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Research Article

Lactuca pygmaea (Asteraceae, Cichorieae) - a new species from India

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ABSTRACT

Lactuca pygmaea Bhellum (Asteraceae, section scariola F. W. Schmit) - a new species from India is described and illustrated. It grows gregariously on open and dry places. The field survey was made in the surroundings of Jammu in 2014 and specimens were studied with the help floristic literature. The area of the entire State of Jammu and Kashmir of India lies between 32° 17´ - 36° 58´ N and 73° 26´ - 80° 30´. The new species differs from its closely relative related *Lactuca dissecta* L. in its height being short, leaves entire or remotely lobed, size of phyllaries capitula, pappus, and achenes. Therefore, it represents distinct species of *Lactuca* L. genus.

Keywords: *Lactuca pygmaea*, Flora, Asteraceae, New species, India.

1. INTRODUCTION

During plant exploration in different parts of Jammu, the author came across some plant specimens of *Lactuca* L. These specimens were quite different from the earlier species of *Lactuca* L., collected so far from Jammu of Jammu and Kashmir State (Fig. 1). Perusal of floristic literature, revealed that these specimens do not match with any species of *Lactuca* L. Therefore, these specimens were identified as *Lactuca pygmaea* Bhellum (Asteraceae) as the taxon not reported earlier, therefore described as new species.

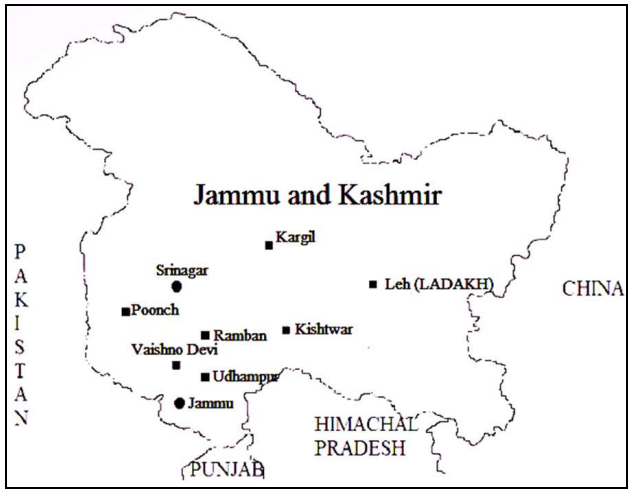

Fig. 1. Map of Jammu and Kashmir State (India).

Genus *Lactuca* L. comprises of 150 species in the world and 24 species in India [1] and 14 species in the Flora of Jammu and Kashmir Jammu and Kashmir State [2]. However the total number of species of *Lactuca* L. is variable by the different authors. The current author of this paper [2-4] undertook the study of tribe Cichorieae and endemic

species of Asteraceae of Jammu and Kashmir. The species of tribe Cichorieae were identified on the basis of morphological variation of florets, cypselae in addition to plant height, leaves shape, size and length and presence or absence of pappus. As many as 11 genera representing 47 species of tribe Anthemideae has already been enumerated from the Jammu and Kashmir [5]. Voucher specimens of the Holotype have been submitted in Herbarium RRLH 22896, IIIM, Jammu (Fig. 5).

2. MATERIALS AND METHODS

During the field visits in different parts of Jammu and its immediate neighbourhood, the author collected some specimens of *Lactuca* L. the smallest member species collected so far from Jammu and Kashmir. These specimens were identified as *Lactuca pygmaea* Bhellum in the Laboratory with the help of taxonomic literature. The illustrations of the plant, floral parts and cypsela were made after studying the various parts of specimens under the Binocular Stereoscope.

3. RESULTS

The present investigation deals with new report of plant species *Lactuca pygmaea* Bhellum from Jammu and Kashmir (India) and compared (Table 1) with its allied species of *Lactuca disssecta* D. Don.

Table. 1. Comparison of *Lactuca pygmaea* Bhellum and *Lactuca disssecta* D. Don characteristics.

Character	*Lactuca pygmaea* Bhellum	*Lactuca dissecta* D. Don
Height	Erect herbs 4-12 cm tall	Prostate or sub-erect, herbs 4-40 cm tall
Leaves	Variable, 5-15 mm long, obovate, oblanceolate, margin remotely dentate	Variable, Oblong, 40-200 mm long, margin multilobed
Capitula	a few, purple, 7-10 mm long, 6-7.5 mm across	many, purple, 10-12 mm long, 3-4 mm
Phyllaries	outer ovate, 2 mm long, inner linear lanceolate, middle 3-5 mm long inner 6-8 mm long	outer ovate or obovate, 2.5-3.5 mm long, inner linear or linear oblong, middle 7-8 mm, inner 9-12 mm long
Pappus	simple, thin, white, 3-4 mm long	simple, white to yellowish white, 3-4 mm long
Achenes	2.2-2.7 mm long, light brown, dilated at tip, beak 3-4 mm long	3 mm long, brown, dilated at tip, beak 3.5-4.5 mm long

3.1. *Lactuca pygmaea* Bhellum sp. nov. Fig. 2 (A-G)

Annual, slender, 3-12 cm tall herbs, juice milky; stem usually solitary, hairy at base, branches a few; leaves thin, basal obovate, oblanceolate, oblong, spathulate, 5-16 x 4-6 mm, margin entire or remotely dentate, apex rounded; upper leaves narrowly arrow shaped, semi-stem clasping, linear or linear lanceolate, apex acute or acuminate; phyllaries 2-3 seriate, outer ovate, 2 mm long, inner oblong, linear, middle 3-4 mm long, inner 6-8 mm long, subequal, tips purple; receptacle flat, naked. capitula pedunculate, solitary or a few, homogamous, 7-10 mm long, borne on top of branches, 6-7 mm across, blue; all florets ligulate, ligules 4-6 mm long, 5-toothed at apex; stamens 5, syngeneous, reaching to the base of stigma, anther basis sagittate; pistil bicarpillary, syncarpous, style hairy; stigma bilobed; achenes obovate, 2.2–2.7 mm compressed, 3-ribbed on either side (Fig. 3) dilated at tip, bright brown, beak elongate, 4-4.5 mm long; pappus simple, white, thin, 3-4 mm long.

3.2. Ecological note

Lactuca pygmaea Bhellum grows gregariously (Fig. 4) on open and dry places and wastelands in association with *Launaea procumbens* (Roxb.) Ramayya & Rajagopal, *Lactuca dissecta* D. Don, *L. scariola* L., *Medicago polymorpha* L. *Melilotus indica* All., *Parthenium hysterophorus* L., *cynodon dactylon* Pers. *Sonchus oleraceus* L. and *Calotis hispidula* F. Muell., *Phalaris minor* Retz.

Etymology: The specific epithet refers to short height of the species.

Flowering and Fruiting: March-April.

Specimens examined: India, Jammu and Kashmir, District Jammu, East of Sainik Colony, Jammu, 25.03.2014 Bhellum (BLB. 15323, RRLH). The location of specimens collected is latitude 32° 40′ 18.07′′ N and longitude 74° 57′ 7.04′′ E.

4. DISCUSSION

Earlier, among other works, e.g. Clarke [6] studied Compositae indicae only with reference to Asteraceae. Asteraceae has been classified on the basis of cladistics study [7]. The changes in climate induce variation in the vegetation differently at intraspecific level on variable altituditional zonation. On the other hand Stebbins studied seriously with wider *Lactuca* L. concept and this knowledge was enhanced with particular reference to Indian species of this genus [5, 8]. The generic concept of *Lactuca* L. has always been a source of disagreement among the different taxonomists [9, 10].

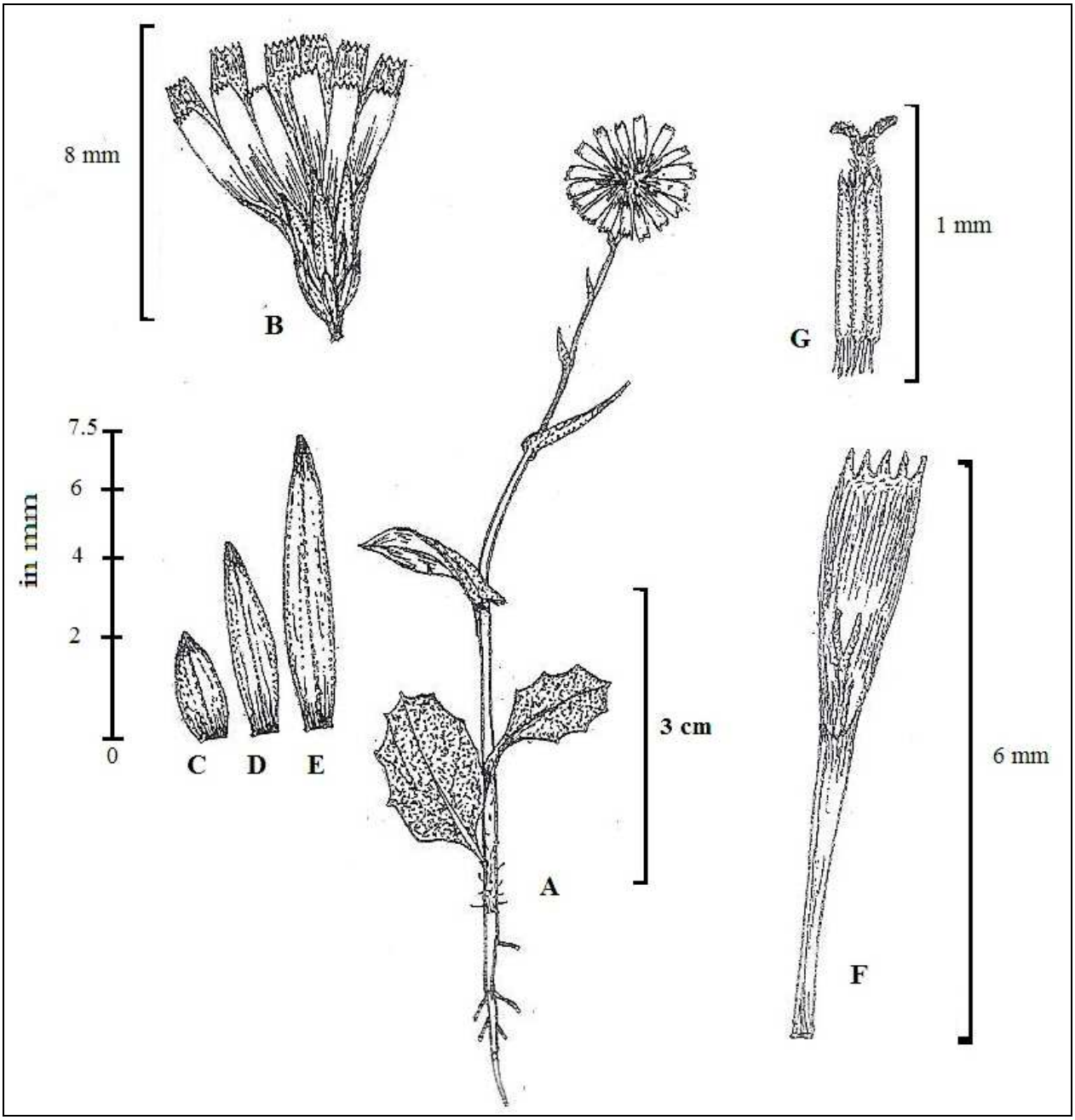

Fig. 2. *Lactuca pygmaea* Bhellum: A, plant; B, capitulum (Lateral view); C, outer phyllary; D, middle phyllary; E, inner phyllary; F, floret; G, anther and stigma.

