 m
Guldara	9	2021	34°44'41.94"N	69°02'24.70"E	1991 m
Guldara	4	2021	34°44'41.94"N	69°02'24.70"E	1991 m
Shakar-dara	4	2019	34°41'51.09"N	69°04'18.87"E	1994 m

Study of morphometric traits

In nature and after releasing the bird from the net, many morphometric traits can be measured, including beak length, wing length, tarsus length, tail length, beak length, width, and height, as well as the wing formula, which includes the measurement of the longest feather length. It is primary and equivalent to giving it zero and the difference in the length of other primary feathers, especially P6 to P10 feathers. In the museum samples that the researcher has more time to measure, other traits can be added, including the length of fingers, knuckles, nail length, the maximum length of feather hair around the tip, etc. In this method, one of the valid identification keys is the identification key of Sanan sparrow species (Dementiev et al., 1996). And the bird atlases of the world and the neighbours whose birds are very similar to our birds.

Molecular method

DNA barcoding using DNA molecular markers has made it possible to identify and classify animals quickly and reliably, especially birds, over the past years. In this method, the sequences of a small part of the mitochondrial genome (mt DNA) are used as an accurate and fast tool for identifying and classifying species. Based on this method, changes, order, number, and sequence of 650 standard gene nucleotides have been defined. Cytochrome oxidase-1 can indicate unique changes for each species. For this reason, this method is called barcode or DNA molecular identifier. COXI protein coding gene generally not only shows more differences at the species level compared to other mitochondrial ribosomal genes but is also more suitable for the diagnosis of very close species (Aliabadian et al., 2009, 2014).

Mitochondrial gene *COX1*

Now-a-days, cytochrome oxidase gene is used in molecular biosystematic studies. Among Kadine genes, this gene shows more differences between sequences than other genes. In recent studies, COX1 has been successful in distinguishing many closely related species in vertebrates and invertebrates. COX1 with 600 to 800 nucleotides in

the mitochondrial cytochrome oxidase gene region has been proposed to determine biodiversity in the world (Rubinoff, 2006).

According to Hebert et al. (2003), the reason for using this gene is the following:

- The presence of strong general primers for this gene, which can repair their 5-end, and these primers make PCR easier.
- It seems that this gene has more phylogenetic signs than other mitochondrial genes. The evolution of this gene is so big and fast that in addition to separating very close species, it also separates phylogeographic groups within a species.
- Baz substitution in the third nucleotide position is high in this gene (Hebert et al., 2003).

PCR Technique

Polymerase Chain Reaction (PCR) is a technique that is widely used in molecular biology. This technique takes its name from one of its key ingredients, DNA polymerase, which is used to make a large number of copies of a DNA strand. With the continuation of the PCR process, the number of initial copies of a DNA fragment is produced in a very large amount to the extent of several million copies, and the final product of PCR is generally called an amplification.

Bayesian analysis

It is a statistical method that uses an optimality criterion based on the state of attributes, with the difference that it does not search to find the best tree. In fact, in this method, based on information such as evolutionary model, branch length, and topology, and using the concept of the maximum likelihood, it draws a tree with the maximum likelihood. Bayesian analysis, like the maximum likelihood tree analysis, searches for a set of acceptable trees (Roderic and Edward, 1998). Bayesian analysis is used in molecular phylogeny, species phylogeny and species divergence time. This method uses a phylogeny tree and a model based on the expected value of the parameters and their maximum probability value, to arrive at a posterior probability distribution for those parameters. The desired model is a tree with a specific topology, specific branch lengths, and a specific spatial model of DNA substitution and a specific distribution rate among nucleotide positions (Philippe et al., 2009).

The three methods mentioned above are discrete clustering methods. In general, the optimal tree method selects the tree or trees that have the least evolutionary changes. The most probable tree method selects the tree or trees that have the most probability among all the trees. Bayesian analysis performs the search by applying the maximum concept and targeting the probability distribution of phylogenetic trees (Hall, 2008).

Sampling method

An invisible net (Mist net) was used to catch specimens of this order in different habitats, and the specimens were captured by playing the sounds of the expected species in each habitat. After capturing the bird, it was separated from the net and before changing the position of its feathers, the necessary pictures were taken from its dorsal, ventral, and lateral surfaces. Then, some feather samples (so that they are not from the primary and secondary feathers of the bird) are separated and placed in a zipped bag. Care was taken that the shaft of the feather does not meet the hand. The number of the sample, the details of the sampling location, including the name of the location, the geographical location, and the gender of the bird were noted on the envelope, and the number and name of the bird were written in a notebook. Then, the tissue sample will be taken from the chest and muscle of the bird, transferred into vials containing alcohol, and the vial will be given a notebook number. Before releasing the bird, its biometrics were done, and the desired traits were measured by calipers with an accuracy of 0.01 recorded in the notebook. Morphometry includes body length, beak, tarsus, wings, and tail.

Sampling tools

Mist net, GPS, photographic camera, binoculars, containers containing alcohol, vials, envelopes, digital calipers, tools for dissection, plastic box, magic ruler.

Separation and storage of tissues for molecular studies: The tissues were kept in Merck alcohol. The size of the tissue should not be large because the water in the tissue dilutes the alcohol, it is better to change the alcohol in the vials containing the collected samples after a few hours. Different tissues such as muscle, liver, and kidney can be used for DNA extraction, but chest muscle tissue is usually used. The tissue pieces were separated from the tissue samples under completely sterile conditions and placed in another vial, and the sample number was noted on it. The parts should be small and large in number. Due to the large volume of DNA extraction and PCR steps, their data were omitted as and when required.

RESULTS AND DISCUSSION

The species identification based on morphological traits and creation of phylogenetic trees based on COX1 gene are presented. In the approach of morphological identification of the samples, the results were obtained based on keys and valid field guides, and in the molecular approach, the results were obtained using the gene sequences available in the NCBI and BOLD gene search engines. In this research, 1240 sequences from the gene bank and 86 sequences from 190 samples of birds captured from Kabul province were analyzed together in a molecular analysis. Finally, determining the position of Passeriformes in the related clades.

Identification of Passeriformes species in Kabul province

In terms of appearance, they are small or medium-sized birds, diverse in tip shape, with 10 to 11 primary feathers, with the first feather not developed, 9 secondary feathers and 10 tail feathers, with feet and 4 toes, with the first toe turned back. The forks are bent to different degrees. There is filling in all of them. Filling is done from the inside to the outside in the primary feathers and from the outside to the inside in the secondary feathers. In king feathers, the middle feathers are replaced earlier than the outer feathers. Their chicks are precocious.

Anatomically, they are birds with 14 cervical vertebrae, no petri-goid appendage, coccygeal gland, 5 true ribs, highly developed brain, no permon muscle, thumb flexor muscle not joined to finger flexor muscle (Dementiev et al., 1998). Passeriforms appeared 36 to 45 million years ago, at the same time as flowering plants and insects (Treplin et al., 2008).

The results showed that 190 specimens of Passerines and non-Passerine birds were captured during three stages during 2019 and 2020 years in 10 districts, 22 stages of field operations in Kabul province. Based on the morphological characteristics and using the valid identification keys of the existing samples, 110 samples belonged to the Passeriform order with 13 families, 23 genera and 35 species. The scientific names of the species by family are listed in Table 2. Also, the distribution map of these species and their characteristics are mentioned below.

Morphological characteristics of the distribution of captured species in Kabul province

Lanius vittatus: This bird is 21 cm long and has a thick body, long tail, and big head. Forehead and eye band are black, tail and neck are gray, and in the male bird, the body is fawn red, the tail is white and gray, the undertail is white, and the breast and sides are pea white, which turns red on the sides. The chin and throat are white, the bill, legs and wings are black with a broad white wing band and the sides and tip of the tail are white. The young bird is generally pale with dark streaks on the sides, greyish white undertail feathers and dark brown wings with a small wing band. It is a species captured from Sarubi, Kabul.

Lanius schach: This bird is 22 cm long and is clearly larger than the Oak-backed Eyestone, but its head is smaller. Also, the tail is longer, the edge of the feathers is pea-colored, the tail and the cover of the feathers on the tail are light orange (in the eye stone behind the oak, the tail is gray, and the cover feathers of the tail are white), the black eye line, which covers the top of the beak and forehead (but narrower). And the base of the feathers is marked with a smaller white spot (wing band), which is

sometimes absent (in an adult bird, the eye stone behind the oak is very distinct). White chin and throat, pea underbody, which is slightly fawn on the sides. The back of the neck is gray, the back is fawn red, and the tail is black. The young bird is paler, dark and grey brown on the back with dark brown spots and brown spots on the breast, underbody and tail. The sample was captured from Sarubi, Kabul.

Oriolus oriolus: This bird is 24 cm long. He is shy and the same size as you, and except when migrating, his voice is heard more than seen. Feathers and wings of the female bird and the immature bird are green, the wings and tail are olive-brown, the tail is greenish-yellow, and the underbody is yellow white with darker streaks. In flight, the tail is relatively short and similar to woodpeckers, its flight is slightly wave-shaped, which changes its flight mode due to the slow tilting of the wings. It sits on the branches and usually at the top of the tree and hides among the leaves. The sample was captured from Sarubi, Kabul.

Corvus monedula: This bird is 23 cm long and is one of the small black crows with gray back of the head and neck and both sides of the face and has colorless eyes and fast movements. The beak and legs are black, in flight, compared to other crows, it is smaller, its wings are faster, and its beak is shorter. It is often seen in a group with a black crow. The captured sample belongs to Herat, but it also exists in Kabul.

Melanocorypha bimaculata: This bird is 16 cm long. And it is similar to the Chekauk, but slightly smaller, and in flight, it can be seen under the gray-brown wings, without the white border at the end of the secondary feathers. The tip of the tail is white, and the outer feathers are pea brown. On the ground, it is similar to Chekauk with the difference that the line under the throat is thinner, the underbody is white, the sides are pea brown, the eyebrow line is white, and the legs are fleshy red. Also, the head has dark and brown streaks more distinct than Chekauk's. The sample was captured from Kabul province.

Alauda arvensis: This bird is 18 cm long and is characterized by a gray earthy color, pale brown wing cover feathers, dark pea streaks on the body and a pea white underbody. Also, the chest with dark brown streaks on a pea background, dark with dark lines on a dark pea background, which at the end of the head, feathers are slightly prominent and turned into a round and short crest that is much smaller than the Chekauk crested crest. The relatively long tail, and the whiteness of its side feathers, can be clearly seen. The underside of the tail is black, and the wings are relatively long and elongated. It walks with a hunched posture and its flight is strong and slightly wavy. In flight, it has both in-situ wings and wing wings. The species is captured from Kabul.

Calandrella brachydactyla: This bird is 14 cm long. Its size is small and pale. The trunk is linear, pale brown, under the trunk is a light pea with black spots on both sides of the throat (adult bird). The beak is hard and pale yellow, the cover feathers of the third largest wing, the white eyebrow line is relatively dark and dark with a black and narrow eye line, which covers the edge of the ear feathers. In an adult bird, the head and both sides of the chest are slightly mottled. It flies in waves and at a low altitude. The bird was captured from Paghman, Kabul.

