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Cultivating onion after paddy – Is it suitable and profitable?

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ABSTRACT

Two varieties of hybrid onion i.e. Orient, a red skin variety and OL, yellow skin variety after field testing on research stations in Wengkhar were tested in farmer's field through research outreach program involving research, extension and farmers in three sites(Thridangbi, 900 m amsl, Chali, 1300 m amsl and Tangmachu, 1700 m amsl) for testing of onion in paddy fallow system and study the cost of production. These varieties maturing between 180-200 days were found to be suitable in paddy fallow system and Orient variety was more preferred by farmers than OL. Economic analysis based on estimates showed that onion can be a profitable crop and indentified cost of transportation, storage and seed as a major factor in net returns.

KEY WORDS: Bulb onion, Orient, OL, rice fallow system

INTRODUCTION

Irrigated wetlands are used mainly for paddy cultivation from June to Nov and are mostly left fallow after paddy harvest except for some parts of Bhutan where winter crops such as wheat chilies and potato are cultivated. For most part of the year, paddy field are either kept fallow or used as a grazing area for cattle. Potential crops such as cole crops, potatoes and chilly cultivation in eastern part of Bhutan has not picked up due to reasons such as lack of irrigation water, labour shortage, knowledge on winter vegetable cultivation, winter vegetables marketing and damage by stray cattle.

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Although this practice is a traditional sustainable land use system allowing fertility build up for paddy for which adequate level of soil fertility management is not provided. The fallow period if utilized can contribute to enhancing productivity of land.

Under these situations, it is becoming important for adaptive research and technology promotions to test and try alternative crops apart from those mentioned above. Tshering et.al, 2010 evaluated six different varieties of onion across low, mid and high altitude and concluded the suitability of onion as a crop after paddy and suggests evaluation of additional varieties and cost analysis.

RNR RDC Wengkhar and the Agriculture Research and Extension Project funded by JICA have also been carrying out on station trials in Wengkhar on hybrid varieties of onion such as OL, Orient (red), Orient (yellow) and are found to be successful. The project through its outreach programs have taken two varieties Orient and OL on farm trials in the paddy fallow system in three sites Thidangbi, Chali and Jangdung in Mongar and Tangmachu under Lhuentse.

MATERIALS AND METHODS

Two varieties of onion (Orient- a red variety and OL- a white variety) were tested in three pilot sites at Thidangbi at 900 m amsl under Saling Geog of Mongar; Chali at 1300 m amsl under Mongar and at Tangmachu at 1700 m amsl under Lhuentse. The crops were cultivated in six farmers' groups with a total membership of 48 farmers covering a total area of about 4.6 acres (Table 1). In addition to the collaborators, some 35 farmers from neighboring villages were also involved as participants during the field days. A field day was organized to create awareness for promotion of the crop in the subsequent season.

Seedlings raised in end of September to early October in the farmer's field were ready for transplanting after 80-90 days from seed sowing (Table 2). Immediately after the paddy harvest i.e. end of Nov to early Dec, collaborating farmers ploughed the fields. The fields were left exposed to natural sun light for few days. Prior to planting, soil was pulverized and a raised bed was prepared. Seedlings were transplanted at a spacing of 20 X 20 cm from plant to plant and row to row in the raised bed.

Tab. 1. Site area and number of farmers

Sites	Total area (ac)	Farmer Collaborators	Field day participants
Chali	4	48	35
Tangmachu	0.1	3	16
Thidangbi	0.5	9	30
Total	4.6	60	81

Suphala (6 kg per site) was provided for top dressing in addition to farm yard manure applied by farmers. Crops were weeded 2 to 3 times manually irrigated twice as recommended in RNR RC Khangma, 2003 and Phuntsho, et.al., 2010. Flower stalks were removed to induce bulb formation. The trial was monitored by conducting field visits by researchers and extension officers.

A field day was organized with the collaborating farmers to assess the crops and to create awareness among other farmers in the locality. Random crop cuts in quadrants of 1 m^2 with at least three replicates were taken. Sample weights which was fresh weight at the time of crop cut was later corrected to its weight after curing by taking samples of weight after curing. Crop cut data was analyzed Genstat for windows 10.1 Rothamstead Experimental station and Windows MS Excel.

A farmers participatory assessment of the crops based on farmers criterion was carried during the field day at the time of harvest in order to understand farmers preference for cultivation of the crop in the subsequent season.

And finally, a financial analysis of cultivating onion was carried out using the average cost of production, the total production calculated as per crop cut data and current market price of onion in the local markets. These costs were then calculated for an acre of land for uniformity and comparison. An approximate constant of 70 % family labor and 30 % hired labor is used to calculate the net returns per man day.

Limitations of the study

The findings of this study is based on only one season and since the activity was mainly to create awareness on cultivating onion after paddy, the study although followed basic recommended practices did not study production parameters such as uniform crop management, soil fertility

level, temperature, day length and irrigation. The inferences drawn were also based on the crop cuts, estimates, field observation and farmers assessment of the crop.

RESULT AND DISCUSSION

Mean Crop yield

Significant yield differences between these varieties were observed at each location (lsd 3.921 in Thidangbi, 0.504 in Chali and 3.62 in Tangmachu). However, the differences were not consistent for varieties. OL yields higher than Orient in two locations while it yielded less than Orient in Chali (Table 2). The lower yield in Chali was mainly due to in adequate crop management practices followed by some farmers as compared to others. Although, it appears that the two varieties give higher yield in higher altitudes as studied by Tshering, et.al., 2010, this trial due to the lower yield in Chali (mid altitude) could not reinforce the increasing trend. However, crop observation in Tangmachu showed larger and more uniform bulb size of OL as compared to other sites.

Comparison of the mean yield across all sites showed that OL yields higher than Orient with an average yield of 12.16 and 8.93 tons per acre respectively. However, an assessment of the % loss in weight after curing showed that OL is prone to damage from rotting and has higher % loss after curing. Only about 48 % of weight is retained for OL as compared to 64 % for Orient as shown in Table 3.

Tab. 2: Yield differences between OL, Orient and Bombay Red varieties of onion in different sites

Sites and altitude	Variety	Mean yield (tons/acre)
Thridangbi at 900 m amsl	OL	13.18[a]
	Orient	8.70 [b]
p value		0.034
lsd		3.921
Chali at 1300 m amsl	OL	6.98 [b]
	Orient	8.36 [a]
p value		0.002
lsd		0.504
Tangmachu at 1700 m amsl	OL	16.32 [a]
	Orient	9.73 [b]
p value		0.007
lsd		3.62

Means with the different superscripts are significantly different.