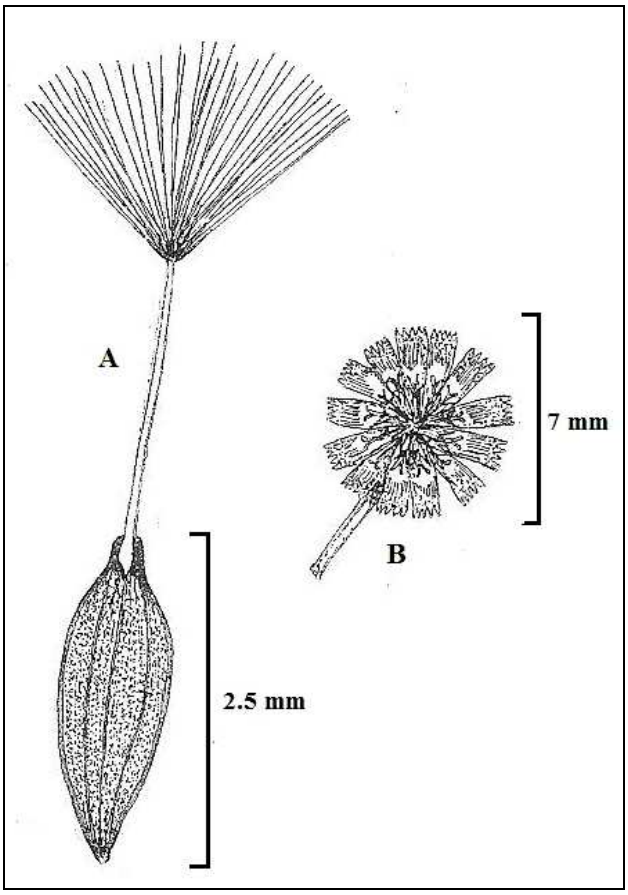

Fig. 3. *Lactuca pygmaea* Bhellum: A, achene; B, capitulum (front view).

Fig. 4. A population of *Lactuca pygmaea* Bhellum.

Fig. 5. Holotype of the specimen submitted in Herbarium (RRLH), IIIM, Jammu.

Tribe Cichorieae of East Tropical Africa was undertaken critically which includes various species of *Lactuca* L. [11]. The style of the members of Asteraceae varies in different divertions with respect to style branches tagentially directed in Heliantheae, Inuleae and Senecioneae and radially directed in Astereae and Anthemideae. The most of the significant references of the style orientation as usually found in literature involves the achene of various members of Heliantheae [12]. In addition to it, Shih segregated a new species *Notoseris* Shih from *Lactuca* L. on the basis of many ribs of achene and well developed secondary ribs in the newly created genus [13-15]. The narrow generic concept accepted by taxonomists [15] which was subsequently followed [7]. Circumscription of *Lactuca* L. in particular studied the achenes, beak, pappi and number of florets per capitulum and favoured broader concept at generic level [16].

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TRANSPARENCY DECLARATION

The author declares no conflicts of interest.

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Research Article

Ethnopharmacological survey of medicinal plants used in the treatment of snakebites in Central Uganda

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ABSTRACT

An ethnopharmacological survey was conducted on medicinal plant species used in the treatment of snakebites in Mukono district, central Uganda, between February and May 2014. Interviews were held using questionnaires and Focus Group Discussions. Data was collected from Herbalists, school children and households who had been afflicted by snakebites. Thirty six plant species from to 25 families were documented reportedly used for treating snakebites. Most of the plant species were in Leguminosae (7) and Solanaceae (3) families. Roots (41%) were the most commonly used plant parts. Bulbs and flowers where the least used plant parts each at 2%. Snakebites are considered an emergency by herbalists advocating a range of plants for immediate utilization to snakebite victims. Most of the plants (29%) were applied as paste on snakebite wounds. These were followed by eating the plant parts immediately and later drinking decoctions made from them (24%). The least commonly used method of administration involved preparing the medicinal plant with milk (2%). The most commonly mentioned plant species by the herbalists were *Musa balbisiana* (5.5%) *Nicotiana tabacum* (5.5%), *Solanum incanum* (5.1%), *Searsia pyroides* (4.7%) and *Imperata cylindrica* (4.2%). The least frequently mentioned plants were *Achyranthes aspera* (0.8%) and *Senna alata* (0.4%). Herbalists and inhabitants of Mukono still rely on the use of plant species as snakebite medicine. Therefore correct plant species use in snakebite treatment could significantly alleviate fatal symptoms resulting from snake envenomation.

Keywords: Snakebite, Medicinal plants, Envenomation, Antivenoms, Herbalists.

1. INTRODUCTION

Snake envenomation is a serious global health, social and economic problem, constituting an occupational hazard mainly in the field of agriculture. Snakebites have been classified as a neglected disease by the World Health Organisation WHO in 2010 [1]. Snakebite afflicts the most impoverished inhabitants of rural areas in tropical developing countries [2-4]. Snakebites remain a primary problem of the poorer rural populations that often suffer from scarcity of antivenin, leading to considerable morbidity and mortality [5, 6].

Globally, more than 5 million persons were estimated to have been bitten by venous snakes annually by 2008 [7]. Out of these, approximately 100,000 developed severe sequelae and 125,000 died [7]. In Kenya, Snow et al. [8] reported that an estimated 19% of the 151 snakebites per 100,000 people were potentially of venomous snakes. In the West African savanna, up to 500 snakebites and between 4-40 deaths per 100,000 population per year, were reported [9, 10].

Snakes are distributed throughout most of the earth's surface with some exceptions including the Arctic and Antarctic [2, 11]. Of the roughly 3000 species of snake known worldwide, only 15% are considered venomous and dangerous. However, most snake bites are caused by non-venomous snakes [12]. The Puff Adder was considered Africa's deadliest snake because of the many human deaths it caused. Other venomous snakes in Africa include black Mamba, Gabon viper and the Egyptian Cobra [12].

The seasonal agricultural activities in Mukono leave the locals highly susceptible to snake bites. This is because snakes are thought to move during the rainy period in the search for prey [2]. Snakebites and scorpion stings have been reported to occur more frequently during the rainy season in India [13]. The incidence of snakebite is inversely correlated with population density of a particular area [14].

Most envenoming is known to occur in South Asia, South East Asia, and Sub Saharan Africa, while the most snakebite deaths of any country are reported in India [7]. A meta-analysis showed that 95% of envenomings occurred in rural areas [14]. However, in many regions of the world, numerous cases of snake bites go undocumented, implying that the number of snakebites reported is lower than the actual number of snakebites occuring [7, 16, 17]. Consequently no, accurate study has ever been done conducted to determine the frequency of snakebites on international level [2, 14], let alone Uganda. There are no statistical reports in hospitals since many snakebite victims die before reaching hospital [18]. This creates a difficulty in estimating needed antidotes, price and distribution policy of the drugs for snakebites in areas there most needed [14]. For example in a study conducted at Gulu Regional Hospital, Northern Uganda [15], none of the patients admitted during the one year study period received snake antivenom since it was unavailable at the hospital.

Although it has been argued that traditional treatment of snakebite delays presentation, distorts the clinical picture, causes bleeding, infection, gangrene, and other complications [4], the global and popular use of medicinal plants has a life saving potential [3, 17, 19]. Between 40 and 80% of snakebite victims in Sub Saharan Africa rely on traditional medicine [20]. Fifty percent of snakebite injuries are not medically treated, and those that seek medical treatment delay by up to 2 weeks. Many plants and traditional herbal medicines used against various effects of snakebite are readily accessible and available in rural areas [16, 17, 21].

Snake envenomings produce both local and systemic effects [19, 21]. However, it has been reported that at least half of the victims bitten by snakes escape without any significant envenoming, thus the bite of a poisonous snake is not synonymous with snakebite poisoning [16]. However, there is overwhelming evidence of the ability of secondary metabolites from different plant species to neutralize the action of snake venoms [1, 2, 19, 21, 22]. For example *Apuleia leicocarpa* and *Phyllanthus klotzschianus* have been shown to offer up to 100% protection from snake bite venom in laboratory mice [19], whereas *Gloriosa superba* has been shown to have significant neutralization of snake venom in a dose dependent manner [3].

Antiserum, the major global therapeutic agent used to manage snakebites is not readily accessible and is very expensive [2, 4, 14, 22]. Antiserum has also been reported not to provide enough protection against venom induced haemorrhage, necrosis, nephrotoxicity and patients often develop hypersensitivity reactions [19, 21]. The plant kingdom therefore provides an alternative to anti-snake venom [5, 6]. Because of inadequacies of biomedical health system, low cost, effectiveness and cultural acceptance, herbal medicines are widely used [3].

Researchers, clinicians, national and regional authorities and community organizations have been urged to work together to impact the availability of reliable epidemiology data on snakebites [5, 13]. This is in accordance to the two tools launched by WHO to help guide the development of appropriate

antivenins and modern methods of health promotion [1, 4]. In Uganda, no study has been conducted to document the medicinal plants used by traditional healers to treat snakebites despite the risk of being lost. The main objective of this study therefore was to document the plant species used in the management and treatment of snakebites in Mukono district.

2. MATERIALS AND METHODS

2.1. Study site

An ethnobotanical survey was conducted in Mukono district, in the villages of Nasuti, Ham Mukasa, Bukasa, Kitete and Kasagalabi in Mukono municipality. Mukono district is located between 0020N-32 45E latitude and 0° 21' 11 N longitude 32° 45' 19 E http://www.horlogeparlante.com/geographical-coordinates-228853.html (Accessed on 30 September 2014, 20:32 hours) and altitude range of 1158 m to 1219 m above sea level [23]. The temperature range is between 21-29^{o}C and annual rainfall between 1100 mm to 1400 mm with two wet seasons and no marked dry season. The district is also popular for the agricultural activities, such as growing sugarcane, tea and coffee among others. The district is characterized by poverty, poor health and transport services [23].

2.2. Data collection

The purpose and objectives of the study were explained to the local authorities and permission was sought from them before the study was begun. The questionnaire was the main tool of data collection used. Prior informed Consent (PIC) was obtained from the respondents before they were interviewed. Interviews were conducted in the local language (Luganda), using semi-structured questionnaires. The survey focused on herbalists, who were the key informants for the study. Two focus group discussions (FDG) were held with traditional healers to obtain in-depth information. All the respondents were asked which plants they used in snakebite treatment, their modes of preparation and administration, and their sources among others. The herbalists were identified through Snowball sampling. After contact was initiated with the herbalist leadership, we were referred to other herbalists who were knowledgeable on snakebites and their treatment. These herbalists in turn referred us to other herbalists they knew with such knowledge. Other participants were randomly chosen from secondary schools using and the general population the lottery sampling technique in order to minimize potential bias and capture the heterogeneity of the people in the area.

2.3 Plant collection and identification

Voucher specimens of the plant species were collected in triplicates, following standard collection procedures as described in Martin (1995). The specimens were taken to the Makerere university herbarium for taxonomic identification. The scientific names of the plant species were validated using the database at http://www.theplantlist.org, Version 1.1 accessed on 30th September 2014.