Eremophila alpestris: this bird is 16 cm long, its body is gray and dark brown, and the back of the neck is pinkish brown, and at the beginning of the neck, a narrow black stripe can be seen that leads to two black crown-like feathers on the sides of the head. The chin is white, the forehead and eyebrow line are white, which covers the border under the eyes and the black band on the chest, the underbody is white, and the legs are blue gray. The immature bird looks spotted and dark. The head markings in the adult bird fade slightly in winter. The seeded species is from Kabul province.

Hirundo rustica: this bird is 16 cm long (including the two-branched tail) and due to the blue-black color of the body, the long two-branched tail (in which small white spots can be seen when the tail is wide), the throat and Oak forehead, dark breast band and pea underparts are easily recognized. The young bird lacks tail branches, but the breast and wing stripes are brown, and the forehead is

pale oak. His flight is strong and magnificent. It often comes close to humans and suddenly returns. It often hunts insects above the ground. The sample is made from Sorubi cable.

Phylloscopus inornatus: This bird is 10 cm long and looks like Hume's leaf beetle (*Phylloscopus humei*) (even without distinguishing from each other). The color of the eyebrow is yellow (although it is often seen as grayish yellow), the body is gray green, the underbody is white, the eyebrow stripe is yellowish-white and continuous and sometimes wide (in red pea color), and a faint line is occasionally seen in the tail. and has two yellow-white wing stripes. The base of the beak is paler than Hume's leaf beetle, which has a dark entire beak. The species is captured from DehSabz, Kabul.

Sitta tephronota: This bird is 14 cm long. Feathers and pillows are similar to small loincloths, but significantly larger. It also has a larger beak, a thicker neck, and a very long and clear black eye band that widens towards the back of the head. Its behavior is similar to that of a small chameleon, but it is often seen on trees or bushes. This species was taken from the straw shop in Kabul.

Acridotheres tristis: This bird is 23 cm long and looks like a gray myna (*A. ginginonus*). But the feathers are reddish brown. The beak and the bare spot under the eye are bright yellow, and in flight, a distinct white spot at the base of the feathers (on both sides of the wing) is clearly visible. In the sitting position, white can be seen on the outer part of the underwing coverts and on the single tail feathers, except for the middle two feathers. The trunk is darker than the underbody and the tail is almost black. Usually seen in pairs or in small groups. His behavior is like a starling, but he is tame and fearless. The bird was captured from Kabul province.

Luscinia megarhynchos: This bird is 18 cm long and its head is black, it is smaller than Mina. This bird is 16 cm long and is very similar to the Spotted Nightingale, but its body is lighter, more pink-brown, and its tail is red, and the white ring around its eyes is often seen more clearly. The underbody is clean and has no stains on the chest and sides. The subspecies that is found in Central Asia and migrates to the East of the Middle East has larger cover feathers and the ends of the feathers on the edge of its wings are lighter and its lower body is also lighter. The eyebrow line is pale, and the rostrum is slightly pink, but mostly gray brown. The young bird is spotted, and the wing has pea spots on the chest and sides and pale spots on the middle cover of the wings. It is a species captured from Sarubi, Kabul.

Luscinia svecica: This bird is 14 cm long and looks like a red breast, but it is a little thinner with longer legs and is often very shy. The eyebrow band is wide and clear, and the base of the tail is red in flight. When sitting, it holds the tail slightly high. In the breeding season, in the male bird, the chin and throat are blue, and the black and red oak lines are separated from the lower part of the breast below. In black and red oak, it is separated from the lower part of the chest. In autumn, the throat turns blue-white. Depending on the breed, sometimes the spot is white, red or absent. Immature birds of both sexes are seen with a pale throat. The young bird is spotted and is distinguished from the red-breasted young bird by its darker appearance and streaked streak and red tail. The captured samples are from Sarubi and Sabz, Kabul.

Passer domesticus: This bird is 15 cm long and is well known. The male bird has gray plumage, oak brown sides of the head, pale gray cheeks, black chin, gray tail, oak brown wings and coverts, and a wide white stripe on the wings. The female bird and the young bird do not have contrasting colors on the head and throat and are seen with a pea-brown body and a pale gray underbody and a pale cream eyebrow stripe, and it is difficult to distinguish them from the female, black-breasted sparrow. The bird is social and reproduces in groups. The bird was captured from Kabul province.

Passer hispaniolensis: This bird is 14.5 cm long and looks like a house sparrow (in some areas it is hybridized with it), but the male bird has a reddish-brown tail, a large black chin, and bright black streaks on the chest and sides. The body is streaked with black, which extends all the way around the underbody, and the cheeks and belly are white. The

female bird and the young bird cannot be easily separated from the female house sparrow. The lower body is whiter and gray streaks can be seen on its chest and sides. It is a social bird and is seen in groups mainly during migration and transit. The sample was captured from Kabul province.

Passer montanus: This bird is 14 cm long and is smaller and prettier than the house sparrow, with a dark oak brown and two small black spots on its white cheeks, and the black on its chin is smaller. It also has two white wing stripes, and its tail is grey-brown. The sample was captured from DehSabz, Kabul province.

Motacilla flava: This bird is 16.5 cm long. The color of the head in the male bird is different according to the breed (8 breeds have been seen in the Middle East region). The flava breed is seen with a dark blue-gray color and no obvious color change in the trunk, but in contrast with the dark gray ear feathers, and usually lacks an eyebrow line or white chin and throat. The female bird, in all breeds, is browner with less yellow underbody and reddish. During migration, it is difficult to distinguish races from each other. Race interbreeding among subspecies has created a variety of colors in this bird. The seeded sample is from Sarubi, Kabul.

Anthus campestris: This bird is 16.5 cm long and is distinguished from smaller pipits by its relatively long tail, legs and beak, slightly striped trunk, and almost unspotted chest, with a simple sandy color. The young bird, in the first autumn, has a striped chest and no side stripes. Its wavy flight pattern is reminiscent of the yellow-bellied tern's tail. It walks quickly on the ground and makes sudden stops, so that, in that case, it keeps the body slightly upright. The sample captured is from GolDareh, Kabul.

Anthus pratensis: This bird is 14.5 cm long. Its size is more delicate than the tree pipet and its tail is slightly longer. Its voice is the best feature of this tree pipit bird. In the first autumn, it is distinguished from the Red-throated Pipit by its unstriped bill (the red-throated Pipit has many stripes), the mantle with fewer stripes, the rump with a less pronounced appearance, and the eyebrow line that is not well defined. Chest with black streaks, pea-white underbody, white undertail and tail feathers, fleshy brown legs, and relatively long rear toenails. Lesser than a pipit, it sits on a tree and its flight is wavy and irregular, and on the ground, it nervously wags its tail. The sample was captured from DehSabz, Kabul.

Serinus pusillus: This bird is 12 cm long. It is small and pure, with black streaks, a smoky brown head and chest, and a bright orange spot on the forehead. In flight, the body is dark, but the underbody and underwings appear pale. The young bird has a clean orange face and black and white striped body and underparts. Outside of the breeding season, it is seen in small groups on the ground or in the grass of seeded weeds and is very lively. The sample was captured from Kabul shop.

Carduelis carduelis: Appearance characteristics: This bird is 14 cm long and is seen with beautiful coloring and is recognized by the bright red and black and white color of the head, the yellow streak in the black wings and the white tail, which can be seen well in flight. The young bird (all breeds) has a pale gray proball and an unmarked head, and is identified only by its wing band, white bill, and voice. This bird is seen in small groups outside the breeding season. The sample was captured from Kabul city.

Carpodacus erythrinus: Appearance differences: This bird is 14 cm long and is the same size as the red-breasted plover. The beak is relatively strong, the body is big, and the head is round. The immature bird is recognized by its red head, breast, and tail, which contrasts with the brown rump. The female bird and the young male bird, in the first summer, are paler and olive-brown in color. The upper body is slightly streaked, the lower body is much more streaked, dark in color with two white or pea stripes. The head is uniformly dark, and the eyes are also dark, and it is often singing at a certain point, its tail is heart-shaped at the end and its flight is wave-shaped. The sample was captured from Kabul city.

Emberiza leucocephalos: This bird is 16.5 cm long and looks like a yellow lemon feather. In the male bird, a white spot with a black border can be seen on the bill and chin, but the head and throat are oak. Its color becomes lighter in winter. The female bird is gray-brown in color, dark, chest, flanks and front line, with a fawn tail similar to lemon feather. There

is a little white in the tail and a little oak color in the white of the throat, which is more in the female bird. Instead of yellow, the young bird has a white color on the belly and streaks on the primary wing feathers. The beak has two colors: the upper half of the tip is dark gray, and the lower half of the tip is pale gray. In the male bird of some intermediate breeds, the head, rump, and wing feathers are scattered with reddish-yellow, while, in others, the head is white. The species is captured from Kabul city.

Emberiza cia: This bird is 16 cm long. In the male bird, the head is a mixture of black streaks on a smoky gray background with a streakless gray throat. The front is distinctly streaked with gray. The gray color of the bill and the white edges of the tail distinguish this bird from the African mountain tern and the house tern. The female bird is paler, with mixed colors and feathers. But basically, it looks like a male bird. The young bird is similar to the lemon-yellow young bird (both have brown oak bill). But the lemon yellowfin at any age has a large orange belly. The sample was captured from Kabul city.

Emberiza stewarti: This bird is 16.5 cm long. The color of the head in the male bird varies according to the breed (8 breeds have been seen in the Middle East region). The flava breed is seen with a dark blue-gray color and no obvious color change in the trunk, but in contrast with the dark gray ear feathers, and usually lacks an eyebrow line or white chin and throat. The female bird, in all breeds, is browner with less yellow underbody and reddish. During migration, it is difficult to distinguish races from each other. Race interbreeding among subspecies has created a variety of colors in this bird. The species is captured from Shakar Dara in Kabul.

Emberiza melanocephala: 16 cm; in the male bird, the ventral surface is uniform yellow, the head is black with a yellow collar, and the back is oak. There is no white color on its tail, its head is brown in autumn. The back surface of the female bird is olive brown with streaks. Among other yellow-breasted, yellow-breasted yellowtails, it is characterized by a ventral surface without streaks, and the cover of the feathers under its tail is yellow (refer to the red-headed yellowtail).

Emberiza bruniceps: 16 cm; the male bird is easily recognized by its oak head and chest and bright yellow belly and tail. The female bird is very similar to the black-headed yellowtail female, but instead of the oak color, its back has a trace of green color, and the undertail feathers are white (not yellow). An immature bird is indistinguishable from an immature black-headed butterfly. It is a species captured from the sugar of the Kabul valley.

Emberiza hortulana: 16 cm; It is distinguished by its pinkish pea-like ventral surface and yellow throat from the yolk of other moths. It has a pale olive-green head and chest, pale yellow throat, and olive stripe. Close up, the thin and yellow eye ring and pink beak can be seen, the dorsal surface is brown with black streaks. The female bird is lighter in color, its head is not very green, and dark and fine streaks can be seen on its chest. The immature bird is browner and streaked on the ventral surface, but its yellow eye ring and pink beak distinguish it. The sample was captured from Kabul city.