Tab. 3: % Weight loss and weight retained after curing of onion

Varieties	Average weight at crop cut (kg)	Average weight after curing (kg)	% weight loss in curing	% weight retained after curing
Orient (Red)	13.12	8.43	36	64
OL (white)	14.4	6.9	52	48

Suitability in rice fallow system

OL and Orient varieties takes about 180-200 days to mature can fit well under the rice fallow system in the study sites. Rice is sown in May and harvested in October and onion is sown in October and can be ready by end of May or latest by first week of June. The two cropping system is found to superimpose in May and June but is manageable since these two months are used for nursery raising requiring small plots of land that can be arranged elsewhere. Ideally, short duration varieties maturing in some 180 to 200 days would be more suitable for promotion in rice fallow system.

Farmers who took part in the trial also reports that onion is less damaged by stray animals much as in other crops during the fallow phase. And with stable price of onion at the local markets, bulb onion cultivation (particularly red varieties) can stand a better chance to be promoted as crop in the rice fallow system. Thickly sown crops can be rouged and sold as green vegetable like spring onion especially in late march and April when green vegetables are scarce.

Effect of variety on bulb size

Bulbs from one site in Thidangbi was segregated in three different categories which showed that OL yellow has equal proportion of large and small bulbs (24 and 26 % for large and small respectively) and greater proportion (50%) of medium bulbs weighing between 150-399 g per bulb. Orient red bulbs were mostly (77%) smaller size weighing < 150 g per bulb and the rest (23%) falling in the medium category of bulbs weighing between 150 to 399 g per bulb (Table 4). Although data was not collected from other sites, similar instances were observed.

Tab. 4: Total number of bulbs and % bulb composition in 1 m^2 plot at Thidangbi 900 m amsl

Variety	Large > 400 g		Medium 150 - 399 g		Small <150 g		Total bulbs	
OL	5	(24)	10	(50)	6	(26)	21	(100)
Orient	0	(0)	6	(23)	19	(77)	24	(100)

Note: Figures in bracket shows the % composition.

Farmers' opinion on cultivating onion as an alternative crop after paddy

Assessment of the varieties by the farmers at the time of harvest showed that all farmers who took part in the program were willing to continue onion cultivation. However, the white onion varieties such as OL which are excessively large, sweet and less pungent are not preferred. Instead, red varieties Orient in this trial have higher preference. Farmers adjust their choice depending on the consumer reference since consumers are more accustomed to imported red onion varieties. The lesser preference to OL is also due to its high weight loss after curing and rotting especially in low altitudes. A farmer's comparative assessment of the two varieties is shown in Table 5.

Tab. 5: Farmers Comparison of Red and White Onion varieties, Thridangbi

Criteria	Orient	OL
Appearance and size	Good, medium 60 % medium and up to 400 gms; 40 % greater than 400 gms	Good but bulb size is too large (about 3 bulbs weighs a Kg)
Cultivation practices	Manageable	Manageable
Top growth (Hardening of the tops)	High growth, poor bulbs	No stem growth, large bulb
Flowering	Flowers and seed production possible Flowering is not good for production	Do not flower, good for production with out flowering
Pungency	Strong	Mild
Taste	Pungent and hot	Sweet

Smell	Yes and good	No smell
Post harvest	Needs good curing place, gets rotten but not as fast as white, 6 month	Needs good curing place; easy to rot 3-4 ,months
Price in the market/kg	Nu. 15 - 30	Nu. 15 - 30
Seed availability	Not readily available	Not readily available
Average yield t/ac	8.93	12.16

Although farmers showed interest in cultivating the crop, unavailability of quality seeds is major constraints.

Financial analysis of cultivating onion- Is it profitable?

The crop budget analysis shows the net returns per acre ranged from Nu. 60,000 to 126,450 and net family labour returns between Nu.309 to Nu.1046. Based on the results, it was found out that onion cultivation is a profitable crop as shown in Table 6 and 7. Although OL yields higher than Orient, consumers' preference for Orient (red) onion than OL makes Orient a better choice for farmers. With current market price of Nu.15 - 25 per kg and with low home produced onion in the market, Orient variety and other OP varieties can be an option.

Tab. 6: Financial crop budget per acre for Onion Orient in outreach sites

Gross value of Production	Thidangbi	Chali	Tangmachu
Main Product (kg)	8,700	8,360	9,730
Price (Nu/kg)	15	15	15
Gross Income (Nu.)	1,30,500	1,25,400	1,45,950
Total Direct cost	**17,750**	**35,200**	**16,750**
Total Cost of Labour (Nu.)	16,500	16,000	20,000
Total COSTS	**34,250**	**51,200**	**36,750**
Net returns (Nu.)	**96,250**	**74,200**	**1,09,200**
Cost of production (Nu/ kg)	3.94	6.12	3.78
Family labour (@ 70% family labour)	115.5	112	140

Net Returns per family labour day (Nu/day/person)	**976**	**805**	**923**
Labour No.	165	160	200
Cost of Labour	100	100	100
Total Cost of Labour	16,500	16,000	20,000
Family Labour	115.5	112	140
Hired Labour (@ 30 % Hired Labour)	49.5	48	60

Tab. 7: Financial crop budget per acre for Onion OL in outreach sites

Gross value of Production	Thidangbi	Chali	Tangmachu
Main Product (kg)	13,180	6,980	16,320
Price (Nu/ kg)	10	10	10
Gross Income (Nu)	1,31,800	69,800	1,63,200
Total Direct cost	**17,750**	**35,200**	**16,750**
Total Labour costs	16,500	16,000	20,000
Total COSTS	**34,250**	**51,200**	**36,750**
Net returns	**97,550**	**18,600**	**1,26,450**
Cost of Production (Nu/ kg)	2.60	7.34	2.25
Family Labour	115.5	112	140
Net Returns per family labour per day (Nu/day/person)	**987**	**309**	**1,046**
Number of Labours	165	160	200
Cost of Labour	100	100	100
Total Cost of Labour	16,500	16,000	20,000
Family Labour (@ 70% family labour)	115.5	112	140
Hired Labour (@30% Hired Labour)	49.5	48	60

The cost of labor especially cost of labor in transporting produce to road head, seeds were major factors in economics of onion as evident from the case in Chali which had the lowest net return and highest cost of production per kg. The remaining sites were accessible by road which did not incur cost for labour in transporting produce to the road head. Other factors are lack of seeds, seed cost and storage can also influence net returns.

CONCLUSIONS

While cultivation of onion after paddy is not a new innovation elsewhere, it is not a common practice in the east. And especially in the case of enhancing productive utilization of fallow period of wetlands amidst shortage of land for production, onion cultivation preferably short duration varieties maturing in 180-200 days can be an alternative crop for the farmers. Since usual choice of crop during the fallow period of wetlands are potato and vegetables, onion is comparatively easier to manage mainly due to less pest and disease incidences and most importantly, onion is less damaged by stray animals.