3. RESULTS

A total of 36 plant species belonging to 25 families were used in snakebite treatment (Table 1). A total of 132 respondents were interviewed, 57 males and 75 females. Herbalists constituted 12.9% of the respondents interviewed. About 9% respondents had no formal education, 28% had reached advanced level education, 46% ordinary level education, 14% primary level of education and below and 3% adult education.

Leguminosae family had the highest variety of medicinal plants, with 7 species. This was followed by Solanaceae, which had 3 species. Compositae, Euphobiaceae and Musaceae had 2 species each whereas the rest of the plant families were represented by 1 plant species each (Figure 1). The most frequently used plant species were herbs 43.2%, followed by shrubs 24.3%, trees 24.4% and climbers 5.4% (Figure 2) Mushrooms were the least used contributing 2.7% of the life forms.

The most commonly mentioned plant species by the herbalists as snakebite medicine were: *Musa balbisiana* (5.5%), *Nicotiana tabacum* (5.5%), *Solanum incanum* (5.1%), *Searsia pyroides* (4.7%) and *Imperata cylindrica* (4.2%). The least frequently mentioned plants were *Achyranthes aspera*

(0.8%) and *Senna alata* (0.4%). From these plant species mentioned the roots were the most frequently used parts in treatment of snakebites at 41%, followed by whole plant (18%), the leaves (16%) and the bark at 14% (Figure 3). The bulb and flowers are less used at 2%. Most of these plant species were collected from the bushes and grazing grounds: others were found planted in gardens, while most trees and herbs growing naturally in the area.

Table 1. Medicinal plants used in the treatment of snakebites in central Uganda.

Family	Scientific name	Local name (Luganda)	Life form	Parts used	Mode of preparation and application	Freq.
Amaranthaceae	*Achyranthes aspera* L.	Kagiri	H	R	Apply paste on bitten part.	2
Anacardiaceae	*Searsia pyroides* (Burch.) Moffett	Akakwansokwanso	S	L & R	Immediately eat the leaves & roots	11
Amaryllidaceae	*Allium cepa* L.	Butugulu	H	Bu	Apply the paste on the bite spot	8
Aristolochiaceae	*Aristolochia saccata* Wall.	Kasero	C	R	Make a decoction and drink	3
Colchicaceae	*Gloriosa superba* L.	Emmereyannamunye	H	R	Apply the paste on the bite spot	7
Compositae	*Aspilia Africana* (Pers.) C.D.Adams	Makaayi	H	L	Squeeze and drink the juice	5
	Microglossa pyrifolia	Akafugakadde	S	R	Immediately eat the roots	10
Convolvulaceae	*Hewittia malabarica* (L.) Suresh	Musotataluma	C	R & L	Apply the paste on the bite spot	6
Euphorbiaceae	*Euphorbia hirta* L.	Kasadasada	H	B	Make a decoction/apply the paste on bite spot	3
	Euphorbia candelabrum Trémaux ex Kotschy	Enkuukuulu	S	R	Plant eaten immediately/ decoction drunk	9
Leguminosae	*Abrus precatorius* L.wall	Olusiiti	S	R	Root powder is drunk in cow's milk.	6
	Cajanus cajan (L.) Millsp.	Ekolimbo/empindi	S	L & R	Squeeze and drink the juice	8
	Glycine max (L.) Merr.	Soya	S	Sd	Immediately eat the seeds	7
	Phaseolus lunatus	Kayindiyindi	H	Wp	Decoction drunk/ paste applied on bitten spot	7
	Erythrina abyssinica DC.	Ejirikiti	T	B & R	Decoction drunk/ paste applied on bitten spot	7
	Erythrina excelsa Baker	Omubajjangabo	T	B	Squeeze & drink juice/apply paste on bitten spot	6
	Senna alata (L.) Roxb.	Ekifura	T	L	Apply the paste on the bite spot	1
Lamiaceae	*Ocimum basilicum* L.	Omujaaja	H	Wp	Make a decoction and drink	2
Lyophyllaceae	*Termitomyces mirocarpus*	Akatiko akabaala	Mu	Wp	Immediately eat the plant	5
Lythraceae	*Punica granatum* L.	Enkomamawanga	T	Wp	Apply the paste on the bite spot	5

Malvaceae	*Grewia damine* Gaertn.	Enkomakoma	H	Wp	Immediately eat the plant	6
Meliaceae	*Azadirachta indica* A. Juss.	Omuttankuyege	T	F	Decoction drunk/ paste applied on bitten spot	3
Moraceae	*Ficus exasperate* Vahl	Oluwawu	T	R & B	Make a decoction and drink	4
Moringaceae	*Moringa oleifera* Lam.	Molinga	T	B & R	Crush or squeeze and drink the juice	3
Musaceae	*Musa balbisiana* Colla	Embidde	T	Sp	Add juice to other plants and drink	13
	Musa × *paradisiaca* L.	Kayinja	T	B	Add juice to other plants and drink	8
Myritaceae	*Morella kandtiana* (Engl.) Verdc. & Polhill.	Ekikimbo	H	R	Decoction drunk/paste applied on the bite spot	7
Oxalidaceae	*Oxalis corniculata* L.	Kajjampuni	H	Wp	Squeeze a juice and drink, the paste is tied on the specific spot of bite.	5
Phyllanthaceae	*Flueggea virosa* (Roxb. ex Willd.) Royle	Olukandwa	S	R	Immediately eat the roots	9
Poaceae	*Imperata cylindrica* (L.) Raeusch.	Essenke	H	R	Immediately eat the roots	10
Portulacaceae	*Portulaca quadrifida*	Bwanda	H	Wp	Add juice to other plants and drink	6
Rosaceae	*Rubus pinnatus* Willd.	Enkenene	S	L	Immediately eat the leaves.	8
Rutaceae	*Citrus limon* (L.) Osbeck	Enniimu	H	Fp	Squeeze and drink the juice	3
Solanaceae	*Nicotiana tabacum* L.	Taaba	H	L & R	Immediately eat	13
	Solanum aculeastrum Dunal	Entengo	S	F & R	Immediately eat the fruit & roots	8
	Solanum incanum L.	Akatengotengo	H	R	Make a decoction and drink	12

Key: B=Bark, Bu=Bulb, Fp=Fruit peels, F=Fruit, L=Leaves, Mu=Mushroom, R=Roots, Sp=Sprouting plant, Wp=Whole plant, Sd=Seed.

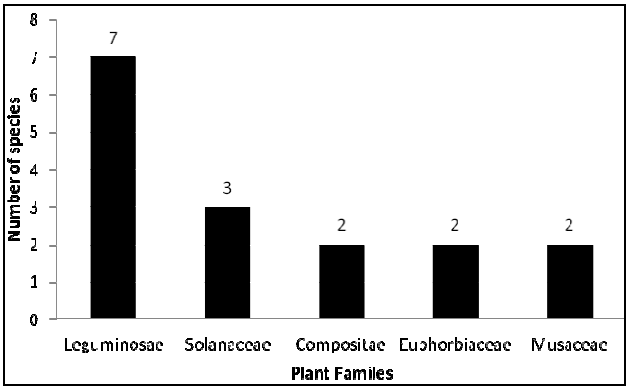

Fig. 1. Plant families used in treatment and management of snakebites.

3.1. Perception of snakes and snakebites

Snakebites were considered a matter of emergency by herbalists; however some herbalists stated that some snakes are not very poisonous such as Pythons which just swallow their prey. Many herbalists attached snakebites with spiritual rituals but the treatment did not include witchcraft. Some of the rituals included avoiding whistling or hissing when a snake is around, avoid unnecessary movement, step ping on your right big toe with the heel of your left foot when you see a snake. They also advised that whenever you kill a snake burn it

as some snakes are ovoviviparous. Still herbalists claimed some snakes to have relations with certain clans or families of people.

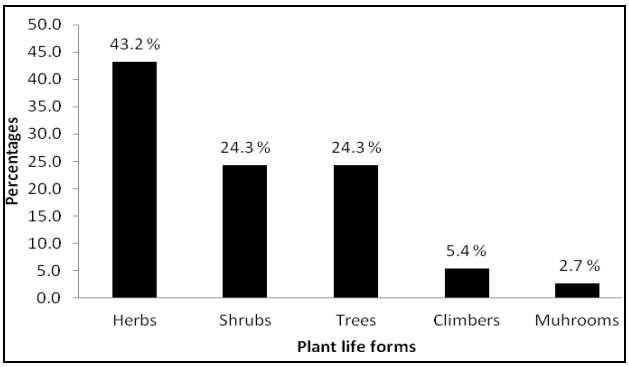

Fig. 2. Life forms of plant species used in treatment and management of snakebites.

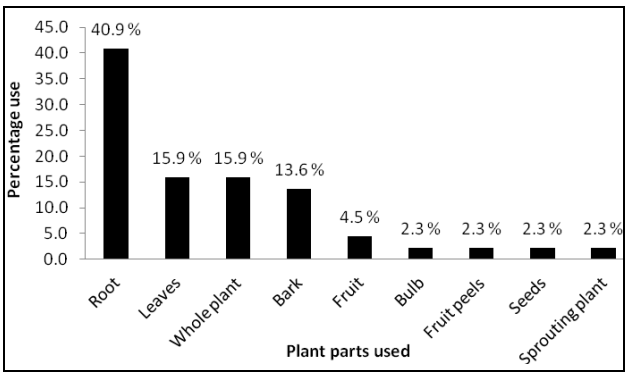

Fig. 3. Parts of the plant species used in snakebite treatment.

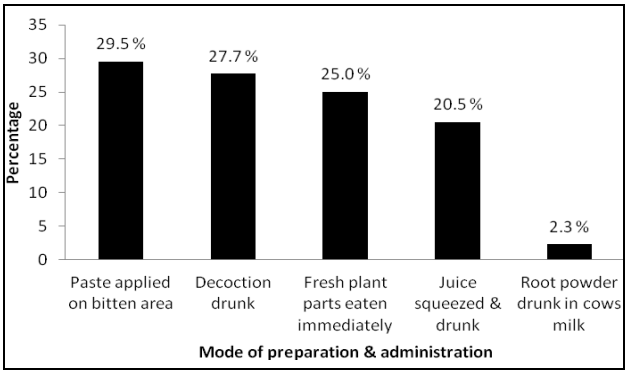

Fig. 4. Mode of preparation and application of plant species used in snakebite treatment.

3.2. Mode of preparation and application of plant species

The plant species applied as paste on the snakebite wounds were 29% followed by the plants recommended by herbalists to victims to immediately eat and make a decoction and drink at 24%. Plant species added to milk are at 2% and these are less commonly used in the mode of preparation of snakebite treatment by Mukono herbalists (Figure 4).

4. DISCUSSION

Out of the 36 plant species documented during this study, 22 have been reported to be used in different parts of the world including Uganda and Kenya for managing snakebites [3, 19, 22, 24-26]. Among the plant species documented, *Achyranthes aspera*, *Abrus precatorius*, *Euphorbia hirta*, *Allium cepa*, *Gloriosa superba*, *Cassia alata*, *Citrus limon*, *Ocimum basilicum*, *Nicotiana tabacum*, *Punica granatum*, *Azadirachta indica*, *Erythrina excelsa*, *Glycine max*, *Musa paradisiaca*, *Moringa oleifera* are reputed to be active against snakebite venom. On the other hand, *Aristolochia cathcartii*, *Erythrina abyssinica*, *Musa balbisiana*, *Searsia pyroides*, *Morella kandtiana*, *Myrica kandtian*, *Solanum aculeastrum*, *Solanum incanum* and *Ficus exasperata* although not explicitly mentioned in the review [19], they had close relatives in the same genus mentioned as active against snakebite venom. Tabuti et al. [24] documented 35 medicinal plant species used in the treatment and management of snakebites in eastern Uganda. Out of these, only 6 species were reported to be used in Mukono, the rest were not mentioned in this study.