Emberiza buchanani: This bird is 16 cm long and looks like a yellow-winged yellow-winged bird with a pink beak and a white eye ring. The head of the adult bird is smoky grey but lacks the gray chest band. The fawn's underparts are brown with a white throat and half-whisker-like stripe, the underbelly and undertail feathers are pea yellow or white. In autumn, the underbody of fawn is pea-colored with gray-brown streaks. The grayish-brown trunk contrasts with the oak color of the Bali strip. Veins in the upper body are either indistinct or absent. The bill is grey-brown, and the flight feathers are more gray-brown than black-brown. The edge of the wings is khaki. The young bird is seen with grayish brown tail and lower body and cover feathers under the tail. The sample was captured from Kabul.

Morphological results of captured species in Kabul province

From all the samples captured, a total of 6 morphological traits were measured with the help of a caliper and a ruler with an accuracy of 1%, 1%, respectively, length, beak length, beak height, beak width, tarsus length, wing length, and tail length. It is given in the table below (Table 2).

Table 2. Measurements taken on species captured from Kabul province and their number

No	Scientific name	Family	Number	en	tb	td	tt	hn	tn	tq
1	*Lanius vittatus*	Laniidae	1	5	90	115	33	9	14	215
2	*Lanius schach*	Laniidae	1	4	90	110	33	9	14	210
3	*Oriolus oriolus*	Oriolidae	1	4						
4	*Corvus monedula*	Corvidae	1	6						
5	*Alauda arvensis*	Alaudidae	1	6	100	75	27	7	17	180
6	*Eremophila alpestris*	Alaudidae	1	6	111	80	25	6	13	185
7	*Alauda gulgula*	Alaudidae	1	5	95	55	18	7	11	153
8	*Calandrella brachydactyla*	Alaudidae	3	6	120	70	30	7	18	175
9	*Hirundo rustica*	Phylloscopidae	1	5	116	115	13	2	6	170
10	*Phylloscopus inornatus*	Sylviidae	2	3	55	35	22	3	10	95
11	*Sylvia curruca*	Sittidae	12	3	65	55	23	3	8	130
12	*Sitta tephronota*	Sturnidae	1	6	82	50	30	5	30	155
13	*Acridotheres tristis*	Sturnidae	1	7	130	85	40	4	20	210
14	*Temeluchus pagodarum*	Muscicapidae	1	6	100	80	36	4	18	180
15	*Luscinia megarhynchos*	Muscicapidae	2	4	70	60	31	4	14	140
16	*Luscinia svecica*	Muscicapidae	2	4	75	60	30	4	14	140
17	*Muscicapa striata*	Phylloscopidae	1	4	67	50	22	4	12	120
18	*Passer domesticus*	Passeridae	12	5	77	60	22	7	10	145
19	*Passer montanus*	Passeridae	8	4	70	50	23	6	10	135
20	*Passer hispaniolensis*	Passeridae	3	5	75	60	22	7	10	145
21	*Motacilla flava*	Motacillidae	2	3	57	65	25	3	10	140
22	*Anthus hodgsoni*	Motacillidae	2	4	95	60	23	6	10	140
23	*Anthus pratensis*	Motacillidae	2	4	95	60	23	6	10	140
24	*Calandrella brachydactyla*	Alaudidae	1	5	90	55	21	5	9	135
25	*Calandrella*	Alaudidae	1	5	90	55	21	5	9	135
26	*Carduelis carduelis*	Fringillidae	2	8	80	55	22	7	12	135
27	*Pyrrhula pyrrhula*	Fringillidae	3	9	80	60	22	9	11	140
28	*Serinus pusillus*	Fringillidae	1	4	75	55	22	7	10	135
29	*Emberiza bruniceps*	Emberizidae	12	7	80	65	22	7	12	150
30	*Emberiza hortulana*	Emberizidae	2	5	80	75	21	5	9	140
31	*Emberiza buchanani*	Emberizidae	8	5	85	75	21	5	9	145
32	*Emberiza leucocephalos*	Emberizidae	1	5	85	80	22	6	10	160
33	*Emberiza cia*	Emberizidae	1	5	80	80	22	6	9	150
34	*Emberiza melanocephala*	Emberizidae	5	7	83	72	24	7	14	165
35	*Emberiza stewarti*	Emberizidae	9	4	85	70	18	4	8	145

(en: beak width, tb: wing length, td: tail length, tt: tarsus length, hn: beak height, tn: beak length and tq: height)

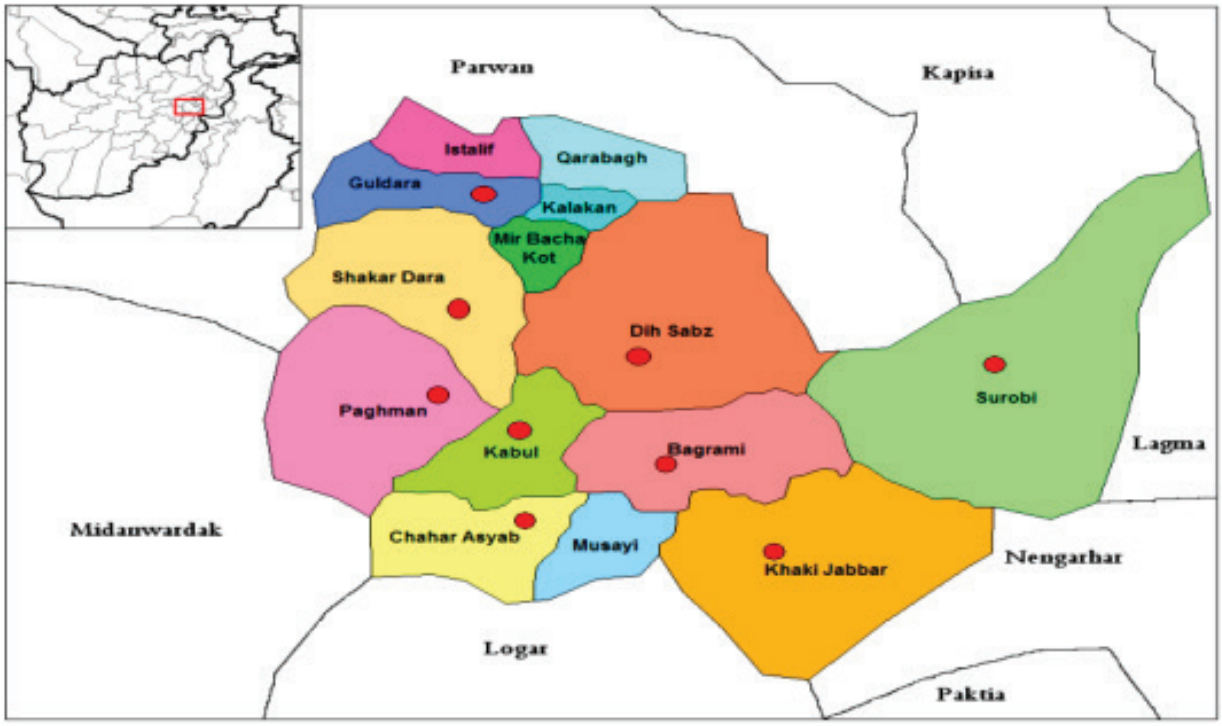

Fig. 2. The abundance and diversity of species captured in Kabul province

Morphological identification of Passeriformes species in Kabul province

Of the 540 species of birds that are mapped in "Birdlife" for Afghanistan, 280 species belong to 221 genera and 36 passeriform families of Afghanistan, including 150 species with 33 families belonging to the passeriform order of Kabul province. 110 sparrow hawks were captured during three stages in 2019 and 2020 in 10 districts and in 22 stages of field operations in Kabul province. They were selected for morphological and molecular study based on morphological traits and using valid identification keys from existing samples. These samples belong to the Passeriform order with 13 families, 23 genera and 35 species. During sampling, 30 species of them were morphometrically, the table of measured traits is given in (Table 2). Among the captured samples, the family of the yellow dragons had the most diversity with 7 species, and the family of the sparrows had the most abundance with 45 individuals, and other families showed less diversity and abundance than these two families (Fig. 2).

Taxonomic status of Passeriform order

Passeriforms include a tectonic group of bird species, which seems to be a successful group and rapidly diverged at the end of the Tertiary period. According to traditional classifications, based on the morphology of the voice box, they are divided into two clades: Suboscine (Tyranni) and Oscines (Passeri), with recent molecular studies, Acanthisittidae was introduced as the third clade and sister group to the previous two clades (Raikow, 1982) and its molecular features (Johansson et al., 2001) have been confirmed.

The Oscines group is the largest group with more than 4500 species and has a global distribution, and probably their basal lineages originated from the Australian region (Johansson, 2008). The division of Oscines into two sister taxa of Corvida and Passerida was done by Sibley and Ahlquist in 1990, in the following years this hypothesis was rejected and Corvida was introduced as a paraphyletic group (Barker et al., 2002; Ericson et al., 2002). Sibley and Ahlquist (1990) defined clades for Passerida that include Passerioidea and Sylvioidea and designated

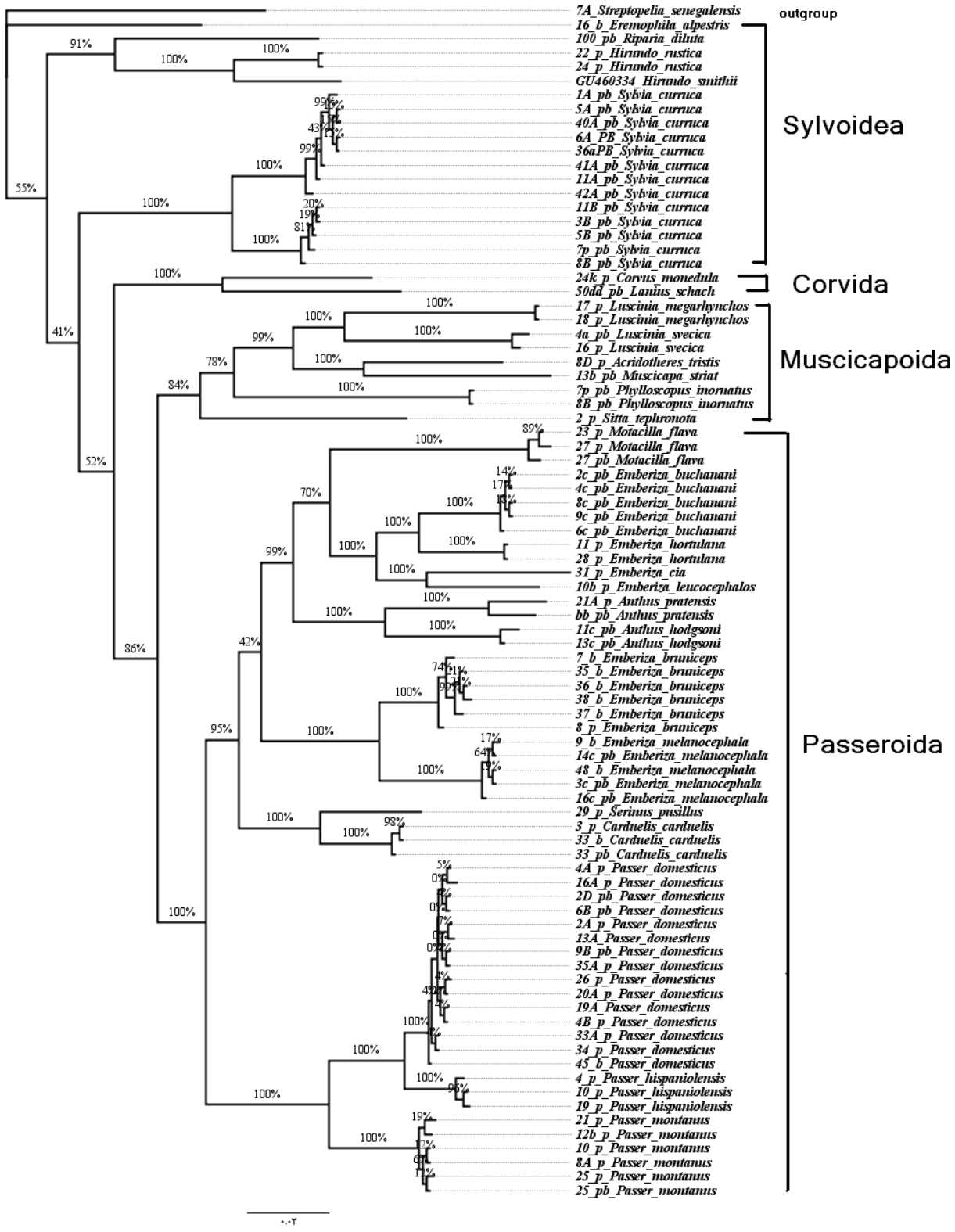

Fig. 3. Bayesian tree of captured passeriform species in Kabul province

Muscicapoidea as the basal group for the other two clades. In addition, conflicting phylogenetic hypotheses are seen for the phylogenetic relationships of lower groups, especially within the Passerida clade and its three superfamilies, including Muscicapoidea, Sylvioidea, and Passeridea (Ericson and Johansson, 2003). Although the hypothesis of Passerida being monotypic has been confirmed by many studies, all species and clades of Passerida have not been covered in the research (Sibley and Monroe, 1990).