The yield potentials of both Orient and OL are promising, current market prices (Nu. 15-25 per kg) are stable and since local onion at the market is limited, farmers have a better chance to sell their produce. OL White despite its high yield is not preferred mainly because of its sweet taste, higher weight loss in curing, prone to post harvest damage and excessively large bulb size. Moreover, consumers have higher preference for red varieties comparatively more pungent, good taste and medium bulb size. Since consumers prefer red onion, Orient is a preferred choice from this trial.
The economic analysis showed that onion cultivation is profitable. However, it is calculated based on estimates and require further study but it has identified the main factors – mainly cost of transportation of produce to road head affecting net returns. Onion cultivation will be more suitable in rice fallow system targeted in areas accessible by road.

Onion seeds are not readily available in the market and therefore, coordinating timely supply of seeds alongside crop promotions is necessary. Seed production especially OP varieties could also be promoted in which selected bulbs planted in October can flower in April and seeds harvested by May. Further research in identifying short duration varieties and further promotion of preferred red varieties such as Bombay red and Pune red Nasik red should be carried out to upscale onion promotion.

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REFERENCES

Phuntsho, L., Gyeltshen, S., Khampa., Tenzin, K., Norbu, U., Penjor, T., Tshering, G., Rai, N.B., Phunsho., Tashi, S., Tshering K, Tshering K., and Tomiyaus, Y (2010). Horticulture Research Trial Report: a compilation of horticulture research trial reports for 2004-2008, RNR RDC Wengkhar Technical Document No. 46/ Ho/ 2009-2010, RNR RDC, Wengkhar, Mongar, Department of Agriculture, Ministry of Agriculture and Forests.

RNR RC Khangma (2003). RNR Technical Recommendations, RNR RC Khangma, DRDS Ministry of Agriculture

Tshering, K., Thapa, L., Chettri, D.D., Dema, S., and Wangmo, K (2010). Bulb Onions, a potential crop for domestic market. RNR Journal Volume 6, No. 1. Council for RNR Resaerch of Bhutan, MoAFs

Kiwifruit – A potential fruit crop for fruit diversification

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ABSTRACT

Kiwifruit is fairly a new crop although its wild strains are found in many parts of Bhutan in the wilderness. Notwithstanding, there is not even a single cultivar being released so far in the country. Against this backdrop, evaluation of the exotic kiwifruit cultivars was conducted at Wengkhar (1650 m amsl).

The cultivars evaluated were four female cultivars viz. Wengkhar Green, Wengkhar Yellow, Semtokha I, Semtokha II, Semtokha III and Semtokha IV. Semtokha I and III are male cultivars and others are female cultivars. The varietal evaluation was conducted mainly to evaluate their performance under Bhutanese condition. Moreover, to assess the consumers' preference for this new fruit over the commonly available fruits, a sensory evaluation of kiwifruit was also conducted. Based on the three years result, among the four exotic cultivars being evaluated, it was observed that Wengkhar Green, Wengkhar Yellow and Semtokha II possess superior fruit quality as compared to Semtokha IV cultivar. Further, based on the sensory evaluation result, it may be concluded that the higher consumer preference for Wengkhar Green over Wengkhar Yellow was mainly due to its larger fruit size and higher TSS content which contributes to fruit taste.

KEY WORDS: Cultivar, Kiwifruit, *Actinidia deliciosa*, Firmness and Total soluble solids

INTRODUCTION

Kiwifruit, which has replaced the old English name of 'Chinese gooseberry', is native to southwestern China where they grow wild on trees and bushes. The kiwifruit was introduced to the United Kingdom,

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Europe, United States, and New Zealand from 1900 to 1910 from China. Commercial plantings were made in New Zealand about 1930 and have become widespread over the last 20-30 years. Kiwifruit (*Actinidia deliciosa* L.) is a berry borne on the vines and belongs to family Actinidiaceae. Kiwi fruit is fairly a new crop in Bhutan although its wild strains are found in many places in Bhutan in wilderness. Few numbers of plants are found grown in the backyard by farmers of Thimphu and Paro Dzongkhags. Kiwifruit is rare in the market; however, in the recent years fruits have been imported and are very expensive. Kiwi cultivation has many advantages like it can be organically grown and transportation is easier and has market potential in India and Bangladesh where there is less potential for Kiwi cultivation. Out diverse agro-climatic condition favors Kiwifruit cultivation than any other fruits. The Semtokha I, II, III and IV were introduced from NMC, Semtokha to Wengkhar with the support from Agriculture Research and Extension Support Project (AREP-JICA) in 2004. Semtokha I, II, III and IV cultivars of Kiwifruit were initially introduced by National Mushroom Centre, Semtokha from Kofu City, Yamanashi Prefecture, Japan in 1985. However, Wengkhar Green and Wengkhar Yellow cultivars were introduced from Japan to Wengkhar with the support from Agriculture Research and Extension Support Project in 2004.

The germplasm consists of four female and two male cultivars (Pollinizer). The germplasm was introduced since agro-climatic condition in the region is favorable for Kiwifruit cultivation. Therefore, the evaluation of kiwifruit cultivars in Wengkhar was conducted. The evaluation of germplasm would indicate the cultivar-specific cultivation practices to be adopted for a given set of agro-climatic conditions.

MATERIALS AND METHODS

Cultivars for evaluation

Both Semtokha and Wengkhar series of Kiwifruit germplasm were introduced and established in Wengkhar in 2004 with the support from Agriculture Research and Extension Support Project (AREP-JICA). The germplasm collection was established at Wengkhar (1650 m amsl) on Completely Randomized Design. The cultivars evaluated were Wengkhar Green, Wengkhar Yellow, Semtokha I, Semtokha II and Semtokha IV. Semtokha I and III were used as the male or pollinizer cultivars while other cultivars the female or main cultivars. There were three vines for each cultivar with the exception of few cultivars. These

cultivars were field grafted on to wild kiwifruit rootstocks (Tomiyasu et al., 2004) in February-March 2004. All the cultivars started bearing fruits in 2007 and fruit quality analysis started from 2007 onward. The experiment was established on terrace system of planting. The vines were trained on trellis or pergola system of training. All cultivars were subjected to similar management in terms of pruning, fertilization, irrigation, fruit thinning, weed control and pest management.

Evaluation date and parameters

The assessment of these cultivars in terms of fruit quality began from 2007 onward. Fruits are harvested at fully matured stage. 10 fully matured fruit samples were randomly collected from each cultivar and kept until ripe. The well ripe fruit samples were analyzed for different quality parameters. The quality parameters analyzed includes; fruit weight in grams, fruit length and diameter in centimeter using Digital Vernier Caliper, total soluble solids measured in degree brix or percentage was determined using Hand Refractometer. The fruit hardness or firmness expressed as $Kgcm^{-1}$ was determined using Penetrometer. Subsequently, a cumulative variety ranking was done based on the mean fruit quality parameters to assess the overall merit of the cultivars. A highest score of 5 was given to cultivar with best fruit quality parameters and a lowest score of 1 was assigned to the cultivar with lowest preferable fruit quality parameters. The sensory evaluation result was correlated with the TSS content of the cultivar.