An earlier study in Mukono district documented 11 medicinal plants used in snakebite treatment [25, 26]. Out of these, *Searsia pyroides*, *Solanum acuelestrum* and *Nicotiana tobaccum* are mentioned in both studies. Others documented the use of medicinal plants in snakebite treatment for example 32 plants in Kenya [17], 23 in India [6], 29 in Colombia [27]. Fabaceae, Solanaceae, Euphorbiaceae were among the plant families with the most plant species used to treat snakebites. These findings are congruent with those of Molander et al. [28] who documented Euphorbiaceae and Fabaceae to be among the top ten plant families used globally for the treatment of snakebites.

The roots were the most utilized plant parts by herbalists of Mukono. This was attributed to the fact that their constant seasonal availability, com-

pared to other plant parts like flowers that might appear at certain times of the year. The roots are also said to contain high concentration of the active compounds by the herbalists. However, the frequent use of roots does not pose considerable threat to the survival of these plants since they are only harvested after someone has been bitten by a snake, which does not occur frequently. On the contrary, the frequent usage of leaves and roots in antivenin preparations has also been reported elsewhere [13, 17, 29]. *Nicotiana tabacum, Solanum incanum* were the most frequently mentioned plant species in snakebite treatment. These plant species were also reported to be widely used elsewhere [17, 24, 29] among others.

4.1. Mode of preparation and application

The majority of antidotes from the plants are from freshly collected plant materials. They were prepared by crushing them and applying them in the form of a paste, poultice or cataplasms. Similar observations have been made by other authors [15, 19, 29] among others. Some of the plant species were both taken orally and applied externally. They were usually chewed and swallowed. Similar methods of preparation of the plant species have also been reported to be used in other parts of the world [6, 21, 30, 31]. Decoctions or infusions were prepared for oral administration whereas poultices were prepared for external application for example *Euphorbia hirta* and *Erythrina abyssinica.*

Some plants were recommended for use immediately after the snakebite as a first aid medicine. For others, decoctions were made and drunk by the victim. Oral administration of herbal remedies used for snakebites has also been reported by other authors [17, 25, 30]. Some plants were said to reduce the toxicity of some poisonous plants while some activated compounds in the main plant. For example Samy et al. [3] reported the use of *Musa paradisiaca* decoctions in India. The combining of different plants while preparing snakebite antidotes was practiced by some healers in Mukono and has also been reported in many parts of the world [21]. Most of the plants were also reported to be herbs and harvested from the wild. Coe et al. [30] reported similar findings. A peculiar method of preparation involved the use of milk. Here the root powder of *Abrus precatorius* was dissolved in milk and drunk as a method of treatment.

4.2. Mode of action

Mors et al. [19] have listed different chemical compounds from plants, which are capable of neutralizing snake venoms, including steroids and triterpenes, particularly sistosterol and its glycosides. Such compounds have been reported in some of the plant species documented in this study including, *Euphorbia hirta, Gloriosa superba and Ocimum basilicum.* Flavonoids have been shown to inhibit phospholipases A_2, which are important components of snakebites whereas tannins are also known to have enzyme inhibiting activities [19].

Some extracts from different anti-venim plant species are known act by inhibiting fibrinogen clotting inhibiting haemorrhagic activity and oedema [19]. Other hypotheses advanced to try and explain the activity of these antivenins include the antioxidant hypothesis [32] chelation [19], enzyme-inactivation [33], protein precipitation [34] among others.

4.3. Conclusion

Traditional healers in Mukono use a wide variety of medicinal plant species in treating snakebites. The roots are the most frequently used parts of plant species used as treatment against snakebites. Phytochemical studies and tests should be done on these plants find the active compounds in these plants, and validate claims concerning their safety and efficacy.

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AUTHORS CONTRIBUTION

NR conceived the research idea, wrote the concept, research proposal and conducted the study. AG participated in the research design and drafting the manuscript. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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Research Article

Diversity analysis of entomopathogenic nematodes against *Helicoverpa armigera* (Hübner) from Tarai region of IGP, India

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ABSTRACT

The Indo-Gangetic Plain (IGP) covers approximately 13% of the total geographical area of India and produces nearly 50% of the country's food grains to feed 40% of the total population of the country. The production of grains was, however, not uniform across the IGP regions because of the spatial variation in land-resource characteristics and socioeconomic in the region. Our study includes diversity analysis of entomopathogenic nematodes from Tarai region of IGP (Gorakhpur, Deoria, Kushinagar and Maharajganj Districts), India. The diversity analysis of entomopathogenic nematodes effective against *H. armigera* from soils of Gorakhpur, Deoria, Kushinagar and Maharajganj regions of Gorakhpur division were carried out. The distribution of entomopathogenic nematodes were positively correlated with soil physiological properties such as soil temperature, soil porosity as well as relative humidity. A total of 36 isolates representing three and two different species of *Steinernematid* and *Heterorhabditid* genera were isolated. Based on morphometric data, the nematode species were identified as *Steinernema abbasi*, *S. masoodi*, *S. seemae*, *Heterorhabditis indica* and *H. bacteriophora*. The distribution frequency of entomopathogenic nematodes was found to be 58.3%, 27.8% and 13.9% from cultivated fields, non-cultivated fields and forests/gardens soils respectively. The diversity index (Shannon, Simpson, Margalef's and Pielou index) was also calculated. The PCA analysis was also carried out by using factor 1 and 2 at 58.60% and 25.92% of the total variances respectively.

Keywords: Diversity index, PCA, Entomopathogenic nematodes, *Heterorhabditis* sp., *Steinernema* sp.

1. INTRODUCTION

The demand of food in world has increased dramatically due to the exponential increase in population. Low agricultural productivity is a major cause of poverty, food insecurity, and malnutrition. However, food production per unit of land is limited by many factors, including fertilizer, water, genetic potential of the crop and pests. Despite the plant-protection measures adopted to protect the principal

crops, 42.1% of attainable production is lost as result of attack by pests [1]. Therefore, accelerated public investments are needed to facilitate agricultural growth through high-yielding varieties with adequate resistance to biotic and abiotic stresses [2]. The noctuid moth, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a cosmopolitan pest of major importance in most areas wherever it occurs, damaging a wide variety of horticultural and agricultural crops [3]. It also able to survive in unstable habitats and adapt to seasonal changes as well as it to attack more than 181 plant species belonging to more than 47 families. On an average a 30% crop loss is reported [4]. In India, this pest causes substantial reduction in the grain yield of leguminous crops and the chickpea crop being the major one [5-9]. The ecological and physiological features like direct attack on fruiting structures, voracious feeding, high fecundity, multi-voltinism, occurrence of overlapping generations and ability to diapause during unfavorable conditions have made this pest a 'bugbear', particularly in Eastern regions of Uttar Pradesh state [4, 10, 11]. It is surprising that, the farmers of this region have almost completely stopped the pulse crop cultivation. This can be witnessed from a progressive decline in area and production of pulse crop during the past three decades. Pest control strategies against *H. armigera* including use of pesticides affect soil health which further slow down crop productivity, therefore nowadays Biocontrol Agent were used to control wide varieties of pests.

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* are obligate pathogens that infect a wide range of insect pests including *H. armigera* [12]. These nematodes are mutualistically associated with bacteria in the genus *Photorhabdus, Xenorhabdus* [13, 14]. The third-stage infective juvenile (IJ) of *H. armigera* carries cells of the bacterial symbiont in its intestine. After entering inside the pest body it releases the symbiotic bacterium that kills the pest host within 48h by septicemia by producing antibiotics that prevent other microorganisms from colonizing the cadaver. Researcher nowadays got intense interest to isolate these nematodes from different regions of the world that are climatically adapted and have the potential for biological control of pests in that area. In addition, many countries refuse to import of exotic entomopathogenic nematodes, because they may have a negative impact on non-target organisms [15].

Keeping all these points, extensive surveys as well as physiological effect on distribution of entomopathogenic nematodes have been conducted at different tarai regions (Gorakhpur division) of IGP, India which demonstrating their wide spread occurrence and providing an indication of species which are indigenous for a given particular area.

2. MATERIALS AND METHODS

2.1. Sampling sites and collection of soil samples

Study site for the sampling at Gorakhpur division includes 4 districts (Gorakhpur, Kushinagar, Deoria and Maharajganj). The four districts had coordinates 26°75'88"N 83°36'97"E (Gorakhpur), 26°74'1"N 83°88'8"E (Kushinagar), 26°30'36"N 83°46′48"E (Deoria) and 27°07′48″N 83°34′12″E (Maharajganj) respectively. Soil samples were collected from the four districts at two successive years (2011-13). Sampling was carried out through random-stratified method. The soil samples were collected in triplicate further brought to laboratory and stored at 15^0C in the laboratory. Within a given site, a sample of ca. 1 kg made up of a composite from three subsamples was taken. Each subsample was obtained using a hand trowel from the upper 0-25 cm within an area of 10 m. Samples were placed in a polyethylene bag to minimize dehydration and transported in a cooler to the laboratory. The hand trowel was sterilized with 70% ethanol before leaving the sampling site.

2.2. Isolation and diversity analysis of entomopathogenic nematodes

Soil samples were divided into two parts and processed within 1 week of collection. From one part, 1 kg soil sample was thoroughly mixed, ca. 240 cc of subsample was placed into a 250-cc plastic container, five last instar larvae of the wax moth *Galleria mellonella* (L.)/ *H. armigera* larvae were placed on the soil, and the container was covered with a lid and inverted. The containers were held at room temperature (25±2^{0}C; 75±5 RH) for a period of 12-18 days. Dead larvae were collected

after 12-18 days of incubation and transferred to White traps to collect the emerging IJs. The IJs were pooled from each sample and were used to infect fresh *G. mellonella/ H. armigera* larvae followed by infection in larvae of *H. armigera* to verify their pathogencity and allow for progeny production for identification. The other part of soil that was not used for baiting for nematodes was used for the analysis of soil physiochemical properties including soil textures, pH, humidity, temperatures using standard procedures [16].

2.3. Vertical distribution of entomopathogenic nematodes

To study the vertical distribution of natural population of EPN, depth samplings were carried out from positive samples by boring during two successive years with fixed interval gaps. Depth sampling consisted of 5 soil samples, each 4 cm in diameter and 0, 5, 10, 15, 20 and 25 cm deep, extracted by using a gouge-auger. Each core sample was divided into 5 cm long sections. The isolation method was used by adding larvae of *H. armigera* in each dish for extracting the nematodes. The dish were kept at 25±2°C and covered with a plastic bag to prevent desiccation. After one week, the larvae were recovered and partially dissected to detect the presence of nematodes, and the number of larvae parasitized by nematodes was recorded at each depth. The vertical distribution of the entomopathogenic namatodes, measured as the percentage of *H. armigera* larvae parasitized by nematodes at each depth.