For example, the sequences of RAGI and RAGII markers have shown that *Paramythia montium,* Toxorhamphus, Oedistoma and Melanocharis, which were classified in Sibley and Monroe (1990) in the Passerida group, belong to the Corvida core (Barker, 2004). Also, molecular studies have shown that taxa that are not included in the Passerida group may be part of this branch. For example, Culicicapa, Chloropsis, Irena and some other species that were traditionally in the flycatcher group have been placed in the Passerida group (Barker, 2004). The species Pseudopodoces humilis, which was previously classified in the Corvini (crows), has been placed in the Passerida family by recent studies. So far, many molecular studies have been carried out on the American sparrow, but the largest number of taxa studied from the American sparrow was in the research considering the number of 173 taxa of the American sparrow (Beresford et al., 2005). Usually, most of the studies have been based on one or more nuclear genes as phylogenetic markers, although the necessity of using several molecular markers has been proven, but so far, a few studies have used the combination of more than two molecular markers (Moore et al., 1999).

Bayesian tree analysis of the mitochondrial COX1 gene

Bayesian tree was drawn using the amplified sequences of COX1 gene from the captured species of Kabul province and gene bank. The placement of genera and species within families and superfamilies is similar to the results of DNA hybridization studies in Sibley and Ahlquist (1990) for sparrows. Passeriformes are a very large monophyletic group whose relationships, at least at higher taxonomic levels, are well known by examining DNA sequences. However, new studies show that many passeriform families in traditional classification are not monophyletic. A more complete understanding of Passeriform phylogeny is possible when more extensive gene sequences are available. Based on DNA hybridization (Sibley and Ahlquist, 1990) and nuclear gene sequences (Barker et al., 2002), three major clades of sparrows have been shown, which conventionally include Sylvioidea, Muscicapoidea, and Passeroidea, which in the final bayesian tree of this research is also shown. The genus Passer with four species, the genus Motacilla with one species, the genus Carduelis with one species, the genus Emberiza with six species and the genus Serinus with one species are placed in the superfamily Passerioiodea. Corvus genus with one species and Lanius genus with one species are included in Corvida clade. Two species of the genus Luscinia with one species of the genus Muscicapa and one species of the genus Acridotheres are placed in the superfamily Muscicapoidea. Two species of the genus Hirundo, one species of the genus Phylloscopus, one species of the genus Ripria, one species of the genus Sitta, one species of the genus Sylvia and one species of the genus Eremophila are included in the superfamily Sylvioidea (Fig. 3).

RECOMMENDATIONS

In the light of results of this study it is recommended to:

- Identify the birds of Afghanistan based on morphological and molecular methods.
- Register reports of the presence and absence of species and extensively investigate the status of reproduction, residence, wintering, or migration of birds.

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Sero-prevalence of hepatitis-E virus in rhesus macaques (*Macaca mulatta*) in and around Kolkata, W.B., India

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ABSTRACT

Hepatitis E virus (HEV) is endemic in many developing countries and becoming a major public health concern that is transmitted through the faeco-oral route. HEV can cross species barriers and infect rhesus macaques and chimpanzees, the most relevant surrogates for human infections. The objective of the study is to assess the prevalence of anti-HEV IgG in rhesus monkey both in free range and in captivity by indirect ELISA and confirmation by western blot. In the present study, serum samples were collected from 100 different individuals of rhesus monkeys both from free range and captive population. The sero-prevalence of hepatitis E virus in rhesus monkeys in and around Kolkata was found to be 47% in captive individuals whereas 23% in free range species. Liver function test of the anti-HEV IgG positive rhesus monkey samples show normal liver condition. Increasing trend of hepatitis E sero-positivity with the increase in age in both captive and free range signifies improper hygiene and sanitation.

Key words: Hepatitis E virus, Kolkata, rhesus monkey, sero-prevalence

INTRODUCTION

Viral hepatitis is caused by infection with one of the five known hepatotropic viruses, which are named hepatitis A virus (HAV), hepatitis B virus, hepatitis C virus, hepatitis D virus, and hepatitis E virus (HEV). The most common clinical consequence of infection with HAV or HEV, is an illness characterized by sudden onset of fever and systemic symptoms, which is followed a few days later by jaundice (Anonymous, 2016). HEV is an enterically transmitted virus that occurs primarily in Asia, Africa, and Central America, where it is the most common cause of acute hepatitis. Hepatitis E infection caused by HEV is an acute and self-limiting hepatitis of a wide range of susceptible domesticated and wild animals like bovines, caprines, swine, rodents, non-human primates, and chickens (Biswas et al., 2020). HEV infection occurs through the faeco-oral route. The virus enters the blood through the gastrointestinal tract, the primary site of viral replication, and reaches the liver where it replicates in the cytoplasm of hepatocytes (Krawczynski and Bradley, 1989).

In Indian subcontinent, HEV is found to be endemic in nature and the prevalence of IgG antibodies to HEV has been studied among pigs (54.6-74.4%), dogs (22.7%), rodents (2.1-21.5%) and cattle (4.4-6.9%) (Arankalle et al., 2001). However, anti-HEV IgG was also detected in Japanese monkeys, cynomolgus monkeys, rhesus monkeys and Taiwan monkeys (Hirano et al., 2003). HEV-specific antibodies and/or the genome of HEV or HEV-related viruses have also been detected in many other animal species, including primates, other mammals, and birds. Genotypes 3 and 4 infections are documented in many domestic, wildlife and zoo animal species (Spahr et al., 2017). Hepatitis E virus antibodies or genes have been

reported to exist in many species of mammals, including monkeys (Huang et al., 2011). HEV-like sequences were detected in seven (29.2%) of 24 Chimpanzees at the studied zoo, suggesting that if the sequences are from real viral particles, the virus may be a new type of HEV using non-human primate as its natural host (Zhou et al., 2014). The prevalence of IgG antibodies to HEV was observed among different Indian non-human primates like wild rhesus monkey 36.7%, bonnet monkey (19.1%) and langur (2%) (Arankalle et al., 1994a). The study was mostly restricted to southwestern part of India. Moreover, the data was fragmentary and did not reflect the distribution of HEV infection of the country.

Though the state of West Bengal is endemic to HEV (Bansal et al., 1998), very few studies have been done so far on the sero-prevalence of HEV in the rhesus monkey population that is the most abundant species in nature and in captivity in Eastern India. The objective of the present study is to assess the prevalence of anti HEV antibody (IgG) in rhesus monkey both in free range and in captivity by an indirect ELISA and confirmation by Western blot.

MATERIALS AND METHODS

The study was conducted in different places in Kolkata, West Bengal and the serum samples of rhesus monkey were collected from species of Animal Rescue Centre (ARC), Forest Department, Govt. of West Bengal, Marble Palace Zoo, Kolkata, and species from different areas of Kolkata under the custody of Madaris. The serum samples for prevalence study of anti-HEV IgG were stored at -20°C prior to analysis. For indirect ELISA, the purified recombinant open reading frame (ORF-2) protein of HEV in aliquots was obtained from National Institute of Cholera and Enteric Diseases, Kolkata, and Rabbit anti-HEV IgG (whole molecule) peroxidase conjugate was obtained from Sigma, USA. SDS-7 marker was used for Western blot analysis. To detect anti-HEV IgG in the serum samples of rhesus macaques, an indirect ELISA was performed using a purified recombinant ORF2 protein expressed in a baculovirus vector according to the method described by Li et al. (2000).

Screening of serum samples

Ninety-six wells flat bottomed micro-titer ELISA plates were coated with 50 µl of diluted ORD2 antigen @1 µl ml^{-1} in PBS-0.1 M. The plates were incubated at 4°C overnight and then washed three times at an interval of five minutes by washing buffer. The antigen sensitized plates were blocked by blocking buffer. The blocking was initiated by incubating at 37°C for 1 hour. The serum samples were diluted at 1:200 in 3% LAH diluents were dispensed in duplicate wells @ 50 µl per well. Then the plates were incubated at 37°C for 1 hour. Ag-Ab reaction was detected by addition of rabbit anti-monkey IgG HRPO conjugate (whole molecule) diluted at 1:3000 in EIA diluent keeping the volume per well was 50 µl and the EIA plates were incubated at 37°C for 1 hour. Then 50 µl of OPD substrate solution was added to all wells and were incubated at 37°C for 10 minutes. To stop the reaction 50 µl of 1 M H_2SO_4 solution was added to each well. The plates were read in an ELISA reader at 492 nm. (Multiskan, Labsyste, Model- 355) to give the O.D. values and the results were calculated. Control wells in duplicate for positive sera, negative sera and conjugate were kept separately in each plate. In each plate, blank wells were kept for estimating the test reagents every time. The samples were analyzed by applying standard protocol of electrophoresis and interpreted by different statistical analysis methods to derive the P value of the samples.

Estimation of anti-HEV IgG reactivity by Western blot analysis

The sera samples were also screened by Western blotting employing the recombinant antigen produced from putative capsid protein ORF2 region (Lee et al., 1994). For immuno-electrophoresis, the protocol described by Laemmli (1970) was followed with vertical mini slab gel electrophoresis system. 12.5% of separating gel solution was poured into the gel casting space between the glass plates until about 70% of the space was filled and layered with n-butanol. After polymerization of the separating gel, n-butanol was drained off by filtering the gel cast. 5% gel solution

was layered over the separating gel-well forming comb into the top of the gel casting area and inserted until both end of the comb stopped at tops of the slide spacer and over layering of n-butanol. The comb was removed after polymerization. The antigen sample was prepared by diluting the ORF2 antigen @ 1 µl/ml in 2X sample buffer. The sample containing equal amount of protein (40 µg μl^{-1}) was applied to each slot and electrophoresis was performed at a constant voltage of 50V at room temperature till the tracking dye reached the lower end of the gel. The data were recorded, and analysis was made by applying statistical methods to derive Fischer's Test value.