Cultivars: (a) Wengkhar Green; (b) Wengkhar Yellow; (c) Semtokha IV & (d) Semtokha II

Sensory evaluation and information collection

The sensory evaluation was also carried out to assess the sensory attributes of kiwifruit cultivars. The sensory evaluation was carried out in collaboration with Regional Agricultural Marketing and Cooperatives (RAMCO), Mongar. The evaluation was carried out during Mongar Tshechu in November, 2009 through the Informant survey. The study employed Informant survey as the main approach wherein the informants were given the fruit samples to taste and thereby collect the

sensory information based on the designed questionnaire. In total, 28 informants ranging from few farmers to consumers of different professional backgrounds were interviewed. The survey questionnaire was formulated in consultation with Regional Agriculture Marketing and Cooperatives and colleagues in RNR RDC Wengkhar. The sensory evaluation mainly focused on general awareness of consumers, their preference and future market prospects of the fruit.

Data Analysis

The year wise fruit quality parameters and sensory evaluation data was compiled in Microsoft Excel. The fruit quality evaluation data compiled in Excel sheet was exported to GENSTAT for Windows version 10, Lawes Agricultural Trust, Rothamsted and analyzed through One Way Analysis of Variance (ANOVA). The sensory evaluation data compiled in Excel sheet was then coded into SPSS (Statistical Package for Social Science software) 16.0 versions. The data was checked for any inconsistencies, computerized and analyzed. The data was analyzed for mean, frequency, percentage, crosstab, descriptive statistics and other related interpretation where necessary.

RESULT AND DISCUSSION

Cultivar evaluation in 2007

The highest fruit weight was observed in Wengkhar Green (135 gm/ fruit, n = 10, s.e = 2.0) followed by Semtokha IV (110 gm/ fruit, n = 10, s.e = 1.3), Semtokha II (107 gm/ fruit, n = 10, s.e = 1.3) and the lowest fruit weight was observed in Wengkhar Yellow (80 gm/ fruit, n = 10, s.e = 1.4). The mean fruit weight of Wengkhar Green was significantly different from Wengkhar Yellow, Semtokha II and Semtokha IV (P = <0.001). However, there was no significant difference between Semtokha II and Semtokha IV according to DMRT, P = 0.05 (Table 1).

Tab. 1: Mean fruit quality parameters of kiwifruit cultivars as observed in 2007

Sample	Fruit Wt. (gm)	Fruit length (cm)	Fruit Dia. (cm)	TSS (%)	Firm-ness
Wengkhar Green	135.0[a] (±2.0)	8.1[a] (±0.16)	5.2[a] (±0.08)	15.9[a] (±0.33)	0.6[a] (±0.14)
Wengkhar	80.0[b]	6.7[b]	5.9[b]	14.5[b]	0.9[a]

Yellow	(±1.4)	(±0.11)	(±0.10)	(±0.29)	(±0.19)
	107.0[c]	6.9[b]	5.1[a]	15.5[a]	0.7[a]
Semtokha II	(±1.3)	(±0.09)	(±0.07)	(±0.21)	(±0.14)
	110.0[c]	6.9[b]	5.1[a]	17.1[c]	0.5[a]
Semtokha IV	(±1.3)	(±0.13)	(±0.07)	(±0.37)	(±0.11)
p value	<0.001	<0.001	<0.001	<0.001	0.23
lsd	4.17	0.36	0.24	0.89	0.42
S.e.d (d.f=36, n=10)	2.06	0.18	0.12	0.44	0.21

*Within the columns means followed by the same letter are not significantly different according to DMRT (P = 0.05). The values in parenthesis are standard error of means, where n = 10 for all cultivars; *Standard errors of differences of means and ** Degree of freedom.*

The fruit length and fruit diameter constitute the size of a fruit. In terms of fruit size Wengkhar Green was observed to have larger fruit length followed by Semtokha II, IV and Wengkhar Yellow. It was observed that there was significant difference between the fruit length of Wengkhar Green and Wengkhar Yellow (P<0.001). However, there was no significant difference between Semtokha II and Semtokha IV according to DMRT at (P=0.05). In terms of fruit diameter, there was significant difference between Wengkhar Yellow and Wengkhar Green, Semtokha II and Semtokha IV according to DMRT at (P=0.05).

The total soluble solids (TSS) content is also an important parameter which indicates the organoleptic maturity. Kiwifruits having 6.2% TSS are ideal for harvesting and delay in harvesting deteriorates storability (Awasthi & Mehta, 2001). Therefore, significant differences were observed between the four cultivars being evaluated in TSS content (P<0.001) (Table 1). However, the effect of cultivar on the fruit firmness was insignificant according to DMRT at (P=0.05) (Table 1).

Cultivar evaluation in 2008

As opposed to 2007 result, the highest fruit weight was observed in Wengkhar Yellow (158 gm/ fruit, n = 5, s.e = 2.1) followed by Semtokha II (112 gm/ fruit, n = 5, s.e = 6.63) and the lowest fruit weight was observed in Wengkhar Green (99.4 gm/ fruit, n = 5, s.e = 3.91). The mean fruit weight of Wengkhar Green was significantly different from Wengkhar Yellow but there was no significant difference between Wengkhar Green and Semtokha II according to DMRT at (P = 0.05) (Table 2).

Tab. 2: Mean fruit quality parameters of kiwifruit cultivars as observed in 2008

Sample	Fruit Wt. (gm)	Fruit length (cm)	Fruit Dia. (cm)	TSS (%)
Wengkhar Green	99.4[a] (±3.91)	6.6[a] (±0.24)	4.84[a] (±0.06)	17.02[a](±0.6)
Wengkhar Yellow	158[b] (±2.10)	7.5[b] (±0.07)	6.12[b](±0.20)	16.36[a](±0.38)
Semtokha II	112[a](±6.63)	6.5[b] (±0.22)	5.33[c](±0.05)	13.76[b](±0.62)
p value	<0.001	0.006	<0.001	0.003
lsd	14.18	0.59	0.39	1.69
S.e.d (d.f=12, n=5)	6.51	0.27	0.18	0.774

*Within the columns means followed by the same letter are not significantly different according to DMRT (P = 0.05). The values in parenthesis are standard error of means, where n = 10 for all cultivars; *Standard errors of differences of means and ** Degree of freedom.*

In case of fruit size, larger fruit length was observed in Wengkhar Yellow followed by Wengkhar Green and Semtokha II. There was significant difference in mean fruit length of Wengkhar Green with that of Wengkhar Yellow and Semtokha II. However, the mean fruit length of Wengkhar Yellow and Semtokha II did not differ significantly according to DMRT at (P=0.05). Similarly, the highest fruit diameter was also observed in Wengkhar Yellow followed by Semtokha II and Wengkhar Green. It was observed that there was significant difference between the three cultivars according to DMRT at (P=0.05) (Table 2).