Statistical analysis

The species diversity within a community or habitat is measured by alpha (α) diversity which has two components: species richness and evenness, and is calculated into single indexes. It was calculated by using software Ecosim (version 5.0.2) [17] (http://www.ecosim.ca/ELCWebApp/ecological_land_classification/ELC eTool.html). The identified species were compared using principal component analysis (PCA). PCA was performed to group or separate samples based on the soil biogeochemical parameters (pH, Organic matter, Soil Temperature, Relative Humidity and soil texture) and the entomopathogenic species in each soil sample respectively. All the data were analyzed in triplicate.

3. RESULTS AND DISCUSSION

The occurrence and distribution of entomopathogenic nematodes were assessed throughout an extensive soil survey in tarai regions of Indogangetic plains of India i.e., Gorakhpur, Deoria, Kushinagar and Maharajganj regions respectively. A total of 11216.1 km^2 sampling area covered i.e., 2873.5 km^2 (Gorakhpur), 2,873.5 km^2 (Kushinagar), 2,535 km^2 (Deoria), 2,934.1 km^2 (Maharajganj) regions (Fig. 1).

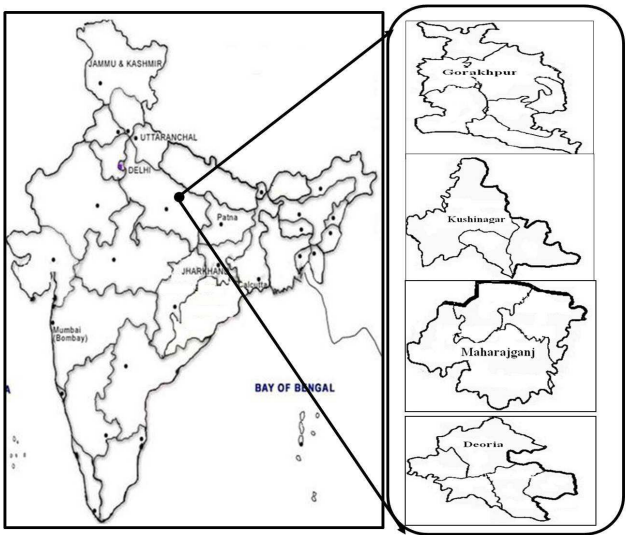

Fig. 1. Map showing the sites of soil sample collection from tarai regions of soil samples of IGP (Gorakhpur regions), India.

Gorakhpur division comes under humid subtropical climate. Annual temperatures of all the districts were reported as 26^0C. The soil samples were taken from three habitats (cultivated field, non-cultivated field and garden/forest) from each district over two year period and were extracted using *H. armigera* bait method in the laboratory. Entomopathogenic isolation results revealed that, the distribution frequency of entomopathogenic nematodes against *H. armigera* also varies as the percentage of nematodes in cultivated fields (58.3%), non-cultivated fields (27.8%) and forests/ gardens (13.9%) respectively. A total of 33.3, 23.8, 23.8 and 19% EPN were procured at cultivated fields of Gorakhpur, Deoria, Kushinagar and Maha-

rajganj districts respectively. Similarly, 30, 30, 20, 20 and 20, 20, 20, 40% EPN were isolated from non cultivated and garden/forest regions fields of Gorakhpur, Deoria, Kushinagar and Maharajganj districts respectively (Table 1). Since long decades, many researchers reported entomopathogenic nematodes from all over world [18-20]. Based on morphometric data, the characteristics of nematode species were compared as described by Stock and Hunt [21] and entomopathogenic nematodes were identified as *S. abbasi*, *S. seemae*, *S. masoodi*, *H. indica* and *H. bacteriophora*. The most common species were found as *S. abbassi*, followed by *H. indica*, *S. masoodi*, *S. seemae* and *H. bacteriophora* respectively. Many researchers also reported above mentioned nematodes as biocontrol agent against *H. armigera* [22, 23].

Soil type is one of the most important factors which affecting the distribution of EPN in the soil. In our survey, the majority of positive samples were reported from sandy-loam followed by loam and least in clay-loam soils respectively (Fig. 2). Sandy-loam harbor maximum number of EPN isolates followed by loam soils might be due to its larger pore size, while clay-loam soils harbor least isolates in survey because of its smaller pore size (Fig. 2). Our survey was also supported by many other researchers [24-29].

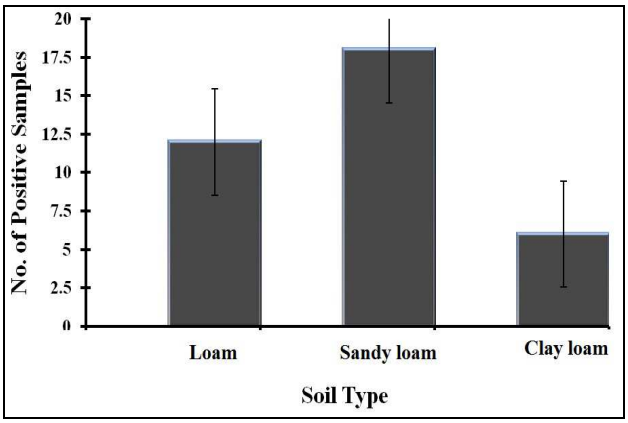

Fig. 2. Recovery of entomopathogenic nematodes from different soil types.

Cultural practice in agricultural soils could also destroy the soil structure and the plant cover. The presence of crop affect EPN population either directly through root action or indirectly through regulate moisture and temperature contents in soil. Many researcher analyses the vertical distribution of EPN in soils [30-34]. The importance for its behavior for the survival of EPN has not yet been studied. Our result shows the pattern of EPN in IGP area which is moist fertile plain of India. The Vertical distribution results revealed that, maximum number of *S. abbasi*, *S. masoodi*, and *S. seemae* population were found at the depth of 0-5 cm, 5-10 cm respectively whereas *H. indica* and *H. bacteriophora* were found more prominent at greater depth i.e., 15-20 cm, and 20-25 cm respectively (Fig. 3). Our survey was also supported by several researchers such as Moyle and Kaya [35], which reported that *Steinernema* prefer surface soils for the search of suitable host, while *Heterorabditis* are more suited for searching the host in the greater depth [36, 37]. Variation in the vertical distribution of EPN might be due to intrinsic and/or extrinsic factors. The intrinsic factors include cultivation cycle of host and presence of different antagonists also [38-40] whereas extrinsic factors include temperature, humidity, structure and organochemical properties of soils [41]. Vertical migration of EPN was also reported to avoid dessication [42]. Several statistical approaches can be used to analyze diversity estimates from the number of species found in relatively small samples [43]. EPN diversity was evaluated by using different diversity indexes such as Margelaf's, Pielou's, Simpsons and Shannon-Wiener indices. All indices indicated a high diversity level of the EPN communities represented by the different entomopathogenic nematode species. The Shannon-Wiener index is determined by species richness, whereas the Simpson index is highly influenced by the abundance of the most common species found in the sample [44] regarding the species richness. Diversity indices were used to determine species richness and evenness into single index, that is, Shannon index, which indicates pattern of diversity, shifting of diversity, and so forth [45]. Our calculations indicated that 1.92, 4.57, 1.56 and 0.96 diversities indexes i.e., Margalef's, Shannon, Simpson and Pielou index were highest in Maharajganj district while lowest in Gorakhpur district i.e., 1.30, 3.33, 1.28, 0.92 respectively, while a total diversity index (α- diversity) of Tarai regions of IGP, India as 3.90, 1.37, 0.83 and 0.99 indexes of Simpson, Shannon, Margalef's and Pielou index were found respec-

tively (Table 2). The implementation of above described techniques will enhance bioprospecting strategies in several respects like presence or absence of species in the environment. The Shannon-Wiener index is determined by species richness, whereas the Simpson index is highly influenced by the abundance of the most common species found in the sample [44] regarding the species richness.

Table 1. Characterization of the soil type and enumeration of entomopathogenic nematodes from Tarai regions of IGP (Gorakhpur Division), India.

Sampling site (Districts with Coordinates)	Sampling Type	Soil texture	pH	Organic matter (%)	Soil humidity (w/w)	Soil temperature (^{0}C)	EPN species
Gorakhpur (26°75'88"N 83°36'97"E)	Cultivated	Sandy loam	7.1 -7.6	0.29	23	26	*S. abbasi*, *S. masoodi*, *S. seemae* and *H. indica.*
	Uncultivated	Loam		0.13	22	25	*S. masoodi*, *S. abbasi* and *H. indica*
	Garden/Forest	Loam		0.34	24	22	*H. indica*
Deoria (26°30'36"N 83°46′48"E)	Cultivated	Sandy loam	7.0-7.6	0.26	23	25	*S. masoodi*, *S. seemae*, *S. abbasi* and *H. indica*
	Uncultivated	Loam		0.12	22	24	*S. abbasi*, *S. masoodi*, and *H. indica*
	Garden/Forest	Clay loam		0.28	24	23	*S. abbasi*
Kushinagar (26°74'1"N 83°88'8"E)	Cultivated	Sandy loam	7.1-7.8	0.32	22	25	*S. abbasi*, *S. masoodi*, *S. seemae* and *H. indica*
	Uncultivated	Sandy-Loam		0.56	21	25	*S. abbasi* and *H. indica*
	Garden/Forest	Clay loam		0.35	24	21	*H. indica*
Maharajganj (27°07′48″N 83°34′12″E)	Cultivated	Sandy loam	7.0-7.5	0.30	20	26	*S. abbasi*, *S. masoodi*, *S. seemae* and *H. indica*
	Uncultivated	Sandy loam		0.16	20	25	*S. abbasi*, *H. bacteriophora*
	Garden/Forest	Loam		0.33	22	23	*H. indica* and *H. bacteriophora*

Table 2. Diversity index of entomopathogenic nematodes from Tarai regions of IGP (Gorakhpur Division), India.

S. No.	Location	Margelaf's (M)	Simpson (1/D)	Shannon (H')	Pielou's (J)
1	Gorakhpur	1.30	3.33	1.28	0.92
2	Deoria	1.36	3.52	1.31	0.94
3	Kushinagar	1.36	3.52	1.31	0.94
4	Maharajganj	1.92	4.57	1.56	0.96
5	Total (α-diversity)	0.83	3.90	1.37	0.99

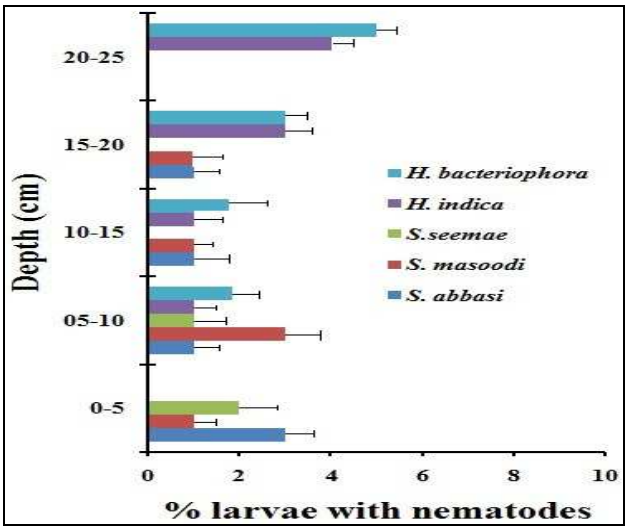

Fig. 3. Presence of *Steiernematid* and *Heterorhabditid* nematodes at five soil depths during various seasons studied (20 samples per season).