Estimation of molecular weight by SDS PAGE

A gel with the protein band was cut and stained with Coomassie blue staining solution followed by destaining with the destaining solution after 6 hours. Molecular weight was determined using standard protein markers. The mobilities of all the proteins were recorded. The Rm values were obtained from electrophoretic mobilities by calculating the ratio of mobility of protein to the mobility of the tracking dye.

Western blot

Transfer of protein from gel to nitrocellulose paper electroblotting

The proteins separated by SDS-PAGE (Laemmli, 1970) were transferred to the NCP according to the method of Svoboda et al. (1985). Rest of the procedure was followed according to Towbin et al. (1979). Electroblotting was performed in Transblot apparatus. The portion of the gel containing protein lanes to be blotted was cut and incubated in western blot transfer buffer for 10 minutes. An NCP membrane (Immobilon-NC) of required size along with 12 pieces of Whatman No. 3 MM chromatography paper slightly larger than the gel were cut and we re equilibrated with transfer buffer. The cathode was located at the highest position and the cover was opened. Transfer buffer was poured on the anode, enough to prevent anode from drying. Six absorbance papers (Whatman No. 3 MM chromatography papers) were layered, one by one, on the anode. The nitrocellulose membrane (NCP) was over layered, and a small amount of buffer was poured on the membrane. The gel was then over layered, and a small amount of buffer was poured on the gel. Care was taken not to trap air bubbles in the gel and the NCP. The cathode was lowered gently to press down the layered material and the cathode was locked. The cathodic and anodic wires were connected to the apparatus and to power supply (cathode to gel, anode to membrane). The transfer of protein was performed at 50 mA. per square centimeters at 4°C for 14 hours.

Post transfer processing

After turning off the power supply the NCP was kept in blocking solution at room temperature for 1 hour. The NCP was then washed (4 × 5 min) with washing solution (PBS-Tween20). The NCP membranes, duplicate in numbers for each sera sample, were probed with selected sera (positive and negative) @ 1:200 in PBS-Tween 20 con taining 1% BSA (diluent). The NCP was incubated at 37°C for 1 hour. Washing of NCP was carried out with washing solution (PBS-Tween20). HRPO conjugated rabbit anti monkey IgG was used @ 1:1000 dilution in diluents to counter stain the corresponding membranes. The NCP membranes were incubated at 37°C for 1 hour. Then, washing of NCP was done as above. Subsequently, the enzyme complexes were detected by addition of DAB (SIGMA, USA) substrate. Reactive protein bands appearing after few minutes were observed. Finally, the enzyme-substrate reaction was stopped by addition of distilled water. Then, the NCP was dried up and preserved.

Estimation of serum biochemical constituents

Estimation of serum total protein, albumin, globulin, and A:G

By using photoelectric colorimeter applying the green filter Serum total protein and Albumin were determined by Biuret method described by Reinhold (1953). Then, Globulin fraction was determined by subtracting Albumin from Total protein. All the values were expressed in g dl^{-1}. The Albumin and Globulin (A:G) ratio was finally calculated.

Estimation of serum alanine aminotransferase and serum aspartate aminotransferase

By applying the method described by Bergmeyer and Bernt (1974) serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were estimated using 2,4 dinitrophenyl hydrazine. By using dilution of pyruvate solution, a standard curve was prepared with the help of the O.D. taken in a photoelectric colorimeter (Systronics) using green filter (520 nm). The enzyme activity was expressed in terms of µg pyruvic acid liberated per mg of protein in serum samples incubated for 30 minutes at 37°C temperature in case of ALT and for one hour at 37°C temperature in case of AST.

Estimation of total bilirubin, conjugated bilirubin, and unconjugated bilirubin

Conjugated (direct) bilirubin, Unconjugated bilirubin, and Total bilirubin were estimated colorimetrically by Jendrassik and Grof's method (Jendrassik and Grof, 1938) and was expressed in mg dl^{-1}.

RESULTS AND DISCUSSION

A total of 100 serum samples of 1 to 9 years old rhesus macaques were collected of which 46 were males and 54 were females. The macaques were restrained from different localities of Kolkata out of which 70 individuals from free ranging areas of Animal Rescue Centre (ARC), Kolkata, 20 individuals from Marble Palace Zoo, Kolkata and 10 individuals from Madaris. All the macaques were found in captivity in different habitats.

The ORF2 ELISA has been performed to optimize baseline reactivity for rhesus monkey sera with the foremost aim of determining 'negative' population. Rhesus monkey sera demonstrated wide variation in level of reactivity, whereas some of them demonstrated low and relatively uniform reactivity. As shown in Fig. 1a, 1b and 1c, the rhesus monkey sera demonstrated widely varying levels of reactivity either in free range or captive sera samples. Analysis of reactivity for low reactive samples showed an approximately normal distribution, as observed with bovine sera samples (FCS) whereas other sera had significant numbers of 'outlying ' considered as highly reactive samples. Therefore, low reactive samples were considered to represent an 'HEV negative' rhesus monkey population in all the cases and the mean + 3 S.D.' of those negative 'cut- off" value for subsequent studies as described by Hirano et al. (2003). In practice, the optical density (O.D.) for a single reference serum on each plate (serving as negative control) was multiplied by a fraction of 1.5, calculated to be equivalent to the 'cut off". The serum samples showing an absorbance value greater than the 'cut off" values was determined to be positive (Choi et al., 2003).

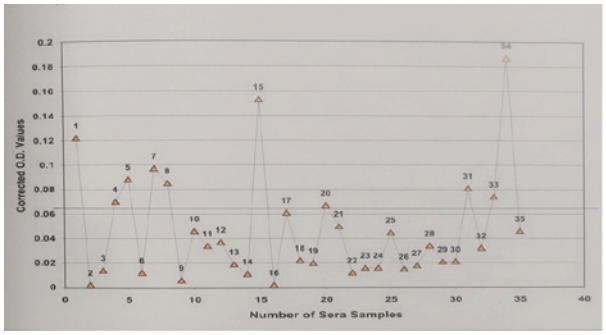

Fig. 1a. Sero-reactivity of Monkey Sera against HEV ORF-2 Antigen

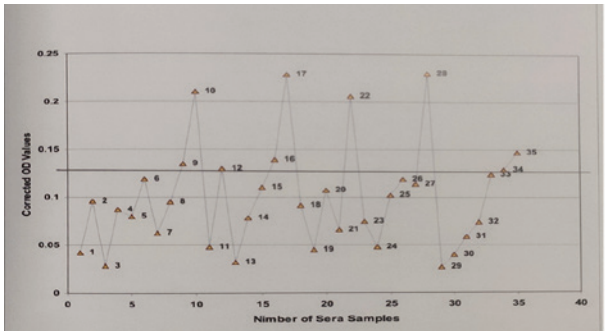

Fig. 1b. Sero-reactivity of Monkey Sera against HEV ORF-2 Antigen

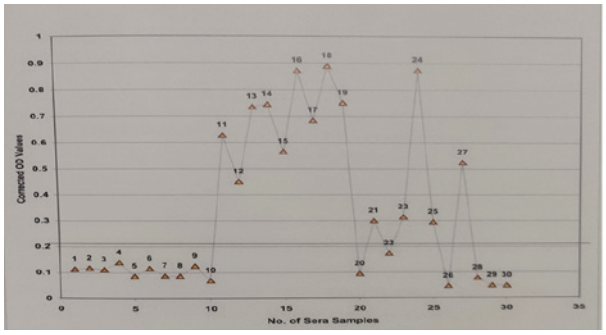

Fig. 1c. Sero-reactivity of Monkey Sera against HEV ORF-2 Antigen

Based on the above calculation, the 'cut off' values for ELISA plates (Fig. 2a and 2b) were found to be 0.065, O.131 and 0.201 respectively. The O.D. values of sample numbers 1-70 (free range) vary from 0.002 to 0.230 and that of sample nos. 71-100 (captive) varies from 0.069 to 0.888. Thus, in al1, 16 nos. of sera from 70 free ranging rhesus monkeys (23%) and 14 nos. of sera from 30 captive rhesus monkeys (47%) were found to be positive.

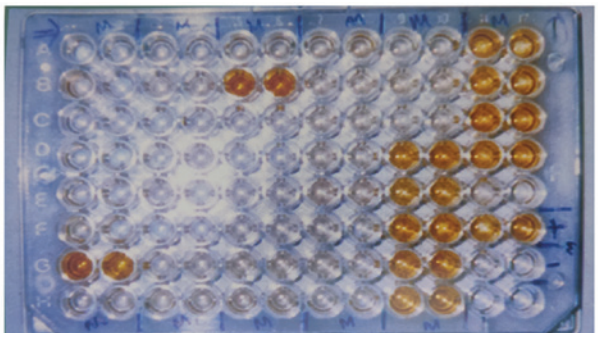

Fig. 2a. ELISA Plate showing positive and negative sera samples

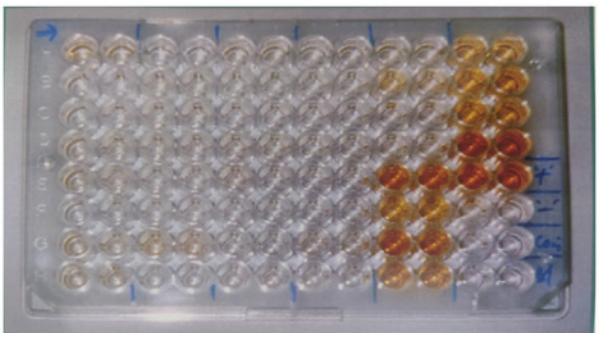

Fig. 2b. ELISA Plate showing positive and negative sera samples

The seropositive rate of anti-HEV IgG increase with age: 33% in 0-3 year old monkeys, 43% in 3-6 year old monkeys and 54% in 6-9 year old monkeys in captive rhesus monkeys whereas in the free range 18% in 0-3 year old monkeys, 23% in 3-6 year old monkeys and 37% in 6-9 year old monkeys (Table 1). No significant differences were observed between males and females.

Table 1. Prevalence of anti-HEV IgG in rhesus monkey of both free ranging and captive

Age	Free ranging (Percentage of positive)	Captive (Percentage of positive)	P Values
0 - 3	5/27 (18%)	1/3 (33%)	P = 0.50 (Fisher's Exact Test)
3 - 6	8/35 (35%)	6/14 (43%)	P = 0.16 (X2 = 1.96)
6 - 9	3/8 (37%)	7/13 (54%)	P = 0.39 (Fisher's Exact Test)
	16/70 (23%)	14/30 (47%)	P < 0.02 X2 = 5.57

This study demonstrates the presence of an antibody reactive to a recombinant HEV protein in rhesus monkeys in and around Kolkata. Although antibody prevalence varied across species and habitats, rhesus monkeys with anti-HEV antibody were found in virtually all the locations sampled. This agrees with the findings of Hirano et al. (2003) in Japanese macaques captured in various geographic regions of Japan. Rhesus monkeys are susceptible to infection with HEV like cynomolgus macaques found experimentally by Erker et al. (1999) and in wild by Hirano et al. (2003). Persistence of IgG anti-HEV antibodies for a long time and protection offered by low titered antibody against re-infection (Arankalle et al., 1999) is also proved by the fact that seroprevalence of hepatitis E virus in the zoo and with the *Madaris* is more than free range (Table 1). The seropositivity of free-range rhesus monkeys (23%) is more than the seroprevalence rate of 3.6% in free range rhesus macaques in Japan (Hirano et al., 2003), where HEV is not endemic and less than that of 36.7% in wild rhesus monkeys in south India (Arankalle et al., 1994b) where HEV is endemic. But again, the seroprevalence of captive rhesus monkeys kept in the zoo and with the *Madaris* is much higher (47%).