Since the total soluble solids (TSS) content is an important parameter which determines the fruit taste and commercial readiness, it was observed that the TSS content of Wengkhar Green and Wengkhar Yellow differed significantly from Semtokha II according to DMRT at (P=0.05) (Table 2).

Cultivar evaluation in 2009

The highest fruit weight was observed in Semtokha II (91.1 gm/ fruit, n = 10, s.e = 3.95) followed by Wengkhar Green (85.3 gm/ fruit, n = 10, s.e = 5.56), Wengkhar Yellow (72.3 gm/ fruit, n = 10, s.e = 1.79) and the

lowest fruit weight was observed in Semtokha IV (66 gm/ fruit, n = 10, s.e = 4.64). The mean fruit weight of Wengkhar Green was significantly different from Wengkhar Yellow and Semtokha IV (P = <0.001). However, there was no significant difference between Wengkhar Green and Semtokha II according to DMRT at (P = 0.05) (Table 3).

Tab. 3: Mean fruit quality parameters of kiwifruit cultivars as observed in 2009

Sample	Fruit Wt. (gm)	Fruit length (cm)	Fruit diameter (cm)	TSS (%)
Wengkhar Green	85.3[b] (±5.56)	6.76[b] (±0.20)	4.86[a] (±0.14)	16.85[b] (±0.35)
Wengkhar Yellow	72.3[a] (±1.79)	4.89[a] (±0.07)	4.75[a] (±0.05)	14.80[a] (±0.53)
Semtokha II	91.1[b] (±3.95)	6.81[b] (±0.08)	5.25[b] (±0.09)	18.94[c] (±0.24)
Semtokha IV	66.0[a] (±4.64)	5.33[a] (±0.22)	4.70[a] (±0.11)	19.72[c] (±0.43)
p value	<0.001	<0.001	0.002	<0.001
lsd	12.11	0.4535	0.2978	1.158
S.e.d (d.f = 36, n = 10)	5.97	0.2236	0.1468	0.404

*Within the columns means followed by the same letter are not significantly different according to DMRT (P = 0.05). The values in parenthesis are standard error of means, where n = 10 for all cultivars; *Standard errors of differences of means and ** Degree of freedom.*

Since the fruit length and fruit diameter determine the fruit size, the highest fruit length was observed in Semtokha II followed by Wengkhar Green, Semtokha IV and Wengkhar Yellow. However, there was no significant difference in mean fruit length between Wengkhar Green and Semtokha II and Wengkhar Yellow and Semtokha IV according to DMRT at (P=0.05). Similarly, the highest fruit diameter among the four cultivars evaluated was observed in Semtokha II followed by Wengkhar Green, Wengkhar Yellow and the lowest in Semtokha IV. The mean difference was significant between Semtokha II and other three cultivars being evaluated (P<0.001) (Table 3).

The highest TSS content was observed in Semtokha IV followed by Semtokha II, Wengkhar Green and Wengkhar Yellow. Interestingly, the

mean TSS content of Wengkhar Green differed significantly from Wengkhar Yellow, Semtokha II and Semtokha IV whereas the mean difference was insignificant between Semtokha II and Semtokha IV according to DMRT at (P=0.05) (Table 3).

Tab. 4: Mean fruit quality parameters of Kiwifruit cultivars (2007-2009)

Sample	Fruit Wt. (gm)	Fruit length (cm)	Fruit Dia. (cm)	TSS (%)	Firmness
Wengkhar Green	106.57	7.15	4.97	16.59	0.6
Wengkhar Yellow	103.43	6.37	5.59	15.22	0.9
Semtokha 2	103.37	6.74	5.23	16.07	0.7
Semtokha 4	88.00	6.14	4.90	18.41	0.50

The Table 4 above shows the mean fruit quality parameters of the four cultivars being evaluated from 2007 to 2009. The mean fruit quality parameters were used for the cumulative ranking of the four cultivars.

The Table 5 below shows the cumulative variety ranking matrix of four cultivars of kiwifruit being evaluated. All fruit quality parameters were considered for cumulative ranking. It was found that the best cultivar among the evaluated kiwifruit cultivars was Wengkhar Green followed by Wengkhar Yellow and Semtokha II.

Tab. 5: Cumulative Variety Ranking Matrix

Kiwifruit Cultivars	Fruit weight (Cm)	Fruit length (Cm)	Fruit diameter (Cm)	TSS (%)	Firm -ness	Total
Wengkhar Green	5	5	1	4	3	18
Wengkhar Yellow	3	2	5	2	5	17
Semtokha II	3	3	3	3	4	16
Semtokha IV	2	1	1	5	1	10

Sensory evaluation of kiwifruit

Varietal preference

The sensory evaluation results revealed that 60% of the respondents (n=28) prefer Wengkhar Green while 32% of the respondents prefer Wengkhar Yellow. The higher preference for Wengkhar Green may be attributed to larger fruit size and higher TSS content. On the other hand, study also showed that 7% of the respondents prefer neither of the cultivars which, clearly indicates that consumers are not familiar with kiwifruit (Figure 1).

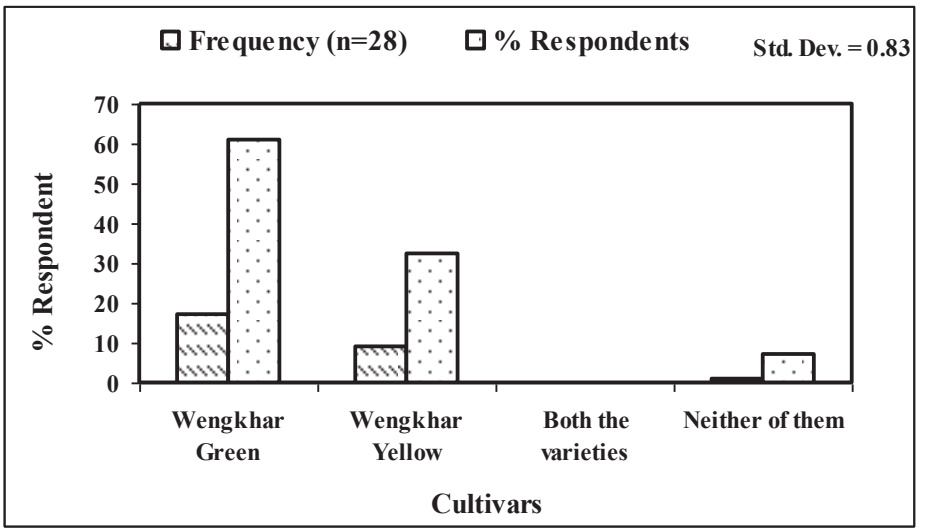

Fig 1: Preference of Kiwifruit cultivars by respondents

CONCLUSIONS

It may be concluded that by promoting the three promising cultivars of kiwifruit being evaluated here, temperate fruits cultivation in the country can be further diversified. Based on the agronomic performance under Wengkhar condition, all four exotic kiwifruit cultivars were found potential. However, it was observed that Wengkhar Green, Wengkhar Yellow and Semtokha II possess performs better as compared to Semtokha IV cultivar. In addition, Kiwifruit has good keeping quality and it can be stored after harvest for two weeks (Loday, P, et. al, 2010). Based on the sensory evaluation result, it may be concluded that the higher consumer preference for Wengkhar Green over Wengkhar

Yellow was mainly due to its larger fruit size and higher TSS content which directly contributes to fruit taste. Hence, it may be concluded that Wengkhar Green, Wengkhar Yellow and Semtokha II are promising cultivars for warm temperate areas ranging from 1200 to 2500 m above mean sea level offering good opportunity for diversification of temperate fruits cultivation as well as diversify farm household income.