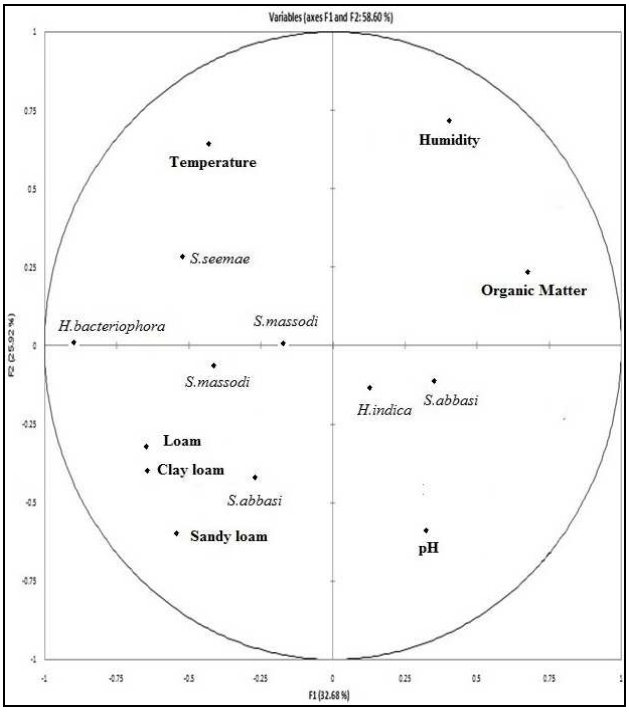

Fig. 4. PCA analysis between the nematodes species and biogeochemical parameters of Tarai regions of soil samples of IGP (Gorakhpur regions), India.

The result of PCA based on soil properties and the principal component factor 1 and 2 explained 58.60 and 25.92% of the total variances. The PCA plot indicates that's *S. seemae, S. masoodi, H. bacteriophora* are closely grouped while their distribution was influenced by temperature and sandy loam soil, while *S. abbasi* and *H. indica* are grouped separately and their distribution is influenced by pH and sandy loam soil type. They remain unaffected with humidity and organic matters (Fig. 4). Soil properties including temperature and humidity may influence the heterogeneity observed in the samples with respect to specific species. Thus it may be implicated that closely associated parameters and a key parameters that influence observed differences in the percentage of specific species. The mapping of EPN in different tarai region of IGP provides us information in understanding the often complex processes. In recent years multivariate techniques such as principal component analysis (PCA) and others have been adopted to demonstrate the relationship between different community composition and environmental factors and have been proven to be more sensitive than univariate methods [46]. Using these techniques, the spatial and temporal variability in EPN community structure along with physio-chemical factors, in Tarai region of IGP have been well documented, and several environmental parameters, such as soil textures, temperatures, humidity and so forth, have been considered to be the key factors driving the changes in community comparison [47, 48]. Other researchers also reported effect of soil physiological parameters such as soil texture**,** soil temperature, pH and relative humidity which limits distribution of EPN in soils [49, 50].

Conclusion

In conclusion, isolation and diversity analysis of entomopathogenic nematodes against *H. armigera* from Tarai regions of IGP, India enable us to understand behavioral as well as ecological requirement in a particular specific niche. On the basis of our investigation, we concluded that the EPN diversity in the Tarai regions of IGP, India is high at both genus and species level. So far, only a few reports were available of systematic investigation of EPN diversity from tarai regions of IGP (Gorakhpur Division), India. The reported nematodes can be important in future bio prospecting in agricultural as well as industrial sectors. Further work emphasis on the determination of its efficacy and potential use of isolated nematodes (reported in the present study) as well as its application in various sectors respectively.

AUTHORS CONTRIBUTION

SPS: conception and design; AKY: development of methodology; SV: Acquisition of data; CPMT: Writing, revised and revision of the manuscript; Administrative, technical or material support as well as study supervision. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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Research Article

Micro- and macrofungal diversity in Langol herbal garden Manipur, India

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ABSTRACT

Studies on the taxonomy and diversity of macro fungi are gaining importance as many macro fungi are becoming extinct and facing threat of extinction because of habitat destruction. Present study deals with the diversity of macrofungi and its association with microfungi in Langol herbal garden, under central forest division Manipur, India. A total of 9 different macrofungal species namely *Amanita vaginata*, *Amanita fulva*, *Boletellus pseudochrysenteroides*, *Cuphophyllus pratensis*, *Lactarious helvus*, *Russula fragilis*, *Xerula radicata*, *Agaricus bisporus* and *Tylopilus felleus* were collected and identified based on their morphological and microscopic characteristics. Soil samples were analyzed for the microflora association with the different mushroom growing soil. A total 51 fungal species belonging to four phylum namely Ascomycota (44), Zygomycota (5), Oomycota (1), and Deuteromycota (1) were identified. Among the species *Aspergillus niger*, *Rhizopus stolonifer* and *Trichoderma harzianum* were the dominant species which were distributed in all the soil samples indicating that soils with these micro fungi were most favourable for the growth of these macrofungi.

Keywords: Diversity, Microfungi, Macrofungi, Relative frequency.

1. INTRODUCTION

Manipur is one of the North Eastern states of India, which is situated between 23.80°N to 25.68°N latitudes and 93.03°E to 94.78°E longitudes. The total geographical area of the state is 22,327 sq. Km, of which, about nine-tenths constitute the hills. It is surrounded by Nagaland on the North, Assam on the West and Mizoram on the South and along the East, and it shares a 398 Km long international boundary with Myanmar. The forest cover of Manipur is 17,219 sq. km, which is 77.12% of the total geographical area of the State. Macrofungi are abundant in these forests on the forest-floor, wigs and branches, rotting plant parts, and in mycorrhizal association with higher plants. They form fruiting bodies of different shapes e.g. such as agarics, boletales, jelly fungi, coral fungi stinkhorns, bracket fungi, puffballs and bird's nest fungi, etc. The first list on Indian Fungi was published by Butler and Bisby [1] and then revised by Vasudeva [2]. Several additional lists appeared in between culminating with the fungi of India [3]. These species in fact can be consi-

dered as the indicators of the forest life support system [4]. The presence or absence of fungal species is a useful indicator to assess the damage or the maturity of an ecosystem. Data on their diversity in different vegetation types is important for planning and managing ecosystem biodiversity [5]. Food values of *Lentinula edodes* (Berk.), *Agaricus bisporous* (J.E. Lange), *Pleurotus sajor-caju* (Fries) and *Volvariella volvacea* (Bull. ex Fries) have been reported by Singh et.al. [6]. 34 species of mushrooms have been reported [7] from Manipur. *Pleurotus ostreatus*, an edible mushroom, which is locally called "Uyen" is sold in the market at Rs. 60-80/kg because of its high demand and high nutritive value [8]. Market survey conducted by Singh et al. reported 33 species of wild edible fleshy fungi [9]. Nutrient values of *Termitomyces eurrhizus* (Berk.) Heim has been studied [10]. Some of the wild edible mushrooms have also been reported from Manipur and Arunachal Pradesh [11, 12]. Fleshy fungal flora belonging to the family Auriculariaceae, Clavariaceae, Cantharellaceae, Tricholomataceae, Pluteaceae, Paxillaceae, Cortinariaceae, Cycoperdaceae, and Sclerodermataceae of Basidiomycotina and Halvellaceae of Ascomycotina have been from Manipur and Meghalaya [13]. Verma et al. [14] recorded ninety five species of higher fungi from a macrofungal survey of the NEH. Among these, 85 species were new records from the NEH region. Although comparatively a small geographical area, Manipur has the distinction of wide range of climate ranging from temperate alpine to tropical, and consequently has a wide range of forests and biodiversity hotspots. Most of these hotspots are inhabited by a large number of ethnic groups, who are economically poor, but hold a treasure house of traditional knowledge on nutritional and medicinal value of these bioresources. Conservation of biodiversity and management of the bioresources are two important issues that need to be addressed at local, regional, national and international levels [7]. A study was conducted to benchmark the diversity of macro fungi with respect to their morphological distribution, habitat and edibility of these species in semi evergreen and deciduous forest of Herbal garden Langol, under Central Forest Division in Manipur.

2. MATERIALS AND METHODS

2.1. Study area

The Langol herbal garden (Figure 1) under Central Forest Division in Manipur, is located at 24°52'15.4N 93°53'90.2E. It is spread over 15 ha. area having *Pinus roxburghii* trees along with medicinal plants species like *Piper longum*, *Smilex glabra*, *Aloe barbadensis*, *Ocimum sanctum*, *Emblica officinalis*, *Paris polyphylla*, *Asparagus racemosus* etc.

Fig. 1. Map of herbal garden, Langol, Manipur, India.

The survey of these fungi were conducted by following the techniques of Susan and Van [15] during rainy season (June to September in 2013) and again from June to July in 2014. Fruiting bodies were wrapped in the wax paper and brought to the laboratory for identification and edibility analysis. The identification was done on the basis of macro and microscopic characteristic following available literatures [16-18]. The soft and hard textured specimens were preserved in 2% and 4% formaldehyde respectively and kept in museum of Forest Protection Division, Rain Forest Research Institute, Jorhat, Assam by assigning identification number. The traditional knowledge on the wild mushrooms were gathered from the local tribes to know their edibility and medicinal values.

2.2. Soil sample collection

The aim of the soil study was to carry out a detailed investigation on the occurrence and distribution of microfungi associated with mushroom growing soil of different macrofungi.

Soil samples were collected from the upper 0 -15 cm depth near the mushroom fruiting bodies. Collected samples were brought to the laboratory and sieved through 2 mm sieve at field moisture conditions.

2.3. Fungal population count

For fungal population analysis, serial dilution plate method [19] was followed using Rose Bengal Agar medium [20], supplemented with streptomycin sulphate. The inoculated Petri plates were incubated in a sterile culture room at 25° ± 1°C. Colony forming units (CFU) were estimated by counting the number of colonies after five days. Fungal colonies formed were calculated on per gram dry soil basis. Fungi were identified according to their macroscopic and microscopic features. Identification at the species level was carried out according to the morphological characters by using standard publications [21-26]. Pure cultures of fungi were maintained in test tube slants containing Czapex Dox agar medium [27] and preserved in deep freezer at -20°C.

2.4. Calculations of occurrence and frequency of fungi

Occurrences and frequencies of fungi occurring in soil were calculated and categorized by the procedures described by Saksena [28] as per formulae given below:

$$\% \text{ Occurrence} = \frac{\text{Number of colonies of individual species in all the quadrates studied}}{\text{Total number of colonies of all the species}} \times 100$$

$$\% \text{ Frequency} = \frac{\text{Number of plates containing particular fungus}}{\text{Total number of plates}} \times 100$$

Categorization of frequency classes:

Class	% Frequency	Category
I	1-20	Rare
II	21-40	Occasional
III	41-60	Frequent
IV	61-80	Common
V	81-100	Dominant

Categorization of soil sample classes:

Amanita vaginata	I
Amanita fulva	II
Boletellus pseudochrysenteroides	III
Cuphophyllus pratensis	IV
Lactarious helvus	V
Russula fragilis	VI
Xerula radicata	VII
Tylopilus felleus	VIII

3. RESULTS

The Northeast India has a rich mycobiodiversity that is yet to be fully explored. Nine species of macrofungi belonging to 6 families were identified (Table 1, Figure 2). Family Amanitaceae, Russulaceae and Boletaceae had two species while all other families i.e. Agaricaceae, Hygrophoraceae, and Physalacriaceae were found to have only one species each. Out of 9 macrofungi, 7 mushrooms namely *Amanita vaginata*, *Amanita fulva*, *Boletellus pseudochrysenteroides*, *Lactarius helvus*, *Russula fragilis*, *Xerula radicata* and *Tylopilus felleus* are considered as inedible while *Cuphophyllus pratensis* and *Agaricus bisporus* are considered as edible. A brief description of these species is mentioned below.