Sixteen (16) out of 70 free range rhesus monkeys (23%) and 14 out of 30 captive rhesus monkeys (47%) were positive for anti-HEV IgG tested by ELISA. These results indicated that HEV is circulating among monkeys belonging to the genus *Macaca*. The difference between the free range and captive monkeys is statistically significant

($P < 0.02$; $x^2 = 5.57$). Hirano et al. (2003) concluded that there is an increasing trend in seropositive rate among the Japanese macaques where the prevalence of anti-HEV IgG is higher in sexually matured adults over 5 years of age. The same finding had been found both in free range and in captivity along with the increase in age. This study demonstrates that the prevalence of anti-HEV IgG is higher in sexually matured adults over 6 years of age than in sexually mature individuals of less than 3 years of age.

Analysis of western blot of the serum samples

Assessment of the molecular weight of the HEV ORF2 antigen by SDS page is shown in Fig. 3a and 3b. All the positive representative rhesus monkey samples, exhibiting anti-HEV IgG reactivity in ELISA showed predominant and specific reaction to recombinant antigen prepared from capsid protein ORF2 in western blot. These reactivities of the selected positive serum samples were compared with sera from negative population also, where no band for anti-HEV reactivity appeared against the ORF2 antigen. The result for the reaction of positive and negative sera samples in Western immunoblot is shown Fig. 3A and 3B. These results co-related with the findings of Chandler et al. (1999) and Tam et al. (1991).

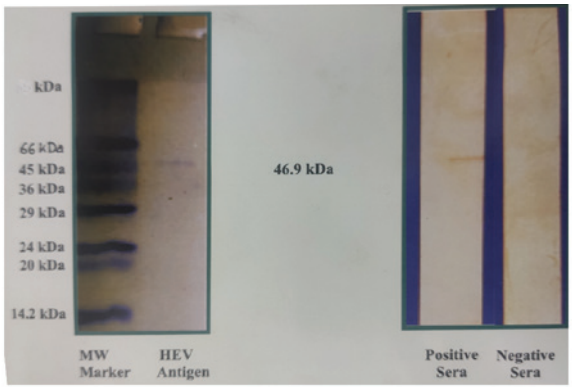

Fig. 3a. SDS-PAGE (12.5% Gel) showing peptide profile of ORF-2 Antigen of Hepatitis-E virus and Western Immunoblot of Positive and negative anti-sera.

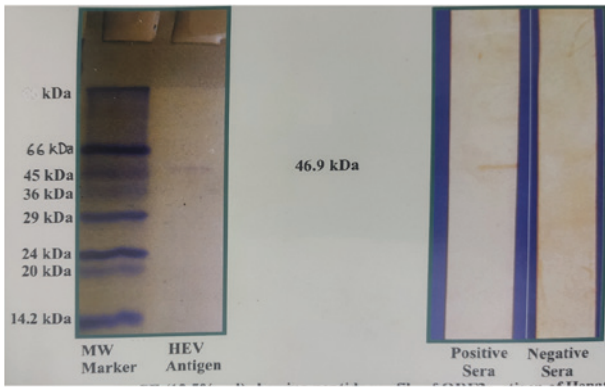

Fig. 3b. SDS-PAGE (12.5% Gel) showing peptide profile of ORF-2 Antigen of Hepatitis-E virus and Western Immunoblot of Positive and negative anti-sera.

SDS -PAGE of ORF2 antigen showed polypeptide profile of MW of 46.9 kDa against the marker antigen showing polypeptide profile of MW from 66 to 14.2 kDa. The representative positive sera sample showed one band of 46.9 kDa when the ORF2 antigen was resolved in 12.5% SDS PAGE and it was found to be immunoreactive when analyzed by Western blot.

The issue of persistence of anti-HEV antibodies is of great concern in countries like India where hepatitis E is highly endemic. HEV antibodies elicited by either natural or experimental infection do not distinguish between different strains of the virus and therefore a single serotype of HEV appears to be in circulation (Arankalle, 1994b; Bradley, 1988).

Results of level of serum protein and serum bilirubin

Depending on the type of test system used for detection of IgG anti-HEV antibodies, disappearance of antibodies was noted up to 6-12 months (Goldsmith et al., 1992), 1-4 years (Favorov et al., 1992) and up to l4 years (Khuroo et al., 1993). Moreover, experimental infection of rhesus with HEV and based on recombinant baculovirus derived frown ORF2 protein-based ELISA, a long-lasting IgG anti-HEV antibodies has been observed for 7 years by Arankelle et al. (1999). In this study there is no rise in ALT levels of all the sero-positive HEV rhesus monkeys. As such, it can be it can be inferred that the captive sero-positive population were already infected previously. The mean (±S.E.) of serum globulin of the rhesus monkeys in the study is found to be higher than the normal values

as reported by Wallach and Boever (1983). Kaneko et al. (1997) reported that with increasing age, the plasma proteins are seen to increase above the normal adult levels because of a small decrease in albumin and a progressive increase in the globulins. The level of serum bilirubin in the rhesus monkeys both in free range and in captivity is found to be within the normal limits which indicate the normal liver function.

CONCLUSION

Association of primates with human being dates to the evolution of man on earth. Among the primate population, rhesus monkey is the most abundant species in India. This study documents that HEV is infecting rhesus monkeys in natural environment both in free range and in captivity. It is noteworthy and alarming that a high prevalence of anti-HEV IgG is observed in rhesus monkeys in captivity (47%) compared to that in free range (23%). This may be because the monkeys in captivity are getting infection leading to a higher percentage of HEV infected population either from the persons handling the animals or due to the captive environment where there is a source of HEV infection. In captive environments, especially in the zoos, the animal houses are cleaned manually by the keepers which lead to animal human interaction. Moreover, lack of proper sanitation is a vital cause for spread of hepatitis E infection. In Kolkata, there is a good population of monkey keepers showing road shows with rhesus monkeys. These so called 'madaris' keep the monkeys confined in their houses and share the same food and drinking water. Proper hygiene is not at all maintained in these cases. These are the probable causes of HEV infection and subsequent development of anti-HEV IgG in the monkeys as found in the present study.

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Effect of normal saline diluted Wova FH, on spawning performance and larval rearing of Indian major carp *(Labeo rohita)*

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ABSTRACT

The age range of brood fishes of 2 to 3 years, 1000 to 2000 g weight, and farm-raised *Labeo rohita* brooders were chosen, and stocked at 1500–2000 kg ha^{-1}. Before stocking, the pond was treated with lime (200 kg ha^{-1}) to balance the pH of the water, bleaching powder (200–300 kg ha^{-1}) for eradication of predatory and weed fishes and cow dung as fertilizer (10,000 kg ha^{-1} $year^{-1}$). The pond was fertilised with @1000 kg ha^{-1} of raw cow dung, 25 kg ha^{-1} of urea, and 25 kg ha^{-1} of single super phosphate. A semi-balanced feed composed of fish meal (10%), ground nut oil cake (35%), soybean oil cake (20%), wheat flour (10%), rice bran (24.8%), and vitamin mineral mixture (0.2%) were given to the brooders @3% body weight. Growth of brooders and water quality of the ponds were monitored at monthly intervals. Induced breeding trials, Wova-FH was diluted with normal saline to a level of 25%. Induced breeding of carps with comparable effectiveness and efficiency to generic Wova-FH were carried out. Economic analysis of spawn production indicated no significant difference in CB ratio between T_0 and T_1, whereas other two treatments showed significantly reduced CB value. Therefore, Wova-FH diluted with normal saline at a rate of 25% could be used to induce the *L. rohita* with a profit comparable to that of the control fishes. This could cut down on the farmers' expenses while ensuring a bigger production level at a reasonable price.

Key words: Feeding, induced breeding, pond fertilization, spawning performance

INTRODUCTION

One of the world's top producers of fish, India contributes 7.58% of the total global production. Given that, it provides roughly 1.24% to India's Gross Value Added (GVA) and 7.28% to agricultural GVA during 2018–19 (Department of Fisheries, 2020). The fisheries industry, which is referred to as the country's sunrise sector, has experienced tremendous growth with an average annual growth rate of 10.88% on a sustainable basis over the course of the past five years. Aquaculture is a promising industry that is rapidly increasing its output, making it the fastest-growing food production industry globally (Maulu et al., 2021) and making a considerable contribution to the nation's economic growth. The total inland

fish production of the country during 2019-20 stands at 10.43 million tonnes with about 57% contribution from Indian major carps (IMCs) (Department of Fisheries, 2020). One of the main barriers to increasing farm productivity and output is the inadequate supply of high-quality seed of the main cultivable species in the quantities required when needed. The total seed production of the country during 2019-2020 was 52,170.6 million fry which is insufficient to meet the projected target production of 22 million tonnes of fish by 2025 (Department of Fisheries, 2020).

The most promising and reliable method for ensuring the year-round availability of high-quality fish seed and the long-term viability of the aquaculture business is artificial fish reproduction. It entails the use of synthetic or natural hormones to stimulate spawning or ovulation in farmed fish. In India, the production of fish seeds is a lucrative sector with a 167% return on variable input costs (Kumar et al., 2008). It is a capital-intensive industry that necessitates expensive infrastructure, huge money, and technological know-how. The effectiveness of the hatchery operation is largely dependent on the cost and availability of synthetic hormones like ovaprim, ovatide, and wova-FH in appropriate amounts. Biswas et al. (2021) reported that the cost of inducing agent contributes about 11% of the total operating cost of a freshwater fish hatchery at Jashore, Bangladesh. Operational costs for timely production of high-quality seed are a key worry for rural farmers who lack resources, and breeding failure from employing hormones results in a loss of both capital and yield.

Wova-FH is a highly potent, less viscous, low priced and ready to use formulation consisting of salmon gonadotropin releasing hormone (sGnRH) and a dopamine antagonist (domperidone). The viscosity of the solution has been specifically maintained at a level that makes the hormone administration easy. Though no literature is available on the effect of diluted hormones on reproductive performance of Indian major carps, few works have been done on the reproductive performance of catfish, *Clarias gariepinus* (Olumuji and Mustapha, 2012; Tiamiyu et al., 2015; Assan et al., 2020) and *C. anguillaris* (Maradun et al., 2019).