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REFERENCES

Chadha, K. L. edt. Handbook of Horticulture. Indian Council of Agricultural Research (2002), New Delhi.

Phuntsho, L. (2010). Horticulture Research Trial Report: a compilation of horticulture research trial reports for 2004-2008, RNR RDC Wengkhar Technical Document No. 46/ Ho/ 2009-2010, RNR RDC, Wengkhar, Mongar, Department of Agriculture, Ministry of Agriculture and Forests.

Renewable Natural Resources Research Centre. (2009). Temperate fruits cultivation manual, AREP-JICA/ RNRRC Wengkhar, CoRRB, Ministry of Agriculture and Forests, Mongar.

Renewable Natural Resources Research Centre. Wengkhar. (2008). National Horticulture Research Program Report, 9th Five Year Plan, July 2002 to June 2008, RNR RC Wengkhar, CoRRB, Ministry of Agriculture.

Renewable Natural Resources Research Centre. (2008). Annual Report 2007 - 2008, RNR RC, Wengkhar, Mongar, CoRRB, Ministry of Agriculture.

Evaluation of Asian pears – A potential varieties to diversify warm temperate fruits

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ABSTRACT

Though Bhutan's rich and diverse agro-ecological zone harbors a vast number of pear species however, pear species found in Bhutan are predominantly Asian pear species. Although the diverse agro-climatic condition favour the production of Asian pears however the cultivation of improved Asian pears are limited. Therefore, this study was conducted to explore the possibility of cultivating improved pear cultivars at commercial scale and at the same time give an alternative to farmers to reap extra income through cultivation of improved cultivars of Asian pears. Asian pear germplasm was established at Wengkhar in February 2002 with the technical support from IHDP and AREP-JICA project for Mongar and Lhuentse Dzongkhags. Initially, there were 17 cultivars in the germplasm. However, due to poor performance under Wengkhar condition, two cultivars were discarded from the germplasm. Although, the germplasm block consists of Hosui, Kosui, Wengkhar Lhee 1, Niitaka, Shinko, Atago, Meigetsu, Yakumo, Okusankichi, Kikusui, Nijisseiki, Thimphu JICA and Jakar Pear. However, only ten exotic cultivars were evaluated in comparison to the released check cultivars. The released cultivar Kosui was used as a check cultivar. The test cultivars were evaluated for their fruit quality and yield performance under our condition. It was found that test cultivars can be potentially cultivated at commercial scale under our condition given the performance of these cultivars in terms of fruit quality and yield performance as compared to check cultivars.

KEY WORDS: Agro-ecological zone, Cultivars, Fruit quality, Germplasm, Evaluation, Total Soluble Solids (TSS), Yield performance

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INTRODUCTION

Pear fruits are believed to have originated in temperate Europe and West Asia regions (IHC, 1994). Asian Pears belong to the family Rosaceae and sub-family Pomoideae. The genus *Pyrus* is comprised of about 22 species found across Asia, Europe, and northern Africa. However, the two major species commercially cultivated are European Pear (*Pyrus communis* L.) which does not occur in nature by itself, and it is believed to be a hybrid of *P. caucasia* and *P. nivalis* It is shaped like a bell (pyriform) and has a fleshy pedicel. On the contrary, Asian Pear (*P. pyrifolia* (Burm. f.) Nak. [Syn. *P. serotina* L.]) which, is also known by many other names like Japanese Pear, Oriental Pear or Nashi Pear. It is round in shape and has non-fleshy pedicel. In Bhutan pears have been grown for ages although most of the cultivars are found to be of poor in fruit quality thereby rendering them unproductive and un-marketable. Pear trees are found in almost every geogs across the country ranging from 955 to 2700m above the mean sea level although not on a commercial level. It is expected to contribute towards the income generation of our farmers besides improving their nutritional status. According to the World Health Organization, a working man should take at least 60 gm of fruits per day.

The most commonly grown Pear in Bhutan is Himalayan pear (*Pyrus pashia* Hamilton ex D. Don), which is distributed across the Himalayas from Pakistan to Vietnam and from the southern provinces of China to the northern regions of India (Ghora and Panigrahi, 1995). The Pyrus pashia (Hamilton ex D. Don) is one of the 17 cultivated pear (Pyrus) species, which is exclusively grown in home gardens of smallholder farmers (Krause et al., 2004). Pear cultivation in the country is predominantly of Himalayan and European pears. Later, with the inception of Japanese assisted projects in the country, Asian pears were introduced into the western Bhutan and from thereon the cultivation of Nashi pear picked up gradually. However, the cultivation of improved Asian pears remained largely unpopular in eastern Bhutan. In order to promote the potential improved Asian pear cultivars along with indigenous cultivars and to close the resource gap among the farmers in the east, Asian pear cultivars were introduced from Druk Seed Corporation (now known as National Seed Centre) to Wengkhar in 2002. Subsequently, the germplasm of Asian pear cultivars was established in 2002. Initially, there were 17 Asian pear cultivars in the germplasm; however, due to the poor performance, 4 cultivars were excluded from

the evaluation. Some of the germplasm started bearing fruits in 2005 onwards. Being perennial in nature, it took some years for all cultivars to come into fruiting. The fruit quality evaluation started in 2006 onward. The fruit quality parameters such as fruit weight, size, firmness and total soluble solids (TSS) content were assessed for each cultivar and compared with the test cultivars.

Japanese pears evaluated here can be categorized into four different groups viz. Early: Kosui, Yakumo); Mid: Meigetsu, Hosui, Wengkhar Lhee 1; Late: Niitaka, Nijisseiki, Shinko and Very Late: Okusankichi, Kikusui and Atago. The fruit quality of Asian pears is superior to local pears and the eating quality is very good. Since the local pears are poor in fruit quality with non-marketable fruit qualities and no export outlet, it was felt necessary to look into the possibility of exploring alternative cultivars which can bring additional economic benefits to the rural poor. Therefore, the adaptive research on Asian pear was designed and carried out to assess the performance of Asian pear cultivars under Bhutanese condition.