Amanita vaginata (Bull.) Lam.

Cap grey to grey-brown, 4.5-9.5 cm broad, convex, expanding to plano-convex, centrally depressed with a low umbo and margin decurved; Gills narrowly attached to free, close, thin, white to pallid. Stem 5-11 cm long, 1.2-2.0 cm thick, equal, not bulbous, hollow; universal veil white, membranous, attached near the stipe base and partial veil absent. Spores measure 7.5-10.5 x 6.5-9.4 µm, subglobose to globose, smooth and thin-walled.

Amanita fulva (Schaeff.) Secr.

Cap 3-8 cm; convex, sticky, tawny brown; with a few scattered white patches; Gills: free from the stem; whitish and crowded; Stem 6-14 cm long; 0.5-1 cm thick; without a ring, white volva presents at the base. Spores measure 8.5 x 10.5 µm; smooth; subglobose, hyaline and basidia 4-spored.

Fig. 2. Wild macrofungi at Langol herbal garden, Manipur, India. A. *Amanita vaginata*, B. *Oudemansiella radicata*, C. *Amanita fulva*, D. *Agaricus bisporus*, E. *Tylopilus felleus*, F. *Boletellus pseudochrysenteroides*, G. *Russula fragilis*, H. *Cuphophyllus pratensis*, I. *Lactarius helvus.*

Table 1. List of identified wild mushrooms from Langol herbal garden under Central Forest Division Manipur.

Order	Family	Genus	Species	Edibility	ID Number
Agaricales	Agaricaceae	*Agaricus*	*Agaricus bisporus* (J.E.Lange) Emil J. Imbach	Edible	MN/RFRI/00057
Agaricales	Amanitaceae	*Amanita*	*Amanita vaginata* (Bull.) Lam.	Edible	MN/RFRI/00059
Agaricales	Amanitaceae	*Amanita*	*Amanita fulva* (Schaeff.) Secr.	Edible	MN/RFRI/00068
Boletales	Boletaceae	*Boletellus*	*Boletellus pseudochrysenteroides* A.H. Sm. & Thiers	Unknown	MN/RFRI/00072
Boletales	Boletaceae	*Tylopilus*	*Tylopilus felleus* (Bull.)P.Karst.	Non edible	MN/RFRI/00073
Agaricales	Hygrophoraceae	*Hygrocybe*	*Cuphophyllus pratensis* (Schaeff.) Murrill	Edible	MN/RFRI/00056
Russulales	Russulaceae	*Lactarius*	*Lactarius helvus* (Fr.) Fr.	Edible	MN/RFRI/00059
Russulales	Russulaceae	*Russula*	*Russula fragilis* (Pers.) Fr.	Non edible	MN/RFRI/00060
Agaricales	Physalacriaceae	*Xerula*	*Xerula radicata* (Relhan) Dörfelt	Non edible	MN/RFRI/00087

Boletellus pseudochrysenteroides A.H. Sm. & Thiers

Pileus 5-10 cm wide, broadly convex, margin incurved, surface dry, velvety, dark, rose red, whitish to pinkish in the cracks; Pore surface yellow, depressed near the stipe. Pores were angular 1-1.5 mm wide, tubes 8-2 mm deep; Stipe 6-9 cm long, 9-14 mm thick, equal, dry, solid, colour like the pileus, partial velly and annulus absent. Spore measure 11-14 x 5.5-8 µm, subelliptic, ornamented with 9-12 µm, sublongitudinal ridges and pale amber-brown.

Cuphophyllus pratensis (Schaeff.) Murrill

Cap 2-9 cm across, flattened with broad umbo with ochraceous color; Stem 15-40 x 10-15 mm, tapering towards the base; Gills decurrent, widely spaced, pale buff. Spores measure 4.5-6.5 x 4.5-5 µm, ovoid to subglobose.

Lactarious helvus (Fr.) Fr.

Cap 3.5-11 cm; convex with an inrolled margin, dry and smooth; Gills attached to the stem, close and yellow in color; Stem 2.5-8 cm long and 2 cm thick; equal; dry and smooth. Spores measure 5.5-8.5 x 5-7.5 µm; ellipsoid; ornamentation up to 1 µm high.

Russula fragilis (Pers.) Fr.

Cap 2-6 cm across, convex, depressed with purple-violet color; Gills adnexed, pale cream; Stem 20-40 x 5-12 mm, white, club-shaped. Spores measure 6.5-8.5 x 5-7.5 µm; globose with warts up to 0.3 µm high.

Xerula radicata (Relhan) Dörfelt

Cap 3-8 cm across, bell-shaped to convex then flattened with a broad umbo, olive-brown, radially wrinkled, slimy; Stem 70-210 × 5-8mm, concolorous with cap colour. Gills were broad, white. Spores measure 10-14 × 10-12 µm; elliptic.

Tylopilus felleus (Bull.) P. Karst.

Cap 5-10 cm, fulvous brown and smooth; Stem 60-100 long creamy color, Pores similarly coloured. Spores measure 10-14 x 4-5 µm; sub-fused.

Agaricus bisporus (J.E.Lange) Emil J. Imbach

Cap 4-10 cm, broadly convex with incurved margin, surface dry, with light brown, innate scales over a pallid; flesh thick and white; Gills free, purple-brown to blackish-brown; Stem 2-5 cm x 2.5 cm, equal to slightly bulbous at base; smooth; veil white, cottony-membranous, forming a medial to superior ring. Spores measure 5-7.5 x 4.5-5.5 µm, elliptical and smooth.

Table 2. Frequency and occurrence of different fungi associated in mushroom growing soil in Langol herbal garden under Central Forest Division Manipur, India.

Sl. No	Name of fungi	Soil sample															
		I		II		III		IV		V		VI		VII		VIII	
		F	O	F	O	F	O	F	O	F	O	F	O	F	O	F	O
1	*Acropkialophora nainiana*	III	8.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	*Alternaria brassicae*	II	3.8	-	-	II	3.6	I	2.5	-	-	-	-	-	-	-	-
3	*Aspergillus flavus*	IV	18.0	II	4.6	II	3.7	II	8.6	I	2.1	I	2.6	-	-	II	4.2
4	*Aspergillus fumigates*	-	-	II	4.2	-	-	-	-	II	4.6	-	-	-	-	-	-
5	*Aspergillus nidulans*	-	-	-	-	-	-	-	-	-	-	-	-	II	4.2	-	-
6	*Aspergillus niger*	IV	16.2	III	11.4	II	4.2	V	28.4	IV	16.2	IV	18.6	III	11.5	III	11.2
7	*Aspergillus oryzae*	-	-	-	-	-	-	II	10.0	-	-	-	-	-	-	-	-
8	*Aspergillus tamari*	II	3.7	II	3.8	I	2.4	-	-	-	-	I	2.4	-	-	-	-
9	*Aspergillus terreus*	-	-	-	-	-	-	-	-	-	-	II	2.3	-	-	-	-
10	*Aspergillus versicolor*	II	4.2	II	4.6	-	-	-	-	II	4.2	II	4.6	-	-	-	-
11	*Aspergillus wentii*	-	-	II	3.6	-	-	II	3.4	-	-	-	-	II	3.5	-	-
12	*Chaetomium bostrychoides*	II	3.2	-	-	-	-	-	-	II	3.4	-	-	-	-	-	-
13	*Chaetomium globosum*	-	-	-	-	-	-	I	1.6	-	-	-	-	-	-	-	-
14	*Choanephora cucurbitarum*	III	10.2	-	-	-	-	-	-	-	-	-	-	II	8.6	-	-
15	*Cladosporium berbarum*	II	4.2	-	-	-	-	II	2.8	-	-	-	-	II	3.7	-	-
16	*Cladosporium cladosporioides*	II	3.2	II	3.6	-	-	I	2.4	-	-	-	-	-	-	-	-
17	*Cladosporium herbarum*	-	-	-	-	I	2.4	I	1.2	-	-	-	-	-	-	-	-
18	*Cladosporium macrocarpus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	1.5
19	*Cladosporium oxysporum*	V	28.2	-	-	III	13.0	II	2.4	-	-	I	1.5	-	-	III	12.0
20	*Cochliobolus sativus*	-	-	-	-	-	-	-	-	I	1.5	-	-	-	-	-	-
21	*Cunninghamella echinulata*	V	27.5	II	3.6	III	12.5	-	-	II	2.8	II	2.4	I	1.2	II	2.8
22	*Cunninnghumella elegans*	-	-	-	-	-	-	-	-	-	-	III	10.2	-	-	II	7.8
23	*Curvularia pallascens*	-	-	-	-	-	-	-	-	-	-	II	6.2	-	-	-	-

Sl. No	Name of fungi	Soil sample															
		I		II		III		IV		V		VI		VII		VIII	
		F	O	F	O	F	O	F	O	F	O	F	O	F	O	F	O
24	*Cylindrocladium scoparium*	-	-	-	-	-	-	-	-	-	-	II	6.4	-	-	-	-
25	*Fusarium moniliforme*	III	10.8	-	-	-	-	-	-	III	8.2	-	-	-	-	-	-
26	*Fusarium oxysporum*	V	28.2	III	11.5	II	6.2	II	3.5	-	-	-	-	V	26.2	III	10.2
27	*Fusarium redolens*	-	-	-	-	-	-	-	-	I	1.2	-	-	-	-	-	-
28	*Fusarium semitectum*	I	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	*Fusarium solani*	V	20.2	-	-	-	-	V	20.5	III	10.2	III	11.5	-	-	-	-
30	*Fusarium solenoid*	III	8.2	-	-	-	-	-	-	-	-	-	-	III	11.5	-	-
31	*Fusarium sporotrichioides*	-	-	I	1.2	-	-	-	-	-	-	-	-	-	-	-	-
32	*Gliocladium catenulatum*	II	5.3	-	-	-	-	-	-	-	-	-	-	II	6.5	-	-
33	*Gliocladium roseum*	-	-	-	-	III	8.2	-	-	-	-	-	-	-	-	-	-
34	*Gongronella butleri*	-	-	-	-	-	-	-	-	-	-	I	1.2	-	-	-	-
35	*Gymnoascus ressii*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	3.5
36	*Humicola fuscoatra*	-	-	-	-	-	-	I	2.5	-	-	-	-	-	-	-	-
37	*Humicola grisea*	-	-	-	-	-	-	-	-	-	-	I	1.4	-	-	-	-
38	*Nigrospora sphaerica*	III	10.2	I	1.6	-	-	-	-	-	-	-	-	-	-	-	-
39	*Penicillium funiculasum*	V	22.1	II	6.2	II	5.3	-	-	III	10.2	II	6.4	I	2.5	I	1.6
40	*Penicillium vermiculatum*	-	-	-	-	-	-	I	1.2	-	-	-	-	-	-	-	-
41	*Periconia digitata*	-	-	-	-	-	-	-	-	-	-	I	1.2	-	-	-	-
42	*Pestalotiopsis theae*	II	8.2	-	-	I	1.2	-	-	-	-	-	-	I	2.5	-	-
43	*Pestalotiopsis versicolor*	-	-	-	-	-	-	-	-	II	4.3	-	-	-	-	-	-
44	*Rhizopus nodosus,*	-	-	-	-	III	10.2	-	-	-	-	-	-	-	-	I	1.2
45	*Rhizopus stolonifer*	V	20.2	IV	18.6	I	1.4	V	22.2	III	11.4	I	1.4	II	6.4	II	4.3
46	*Tetraploa aristata*	-	-	-	-	-	-	-	-	II	4.3	-	-	-	-	-	-
47	*Trichoderma harzianum*	V	27.5	V	26.3	II	10.0	IV	16.7	IV	16.7	V	27.5	III	10.2	II	11.2