To lower the price of fish breeder hormones used in the induced breeding and the overall cost of fish production along with attaining high spawning success, the current study was designed to evaluate the effect of Wova-FH diluted with normal saline at 25%, 50%, 75% and 100% on spawning response and larval rearing of Indian major carp, *Labeo rohita.*

MATERIALS AND METHODS

Collection and maintenance of brood fish

The age range of 2 to 3+ years, 1000 to 2000 g weight, and farm-raised *L. rohita* brooders were chosen, and they were stocked at 1500–2000 kg ha^{-1}. Before stocking, the pond was treated with lime (200 kg ha^{-1} $month^{-1}$) to balance the pH of the water, bleaching powder (200–300 kg ha^{-1} $month^{-1}$) for eradication of predatory and weed fishes and cow dung (10,000 kg ha^{-1} $year^{-1}$). Following stocking, the pond was fertilised with 1000 kg ha^{-1} of raw cow dung, 25 kg ha^{-1} of urea, and 25 kg ha^{-1} of single super phosphate. A semi-balanced feed composed of fish meal (10%), ground nut oil cake (35%), soybean oil cake (20%), wheat flour (10%), rice bran (24.8%), and vitamin mineral mixture (0.2%) were given to the brooders@ 3% body weight (Singh et al., 2000). Growth of brooders and water quality of the ponds were monitored at monthly intervals.

Experimental design

Each ml of Wova-FH comprises 20 µg of a salmon gonadotropin releasing hormone analogue and 10 mg of Domperidone, manufactured by M/s. USV Limited in Mumbai. As a diluent, normal saline solution (Sodium Chloride, 0.9% w/v, Baxter) was used in the experiment which is manufactured and marketed by Baxter Healthcare Corporation, USA

Five different treatments were used in the experiment such as T0 (100% Wova-FH), T1 (25% diluted Wova-FH with normal saline), T2 (50% diluted Wova-FH with normal saline), T3 (75% diluted Wova-FH with normal saline) and T4 (100% normal saline). The breeding programme was carried out at College of Fisheries (OUAT),

Rangailunda, Berhampur, Odisha, and a complete randomized design was followed where each treatment was conducted in three replications.

Captive breeding

For three years, from 2016 to 2018, captive breeding of the experimental fishes was done with the commencement of the south-west monsoon during the months of June, July, and August. The secondary sexual characteristics of the male and female brood fishes were used to identify and separate them at the time of breeding. They were stocked in breeding hapa (2 × 1 × 1 m) made of muslin cloth with a lid on top after sexual segregation. One female and one male fish of identical size made up the breeding group of fish. Before induced breeding, the fishes were acclimatized for 24 hours in the same breeding hapa. Brooders in treatment groups were administered with a single dose of hormone preparation intraperitoneally using a hypodermal BDH syringe of 2 ml with needle number 22 (Table 1). The injected brooders were immediately released into the breeding hapas.

Water quality parameters

The water samples were collected from brood stock ponds at monthly intervals to estimate pH, EC, dissolved oxygen, total alkalinity, and total hardness. The water samples from each nursery rearing tank during larval rearing was also collected at an interval of 5 days to estimate pH, EC, dissolved oxygen, total alkalinity and total hardness, free CO_2, total ammonical nitrogen (NH_3-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and phosphate phosphorus (P_2O_5-P) as per the standard methods (APHA, 1998).

Breeding performance

Latency period: is the time gap between time of hormonal administration to the time of first visible spawning by fish and is expressed in hours.

No. of eggs laid: The number of eggs laid was estimated by taking 20 ml of the water hardened egg and counting in a consecutive manner for four sub samples. The water hardened eggs were strained by using a strainer to expel water from the sample. After finding out the average number eggs in the samples, the same number is computed to one liter and finally to the total volume of water hardened eggs collected to arrive at the total number of eggs laid (Behera et al., 2010).

Fertilization (%): The fertilization percentage of eggs in each set, under different treatments was estimated following the formula given by Garg et al. (2002).

$$\text{Fertilization (\%)} = \frac{\text{No. of incubated eggs-no of dead/opaque eggs}}{\text{Total no of incubated eggs}} \times 100$$

Larval rearing

Eggs were incubated in a pool, and after the eggs hatched, the hatchlings were moved to nursery ponds of size 5 × 3 × 1.5 m at College of Fisheries, OUAT, Rangeilunda, where they were raised at a stocking density of 10 million ha^{-1}. Before stocking, the dead spawn was removed as far as possible and the number of spawn was counted. For this, first the number of spawn in an aluminum sample cup of 10 ml capacity was counted and then the number was converted to total volume of spawn collected. The hatching percentage was estimated by the formula given by Rath (2000).

$$\text{Hatching (\%)} = \frac{\text{Number of hatchlings}}{\text{Total no of fertilized eggs}} \times 100$$

The nursery tanks were given a 10 cm soil base prior to being stocked, and filtered pond water was added until they reached a depth of 1.0 m. The level was then maintained throughout by occasional filling. All the tanks were fertilised according to the recommended dosages of 750 kg ha^{-1} groundnut oil cake, 200 kg ha^{-1} raw cow dung, and 50 kg ha^{-1} single super phosphate in split doses, with 50% of the recommended dosage applied as a base dose four days prior to stocking and the remaining 50% applied in two additional splits after stocking of spawn, i.e. on the sixth and eleventh days (Jena et al., 1998). A drag net with a 1/8" mesh size was used to eliminate predatory insects from the tanks. The spawns were fed with finely powdered ground nut oil and rice bran (1:1) is given 4 times the weight for first 5 days and then the quantity is doubled from 6^{th} to 14^{th} day. Mean initial weight (g) of the spawn were recorded by taking measurement

of 50 samples. Growth in terms of weight was further assessed through periodic samplings at 5-day intervals. Mean increment in weight were computed from random samples of 25 animals from each tank. The health status of the fry was also assessed during the sampling. The net weight gain of the spawn treatment wise was calculated at the end of the rearing period by the following formula:

Net Weight Gain (g) = Final weight of fry (g) - Initial weight of fry (g)

The survival of spawn released and reared in the cement cisterns was estimated by using the following formula:

$$\text{Survival (\%)} = \frac{\text{Fry survived at the end of rearing}}{\text{Total spawn released}} \times 100$$

Economic Analysis

The CB ratio of the breeding experiment has been estimated by the following formula :

$$\text{BC ratio} = \frac{\text{Gross return (total selling price of spawn)}}{\text{Gross cost of Production (variable cost)}} \times 100$$

Statistical analysis

The data were statistically analyzed by statistical package SPSS version 20.0, in which data were subjected to one way ANOVA and Duncan's Multiple Range Test to determine the significant differences between the mean values. Comparison was made at the 5% probability level.

RESULTS AND DISCUSSION

Breeding performance

The water quality parameters in different treatments (Table 2) did not show any marked variations among the treatments and found to be within the range for hatchery operation of Indian major carps (Gupta et al., 2008). Similarly, the water quality parameters during nursery rearing of the spawns did not show any distinct variations among the treatments. The values of all the important parameters were found to be within the optimum range for spawn growth (Jena et al., 1998; Das et al., 2005; Biswas et al., 2006).

The percentage of spawning response showed significant difference ($P<0.05$) among the treatments in the present study. No significant difference was observed in rohu with respect to % spawning response among T0 (control) and T_1, where Wova-FH was diluted at 25% with physiological saline. But as the dilution increases to 50% and 75%, it was observed that the % of spawning response decreases significantly. A spawning response percentage of 85.15 ± 6.42 and 81.48 ± 6.42 was observed in control and T_1 of *L. rohita* respectively (Table 3), which is lower than the spawning response percentage of 95-100% obtained in *Carassius auratus* administered with Wova-FH (Mahadevi et al., 2018). The lower value of spawning response % in the present study might be due to the prevailing dry weather in the monsoon months during the study period. Similarly, Das et al. (2023) reported lower value of spawning response % (80-90%) in catla and mrigal injected with normal saline diluted wova-FH. Significantly ($P<0.05$) lower spawning response in T_2 (62.96%) and T_3 (25.92%) in rohu, indicated that the quantity of hormone administered in these groups are insufficient to induce spawning. Lower doses (10 and 15 μg kg^{-1} BW) of rGnRH administration were reported to be less effective than 20 μg kg^{-1} BW in the induction of final maturity in female goldfish (Mohammadzadeh et al., 2021). Similarly, lower dose of 10 μg kg^{-1} BW was proved to be less effective than 15 and 25 μg kg^{-1} BW in induction of spawning in channel catfish, *Ictalurus punctatus* (Chatakondi, 2017). Therefore, it is confirmed that the dose of hormone administration is a critical factor for the success of reproduction in teleosts. None of the females of *L. rohita* spawned in T_4, administered with 100% normal saline, suggested no biological activity of the normal saline in reproduction of the species, instead they require adequate dose of hormone for final maturity and good spawning.

Results of latency period showed a significantly lower value of 8.44 ± 0.20 h in T0 (control). The significantly highest latency period of 12.33 ± 1.11 h was found in T_3 group (Table 3). This could be supported by the work of Pandey et al. (2015), where they got a latency period of 7-8 hrs in *L. rohita* injected with Wova-FH. It has also been observed from the current study that the quantity

of hormone injected showed an inverse relationship with the latency period. Higher latency period in low doses of hormones indicates difference in mode of action of the hormone. The result of the present study corroborates with the work of Pandey et al. (2002) and Behera et al. (2010) where they reported longer latency period in low dose of synthetic hormone ovaprim and ovatide on induced breeding of *L. rohita* and *L. bata* respectively. Behera et al. (2010) and Das et al. (2016) also observed prolonged latency period at low dose of CPE (crude pituitary extract) and synthetic hormone (ovaprim, ovatide and wova-FH) administration in *Osteobrama belangeri* under captivity. In contrary to the present study, Maradun et al. (2019) revealed no significant effect of diluted ovulin with normal saline at 25%, 50% and 75% level on latency period of African cat fishes, *Clarias anguillaris* and *C. gariepinus* except 100% normal saline administered group where ovulation did not occur. Olumuji and Mustapha (2012) also did not notice significant difference in latency period of *C. gariepinus* with different doses of normal saline diluted ovaprim. This might be because physiological response to exogenous hormone during induced breeding is species specific.

In the current investigation, in *L. rohita* significantly higher number of eggs were laid in T_0 (8.98 ± 1.13 lakh) group injected with undiluted Wova-FH and T_1 (8.42± 0.90 lakh) group where 25% dilution of Wova-FH was done. As the dilution increases to 50% and 75%, the number of eggs laid decreases significantly (Table 3). The result obtained in T_0 can be compared with the study of Dash et al. (2018), where they reported an egg output of 5,56,224 numbers from 4.8 kg females injected with Wova-FH at 0.5 ml per kg body weight. Similar results were obtained for catla and mrigal where number of egg output decreases with increase in dilution of wova-FH with normal saline (Das et al., 2023). In a study, Tiamiyu et al. (2015) reported significantly lower ($P< 0.05$) relative fecundity in *C. gariepinus* injected with ovaprim diluted with normal saline beyond 1:1 and coconut water beyond 1:3 dilution. This might be due to the reduction of potency of the hormone by dilution which leads to reduction in egg output. The number of eggs released by the females of *L. rohita* in T_1 was at par with the control. This might be because the reproductive cycle of Indian major carps is influence by environmental parameters particularly temperature and rainfall (Vass et al., 2009). Increased temperature plays an important role in stimulating maturation of gonads in fishes and accelerated spermiation. But rapid and high fluctuations in water temperature will definitely be detrimental to fish reproduction. However, increased temperature to the comfortable limits may be useful for maturation process of gonad of carps during February-March when the temperature gradually increases and completes prior to onset of monsoon in May-June. This advancement in maturation of gonads of Indian major carps in some hatcheries of Odisha has been reported by Das et al. (2012). A marginal increase in air temperature during the study period has been reported by IMD, 2018. In the current study, as the brooders taken for breeding were in advanced maturity stage, they may require low quantity of exogenous hormone to stimulate the hypothalamus-pituitary-gonadal axis for spawning. This might be the reason for which diluted Wova-FH at 25% level for *L. rohita* showed at par result with control (undiluted Wova-FH) in terms of quantity of egg released. Further, it can also be stated that increase in temperature may lead to stress in fishes. Hence, there is an increase in the corticosteroid level, which may have a stimulatory effect on reproductive performance in teleosts (Pankhurst, 2016). This might be a reason for achieving significantly higher egg output from treatment T_1 and T_2 where dilution of Wova-FH at 25% and 50% was done.