MATERIALS AND METHODS

Cultivars for evaluation

The exotic Asian pear cultivars were obtained from Japan and Druk Seed Corporation (now National Seed Centre), Paro through Agriculture Research and Extension Support Project (AREP-JICA). The released cultivars were brought from Druk Seed Corporation, Paro and used as check cultivar for the evaluation. The cultivars evaluated were Atago, Niitaka, Meigetsu, Nijisseiki, Yakumo, Kikusui, Shinko, Okusankichi, Jakar Pear and JICA Pear. Kosui was used as a check cultivar. The ten cultivars were evaluated at RDC Wengkhar. The one-year old grafted plants were established in February, 2002 and there are four trees per cultivar. All the cultivars came into fruiting from 2006 and fruit quality analysis was carried out from 2006 onward. All the cultivars were managed similarly in terms of training and pruning, fertilization, irrigation, basin making, weed control, insect-pest and disease management.

Evaluation date and evaluation parameters

The evaluation in terms of fruit quality was started from 2006 onward. The cultivars were evaluated at the end of every harvest. Five fruits per

cultivar were used for fruit quality analysis. The following quality parameters of the fruits were analyzed; fruit weight in grams using weighing balance, fruit height & diameter in cm using Vernier calliper, total soluble solids content (in %) using Refractometer. Finally a cumulative cultivar ranking was carried out to assess overall merit. A cultivar with best fruit quality parameters was assigned the score of 5 and a lowest score of 1 was assigned to the cultivar with least preferable fruit quality parameters.

Data Analysis

The data was compiled in MS Excel 2003 and 2007. The compiled data was exported to GenStat Release 10.1 (PC/Windows), Lawes Agricultural Trust (Rothamsted Agriculture Experimental Station) for fruit quality analysis with the Analysis of Variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used to compare the mean differences of fruit quality parameters of the Asian pear cultivars.

RESULT AND DISCUSSIONS

Fruit weight and fruit size

The fruit quality analysis over the years resulted in discarding some of the cultivars due to its poor quality fruits. As a result, 4 cultivars of Asian pear were observed to bear poor quality fruits when compared to other cultivars. There was highly significant differences due to the effect of all the cultivars on fruit weight, fruit size, total soluble solids (TSS) content and fruit firmness ($P = <0.001$). Amongst the ten cultivars being evaluated, Okusankichi cultivar resulted largest fruit with a fruit weight (1206 g/fruit, n = 5, s.e = 65.8) followed by Atago with a fruit weight (855 gm/ fruit, n = 5, s.e = 85.2), Niitaka which gives 604.6 gm/ fruit, n = 5, s.e = 16.4) and Shinko with a mean fruit weight of (510 gm/ fruit, n = 5, s.e = 28.8) as compared to check cultivar (Kosui) which resulted in fruit weight of 227 gm/ fruit, n = 5, s.e = 18.2. It clearly shows that these cultivars differed significantly from check cultivar (Kosui) according to Duncan's Multiple Range Test (DMRT) at $P = 0.05$ (Fig 1).

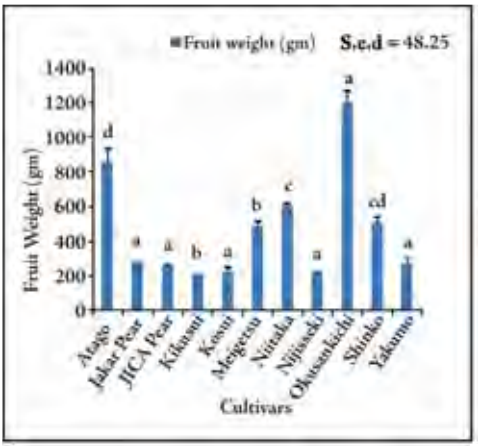

Fig1: Fruit weight of Asian pear cultivars

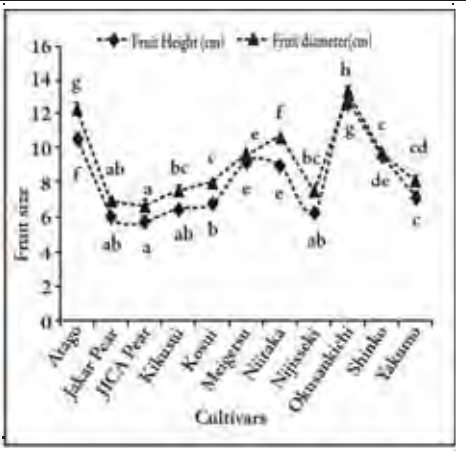

Fig 2: Fruit size of Asian pear cultivars

The fruit height and fruit diameter are also one of the important fruit quality parameters which determine the marketability of any fruit. As such, the fruit size of test cultivars were assessed and compared with the check cultivars. Based on the analysis results, it was observed that there was highly significant difference between cultivars due to the effect of different cultivars ($P < 0.001$).

The fruit height and diameter directly contributes to the fruit weight. Amongst the ten cultivars evaluated, Okusankichi possess highest fruit size with a mean fruit height (12.57 cm, n = 5, s.e = 0.67) and diameter of (13.41 cm, n = 5, s.e = 0.47) followed by Atago with a fruit height of (10.65 cm, n = 5, s.e = 0.37) and diameter (12.35 cm, n = 5, s.e = 0.42), Niitaka with a fruit height of 8.99 cm, n = 5, s.e = 0.25 and diameter of 10.73 cm, n = 5, s.e = 0.09 and Shinko with a fruit height of 9.62, n = 5, s.e = 0.37 and fruit diameter of 9.82, n = 5, s.e = 0.11. On the other hand, Kosui (check cultivar) possess the fruit height of 6.78 cm, n = 5, s.e = 0.19 and diameter of 8.04 cm, n = 5, s.e = 0.15. These cultivars differed significantly from test cultivars according to Duncan's Multiple Range Test (DMRT) at $P = 0.05$. This clearly shows that the fruit size of these cultivars is larger than the Kosui and other test cultivars (Fig 2).

Total soluble solids content and fruit firmness

Total Soluble Solids (TSS) content is the most important parameter that determines fruit quality of Asian pears. Among the ten cultivars being evaluated, the highest total soluble solids (TSS) content was observed Shinko with a TSS content of 12.64%, n = 5, s.e = 0.26 followed by Atago (12.4%, n = 5, s.e = 0.4), Okusankichi (12.28%, n = 5, s.e = 0.34)

and Niitaka (11.88%, n = 5, s.e = 0.39) which were not statistically different from each other according to Duncan's Multiple Range Test (DMRT, $P = 0.05$) but these cultivars are superior to other cultivars (Fig 3). On the contrary, the lowest TSS content was observed in Kikusui cultivar (10.26, n = 10, s.e = 0.19) and the means differed significantly from other cultivars except Jakar Pear, Kosui, Meigetsu, Nijisseiki, Shinko and Yakumo according to Duncan's Multiple Range Test (DMRT, $P = 0.05$) (Fig 3). It clearly indicates that Atago, Niitaka and Okusankichi are sweeter than Kosui owing to higher TSS content (Fig 3).