Sl. No	Name of fungi	Soil sample															
		I		II		III		IV		V		VI		VII		VIII	
		F	O	F	O	F	O	F	O	F	O	F	O	F	O	F	O
48	*Trichoderma koningii*	III	13.8	-	-	-	-	-	-	-	-	-	-	-	-	II	7.8
49	*Trichoderma virens*	-	-	-	-	-	-	-	-	I	1.2	-	-	-	-	-	-
50	*Trichoderma viride*	I V	14.2	-	-	III	10.4	-	-	-	-	-	-	-	-	II	4.6
51	*Volutella concentric*	-	-	-	-	-	-	I	1.4	-	-	-	-	-	-	-	-

F = frequency; O = occurrence. The Roman numbers show the frequency classes and the Arabic numbers represent percentage frequency and occurrence of fungi.

Table 3. Frequency and occurrence of microfungi in Langol herbal garden under Central Forest Division Manipur, India.

Sl. No	Name of fungi	Phylum	Order	Family
1	*Alternaria alternate* Fr. Keissl	Ascomycota	Eurotiales	Pleosporaceae
2	*Alternaria brassicae.* (Berk.) Sacc.	Ascomycota	Eurotiales	Trichocomaceae
3	*Aspergillus flavus* Link	Deuteromycota	Eurotiales	Trichocomaceae
4	*Aspergillus fumigates* Fresenius	Ascomycota	Eurotiales	Trichocomaceae
5	*Aspergillus nidulans* G Winter	Ascomycota	Eurotiales	Trichocomaceae
6	*Aspergillus niger*van Tieghem	Ascomycota	Eurotiales	Trichocomaceae
7	*Aspergillus oryzae* (Ahlburg) E. Cohn	Ascomycota	Eurotiales	Trichocomaceae
8	*Aspergillus tamari* Micheli	Ascomycota	Eurotiales	Trichocomaceae
9	*Aspergillus terreus* Thom	Ascomycota	Eurotiales	Trichocomaceae
10	*Aspergillus versicolor* (Vuillemin) Tiraboschi	Ascomycota	Eurotiales	Trichocomaceae
11	*Aspergillus wentii* whemer	Ascomycota	Sordariales	Chaetomiaceae
12	*Chaetomium diversum* Lodha	Ascomycota	Sordariales	Chaetomiaceae
13	*Chaetomium globosum.* Kunze ex Fr.	Ascomycota	Mucorales	Choanephoraceae
14	*Choanephora cucurbitarum* (Berk. & Ravenel) Thaxt.	Oomycota	Capnodiales	Mycosphaerellaceae
15	*Cladosporium berbarum* (Pers.) Link	Ascomycota	Capnodiales	Mycosphaerellaceae
16	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries	Ascomycota	Capnodiales	Mycosphaerellaceae
17	*Cladosporium herbarum* (Pers.) Link	Ascomycota	Capnodiales	Mycosphaerellaceae
18	*Cladosporium macrocarpus* Preuss	Ascomycota	Capnodiales	Mycosphaerellaceae
19	*Cladosporium oxysporum* Berkely & M.A. Curtis	Ascomycota	Pleosporales	Pleosporaceae
20	*Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur	Ascomycota	Mucorales	Cunninghamellaceae
21	*Cunninghamella echinulata* (Thaxt.) Thaxt. ex Blakeslee	Zygomycota	Mucorales	Cunninghamellaceae
22	*Cunninnghumella elegans* Lendner	Zygomycota	Pleosporales	Pleosoporaceae
23	*Curvularia pallescens* Boidijin	Ascomycota	Pleosporales	Pleosoporaceae
24	*Cylindrocladium scoparium* Morgan	Ascomycota	Pleosporales	Pleosporaceae

Sl. No	Name of fungi	Phylum	Order	Family
25	*Fusarium moniliforme* (Sawada) Wollenw.	Ascomycota	Hypocreales	Nectriaceae
26	*Fusarium oxysporum* Schlecht. emend Snyder & Hansen	Ascomycota	Hypocreales	Nectriaceae
27	*Fusarium redolens* Wollenw	Ascomycota	Hypocreales	Nectriaceae
28	*Fusarium semitectum* Berk. & Ravenel	Ascomycota	Hypocreales	Nectriaceae
29	*Fusarium solani* (Mart.) Sacc.	Ascomycota	Hypocreales	Nectriaceae
30	*Fusarium equiseti* (Corda) Sacc.	Ascomycota	Hypocreales	Nectriaceae
31	*Fusarium sporotrichioides* Sherb.	Ascomycota	Hypocreales	Nectriaceae
32	*Gliocladium catenulatum* J.C. Gilman & E.V. Abbott	Ascomycota	Hypocreales	Hypocreaceae
33	*Gliocladium roseum (*Link) Schroers	Ascomycota	Hypocreales	Bionectriaceae
34	*Gongronella butleri* (Lendn.) Peyronel & Dal Vesco	Zygomycota	Mucorales	Cunninghamellaceae
35	*Gymnoascus reessii* Baran.	Ascomycota	Onygenales	Gymnoascaceae
36	*Humicola fuscoatra* Traaen	Ascomycota	Sordariales	Chaetomiaceae
37	*Humicola grisea* Traaen	Ascomycota	Sordariales	Chaetomiaceae
38	*Nigrospora sphaerica* (Sacc.) E.W. Mason	Ascomycota	Trichosphaeriales	Trichosphaeriaceae
39	*Penicillium funiculosum* Thom	Ascomycota	Eurotiales	Trichocomaceae
40	*Penicillium vermiculatum.* P.A. Dang., Le Botaniste	Ascomycota	Eurotiales	Trichocomaceae
41	*Periconia digitata* (Cooke) Sacc	Ascomycota	Pleosporales	Dothideomycetes
42	*Pestalotiopsis theae* (Sawada) Steyaert	Ascomycota	Xylariales	Amphisphaeriaceae
43	*Pestalotiopsis versicolor* (Speg.) Steyaert	Ascomycota	Xylariales	Amphisphaeriaceae
44	*Rhizopus nigricans* Ehrenberg	Zygomycota	Mucorales	Mucoraceae
45	*Rhizopus stolonifer* Ehrenb	Zygomycota	Mucorales	Mucoraceae
46	*Tetraploa aristata* (Scheuer) Kaz. Tanaka & K. Hirayama	Ascomycota	Pleosporales	Tetraplosphaeriaceae
47	*Trichoderma harzianum* Rifai	Ascomycota	Hypocreales	Hypocreaceae
48	*Trichoderma koningii* Oudem	Ascomycota	Hypocreales	Hypocreaceae
49	*Trichoderma virens* (J.H. Mill., Giddens & A.A. Foster) Arx	Ascomycota	Hypocreales	Hypocreaceae
50	*Trichoderma viride Pers.*	Ascomycota	Hypocreales	Hypocreaceae
51	*Verticillium dahlia* Kleb.	Ascomycota	Hypocreales	Incertae sedis

A total of 51 fungal species (Table 3) were isolated from eight mushrooms collected near the soil samples. The list of fungal species isolated from the different soil samples is depicted in Table 2. These species belong to four phyla viz. Ascomycota (44), Zygomycota (5), Oomycota (1), Deuteromycota (1), and 16 family viz. Nectriaceae (7), Hypocreaceae (5), Bionectriaceae (1), Cunninghamellaceae (3), Gymnoascaceae (1), Chaetomiaceae (4), Trichosphaeriaceae (3), Dothideomycetes (1), Amphisphaeriaceae (2), Mucoraceae (2), Tetraplosphaeriaceae (1), Incertae sedis (1), Pleosporaceae (5), Trichocomaceae (9), Choanephoraceae (1) and Mycosphaerellaceae (5) have been recorded from mushroom growing soil of Langol herbal garden, under central forest division Manipur, India.

Out of 51 microfungal species only seven species which were associated with mushroom growing soil of *Amanita vaginata* (I), *Cuphophyllus pratensis* (IV), *Xerula radicata* (VI) and *Tylopilus felleus* (VII) namely *Cladosporium oxysporum*, *Cunninghamella echinulata*, *Trichoderma harzianum*, *Fusarium solani*, *Rhizopus stolonifer*, *Aspergillus niger* and *Fusarium oxysporum* showed highest frequency and categorized under dominant frequency (V) and exhibited highest percentage of occurrence.

Simultaneously eight fungi namely *Chaetomium globosum*, *Cladosporium herbarum*, *Cochliobolus sativus*, *Fusarium sporotrichioides*, *Humicola fuscoatra*, *Humicola grisea*, *Trichoderma virens* and *Volutella concentric* were categorized under modest frequency (I) due to their lowest percentage of occurrence.

4. DISCUSSION

This region of Northeast India has a rich myco-biodiversity that is yet to be fully explored. This study was an attempt to survey and collect valuable wild forms of mushrooms to know the myco treasure in association and on surface of the forest lands. The nutrients composition of indigenous *Termitomyces eurrhizus*, fungus combs, *Tetmitomyces* growing soil and saw dust of *Phoebe* sp. in Manipur were determined and reported [10]. Some of the wild edible mushrooms have been reported from Manipur and Arunachal Pradesh of North East India [11, 12]. Description of fleshy fungal flora of the NEH India from Manipur and Meghalaya has been made [13] and 34 mushroom species has been reported [7] from Manipur, Baruah et al. reported few Basidiomycetous fungus from Sibsagar District, of Assam, Northeast, India [29].

5. CONCLUSIONS

Despite the importance of indigenous mushrooms in our lives, their production and consumption is still hampered by a general lack of awareness in the society. The present study revealed that *Aspergillus niger*, *Rhizopus stolonifer* and *Trichoderma harzianum* were the only genus which were distributed in all the soil samples collected from mushroom growing areas indicating that soil with these micro fungi are most favourable for the growth of macrofungal species. There is need for a wider study area so as a complete representation of the fungal diversity and beneficial aspects of these significant macro and micro fungal flora can be explored.

AUTHORS CONTRIBUTION

Conception and design: RK; Development of methodology: RK, GM and KK; Acquisition of data: RK, RB; Analysis and interpretation of data writing, review and/or revision of the manuscript, administrative, technical or material support: RK; Study supervision: NS. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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