In the current investigation, significantly the highest per cent of fertilization was observed in T_0 (77.12 ± 3.74) followed by T_1 (75.09 ± 4.11) and lowest % of fertilization of 43.91±23.97 in T_3 where 75% dilution of Wova-FH was made (Table 3). The result of the present study is in accordance with the result of Dash et al. (2018), where they found a fertilization % of 75.23 in *Cyprinus carpio* injected with Wova-FH. But in another study, Kumar et al. (2019) reported a

fertilization (%) of 88.5 and 90.5 in *C. mrigala* injected with Wova-FH at Dharua reservoir, Uttarakhand, and College of Fisheries, Pantnagar, Uttarakhand respectively. This might be due to different environmental parameters prevailing in the areas. The result of the present study is in agreement with the work of Olumuji and Mustapha (2012) who examined the effect of varying doses of normal saline diluted ovaprim on induced breeding of *C. gariepinus*. Maradun et al. (2019) reported significantly ($P<0.05$) high fertilization rate (92.22%) in undiluted ovulin treated *Clarias batrachus* and *Clarias anguillaris* than the normal saline diluted treatments at 25%, 50% and 75%. Though a significantly high value of breeding parameters in terms of fertilization rate, hatching % and survivability were observed in control treated with undiluted ovulin, they suggested using 25% and 50% dilution of ovulin in induced breeding of these cat fishes. The significantly highest percentage of hatching was observed in T_0 (77.18 ± 2.88) followed by T_1 (75.87 ± 3.26) and T_2 (72.81 ± 6.23) in *L. rohita* (Table 3). This result agrees with the study of Kumar et al. (2019), where they reported a hatching (%) of 84.5 and 83 in *C. mrigala* injected with Wova-FH at Dharua reservoir, Uttarakhand, and College of Fisheries, Pantnagar, Uttarakhand respectively. It has been reported that, the embryonic development in teleosts is retarded at low temperature and is accelerated in high temperatures (Hart and Purser, 1995) and may be due to this significantly high % of hatching was recorded in T_1 and T_2 where Wova-FH was diluted at 25% and 50 % level with normal saline. Das et al. (2006) achieved a high hatching rate for *L. rohita* at 31.0°C. During the present study, the water temperature was found to varied from 29.39-30.11°C (Table 2) influence the hatching percentage. However, in the present study, it has been observed that diluting Wova-FH with normal saline at 25% and 50% could result in hatching percentage at par with the undiluted hormone. Thus, it shows that either the normal saline could enhance the hatching percentage and number of spawn recovered or it could be due to the prevailing water temperature during the study period (Hart and Purser, 1995; Vass et al., 2009).

Spawn growth and survival (%)

The growth study was carried out with the estimation of net weight gain (g) of spawn reared for a period of 15 days with an interval of 5 days sampling. The net weight gain (g) of the spawn significantly decreases as the dilution of the hormone increases to 50% and 75%. The highest net wet gain (3.85 ± 0.08) of spawn was observed in T_0 (Table 4), which did not differ significantly from T_1. According to Bobe and Labbe (2010) the quality of an egg is defined as its competence to develop into proper embryo. In induced breeding of a fish, the hormonal administration changes the circulating levels of gonadal steroids hormones which ultimately affect the oocyte maturation and egg ovulation (Nagahama and Yamashita, 2008). The type and adequate quantity of exogenous hormone are essential for successful ovulation and crucial for survival and growth of the spawn (Ljubobratovic et al., 2019; Fahmy et al., 2020). Though no literature available on the effect of diluted hormone on spawn growth and survival, the significant decrease in net weight gain and survival (%) of spawn in 50% and 75% diluted Wova-FH treated groups in this study might be due to the low quality of eggs produced.

Economic analysis

The findings of economic analysis in the present study suggested a decrease in percentage share of hormones to variable cost from 10.71% in control to 8.26% in T_1 and 5.56 % in T_2 (Table 5). The is in accordance with the result of Biswas et al. (2021), where they reported that the cost of inducing agent (pituitary gland) contribute about 11 % of the total operating cost of a freshwater fish hatchery at Jashore, Bangladesh. CB ratio calculation in the current investigation indicated that though the contribution of hormone cost to total variable cost reduces in T_1 (25% dilution of Wova-FH) compared to control, there was no significant difference among these treatments with respect to CB ratio. From this it can be inferred that the farmers can use 25% normal saline diluted Wova-FH without compromising profit. In the present study, a CB

Table 1. Treatment wise Dosage of Wova-FH and normal saline for induced breeding of rohu

Treatments	Wova-FH	Normal saline	Dose
T_0	0.5 ml	-	Single dose
T_1	0.375 ml	0.125 ml	Single dose
T_2	0.25 ml	0.25 ml	Single dose
T_3	0.125 ml	0.375 ml	Single dose
T_4	-	0.5 ml	Single dose

Table 2. Water quality parameters of brood stock pond and nursery pond during the study period

Parameters	Brood stock rearing pond	Nursery pond
Temperature (°C)	29.78± 0.30	29.73±0.32
pH	8.48±0.06	8.21±0.08
EC (d Sm^{-1})	1.84±0.02	2.48±0.09
Dissolved oxygen (mg l^{-1})	7.1±0.02	5.81±0.05
Total alkalinity (mg $CaCO_3$ l^{-1})	166.67±1.63	62.49±1.40
Total hardness (mg $CaCO_3$ l^{-1})	449.88±3.83	140.91±1.35
Free CO_2 (mg l^{-1})	-	2.67±0.02
Total NH_3-N (mg l^{-1})	-	0.09±0.01
NO_2-N (mg l^{-1})	-	0.006±1E-18
NO_3-N (mg l^{-1})	-	1.48±0.01
P_2O_5-P (mg l^{-1})	-	0.067±0.002

Table 4. Net weight gain (g) and survival of rohu spawn

Treatment	Initial weight (g)	Weight gain (g)	NWG in 28 days (g)			
			7^{th} day	14^{th} day	21^{st} day	28^{th} day
T_0	0.0014	0.28±0.01	0.66±0.01	1.10±0.03	1.82±0.03	3.85^{a}±0.08
T_1	0.0014	0.31±0.02	0.67±0.04	1.08±0.03	1.78±0.05	3.85^{a}±0.15
T_2	0.0014	0.27±0.05	0.58±0.07	0.93±0.11	1.42±0.18	2.87^{b}±0.55
T_3	0.0014	0.24±0.05	0.53±0.08	0.88±0.11	1.38±0.21	2.92^{b}±0.38

Table 3. Breeding performance of rohu to different treatments

Treatment	No of female (Wt. in kg)	No. of male (Wt. in kg)	Spawning response (No)	Percentage of spawning response (%)	Latency period (hrs)	Quantity of eggs laid (Lakhs)	Percentage of fertilization (%)	No of fertilized egg (Lakhs)	Percentage of hatching (%)	No of spawn recovered (Lakhs)
T_0	5.47±0.32	5.13±0.13	2.56^{a}±0.20	85.18^{a}±6.42	8.44^{c}±0.20	8.98^{a}±1.13	77.12^{a}±3.74	7.03^{a}±1.09	77.18^{a}±2.88	5.52^{a}±1.01
T_1	5.43±0.21	5.48±0.34	2.44^{a}±0.20	81.48^{a}±6.42	9.78^{b}±0.51	8.42^{a}±0.90	75.09^{a}±4.11	6.37^{a}±1.03	75.87^{a}±3.26	4.87^{a}±1.02
T_2	5.59±0.38	5.23±0.27	1.89^{ab}±0.51	62.96^{b}±16.97	10.56^{b}±0.20	6.81^{a}±2.24	64.19^{ab}±12.77	4.52^{a}±2.25	72.81^{a}±6.23	3.35^{ab}±1.89
T_3	5.65±0.39	5.39±0.38	0.78^{b}±0.19	25.92^{c}±6.41	12.28^{a}±1.11	2.72^{b}±0.80	43.91^{b}±23.97	1.54^{b}±0.91	52.11^{b}±16.28	1.04^{b}±0.67
T_4	5.50±0.25	5.33±0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5. Economic analysis of span production of rohu during the study period

Treatment	Weight of female (kg)	Weight of male (kg)	No of spawn recovered (Lakhs)	*Net price of spawn (Rs kg^{-1} bw^{-1})	**Total cost of hormone (Rs kg^{-1} bw^{-1})	*** variable cost (Rs kg^{-1} bw^{-1})	Total cost of production (Rs kg^{-1} bw^{-1})	Percentage share of hormone to variable cost (%)	CB ratio
T_0	5.47±0.32	5.13±0.13	5.5246±1.01	1009.98±124.48	28.80±0.00	240.00±0.00	268.80±0.00	10.71±0.00	3.76^{a}±0.46
T_1	5.43±0.21	5.48±0.34	4.8776±1.02	898.27±167.45	21.60±0.00	240.00±0.00	261.60±0.00	8.26±0.00	3.43^{ab}±0.64
T_2	5.59±0.38	5.23±0.27	3.3524±1.89	599.71±288.47	14.40±0.00	240.00±0.00	254.40±0.00	5.66±0.00	2.36^{b}±1.13
T_3	5.65±0.39	5.39±0.38	1.0496±0.67	185.59±109.75	7.20±0.00	240.00±0.00	247.20±0.00	2.91±0.00	0.75^{c}±0.44

ratio of 3.85 ± 0.08 in T0 and 3.76±0.46 in T1 was found in spawn production of *L. rohita*. In a study, Biswas et al. (2021) reported a lower CB ratio of 1.59, 1.51 and 1.46 during May, June and July in Ma Fatima fish hatchery, Jashore, Bangladesh. The lower CB ratio obtained in this study might be due to inclusion of fixed cost along with the variable cost during the calculation of the CB ratio, whereas in the present study only the variable cost is taken for calculation of the CB ratio. This can be supported by the work of Kunguma et al. (2019), where they found a CB ratio of 2.32 on variable cost and 1.15 on total cost on production of Indian major carps of Cauvery delta zone, Tamil Nādu.

CONCLUSION

In the current investigation, Wova-FH diluted in normal saline to a level of 25% will induce breeding with comparable effectiveness and efficiency to generic Wova-FH without affecting breeding results. Economic analysis of spawn production indicated no significant difference in CB ratio between T_0 and T_1, whereas other two treatments showed significantly reduced CB value. Therefore, Wova-FH diluted with normal saline at a rate of 25% might be used to induce breeding of *L. rohita* with a profit comparable to that of the control. This will cut down on the farmers' expenses while ensuring great production at a reasonable price.

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