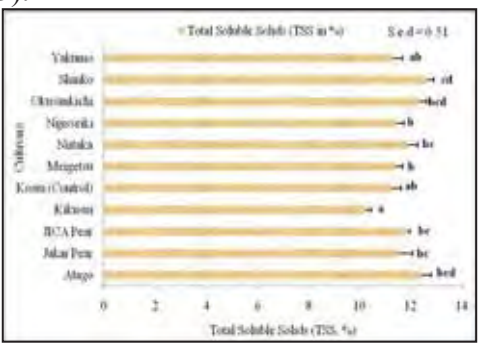

Fig 3: TSS content of Asian pear cultivars

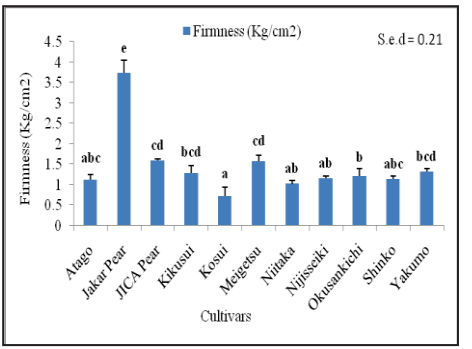

Fig 4: Fruit firmness of Asian pear cultivars

The firmness of the fruit is a vital quality parameters which determines the shelf life of the fruit and hence the transportability. This parameter was assessed since pear fruits are perishable fruit and need to identify potential cultivars with high firmness to ensure longer shelf life. Based on the results, the highest fruit firmness was observed in Jakar Pear with (3.74 kg/cm^2, n = 5, s.e = 0.31) followed by JICA Pear with (1.59 kg/cm^2, n = 5, s.e = 0.03), Meigetsu with a firmness of (1.57 kg/cm^2, n = 5, s.e = 0.14) and Yakumo with a firmness of 1.31 kg/cm^2, n = 5, s.e = 0.08). On the other hand, lowest firmness was observed in Kosui (0.71 kg/cm^2, n = 5, s.e = 0.22) which differed significantly from all cultivars evaluated except Atago, Niitaka, Nijisseiki, Okusankichi and Shinko cultivars according to Duncan's Multiple Range Test (DMRT) at $P = 0.05$. The results clearly indicate that exotic cultivars possess higher shelf life than the check cultivar (Kosui) (Fig 4).

Tab. 1: Cumulative Cultivar Ranking Matrix

Cultivars	Fruit weight (Kg)	Fruit height (cm)	Fruit diameter (cm)	TSS (%)	Firmness ($Kgcm^{-2}$)	Total
Atago	5	5	5	5	2	22
Jakar Pear	1	1	1	3	5	11
JICA Pear	1	1	1	4	3	10
Kikusui	1	1	2	1	2	7
Kosui	1	1	3	3	1	9
Meigetsu	3	5	2	3	3	16
Niitaka	5	5	5	4	1	20
Nijisseiki	1	1	2	3	2	9
Okusankichi	5	5	5	5	2	22
Shinko	4	5	5	5	2	21
Yakumo	1	2	4	3	2	12

Table 1 shows the cumulative cultivar ranking matrix. We have considered all the fruit quality parameters. Based on the cultivar ranking exercise, it was observed that the superior cultivars among the exotic cultivars being evaluated were Atago, Okusankichi, Shinko, Niitaka, Meigetsu, Yakumo and Jakar Pear. This indicates that the new exotic pear cultivars are superior in fruit quality to Kosui cultivar (Control) (Table 1).

Agronomic and horticultural characteristics

Horticultural characteristic include fruit size, colour, texture and eating quality whereas the agronomic characteristic includes time of maturity and insect-pests and disease tolerance. Based on the maturity and harvesting time of ten cultivars being evaluated here, these cultivars were categorized into different types viz. early maturing, mid maturing, late maturing and very late maturing cultivars (Table 2). Based on the visual observation as well as quality analysis, the Okusankichi cultivar resulted in largest fruit followed by Atago, Niitaka and Shinko. On the contrary, smallest fruit was observed in Kikusui cultivar. However, the fruit size is larger than the Kosui (Check cultivar). As opposed to vital horticultural characteristics, farmers' feedback also indicated that all exotic cultivars being evaluated here possess attractive fruit colour. Similarly, all cultivars have good eating quality due to high juice content and TSS content. The superior agronomic and horticultural characteristic of these cultivars also indicates good performance under our condition.

Table 2: The horticultural characteristics of Asian pear cultivars

Cultivars	Fruit colour	Fruit size	Harvest time	Eating quality	Remarks
Kosui (Check)	Golden brown	Medium	End July to Mid August	Excellent	Early maturing
Yakumo	Yellowish green	Medium	First to mid August	Excellent	Early maturing
Kikusui	Yellowish green	Medium	First to mid August	Excellent	Early maturing
Shinko	Russet brown	Medium to large	Mid to end September	Excellent	Late maturing
Meigetsu	Yellow brown	Medium	Mid to end September	Good	Late maturing
Niitaka	Russet brown	Large	Mid to end September	Excellent	Late maturing
Atago	Brown	Very large	First to mid October	Excellent	Late maturing
Okusankichi	Light brown	Very large	Mid to end November	Excellent	Very late maturing
JICA pear	Light brown	Medium	Mid to end August	Good	Mid maturing
Nijisseiki	Greenish yellow	Medium	End August to first September	Good	Late maturing
Jakar pear	Light brown	Medium	First to mid August	Good	Mid maturing

CONCLUSION

It may be concluded that by using the ten exotic Asian pear cultivars evaluated here, Asian pear cultivation can be further diversified in the country. All the exotic cultivars have been categorized into different types based on the maturing time of fruits as Early, Mid, Late and Very late maturing cultivars. Although all the exotic cultivars were found to be superior to Kosui cultivar (Control); however, Atago, Niitaka, Shinko and Okusankichi showed superior quality performance. These four cultivars were found to have superior fruit characteristics. Okusankichi has the largest fruit size followed by Atago, Niitaka and Shinko.

Nevertheless, fruit size of Niitaka and Shinko are medium to large but larger than other cultivars being evaluated. This clearly indicates that these cultivars can be promoted at large scale. Therefore, Atago, Shinko and Niitaka may be proposed for release so that National Seed Centre can multiply for commercial cultivation.

With the promotion of Atago, Shinko and Niitaka, farmers will be able to fetch premium price for their crop because these cultivars mature late to other temperate fruits.

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REFERENCE

Chadha, K.L. Edt., Handbook of Horticulture, Directorate of Publications and Informations, ICAR, Pusa, New Delhi.

Department of Agriculture-DoA (2000 & 2004). *Agriculture Statistics, Vol I & II*. Department of Agriculture, MoA, Thimphu, Bhutan.

International Horticulture Congress' Publication (1994). *Horticulture in Japan.* International Horticulture Congress.

Phuntsho, L., et al. (2005). *Local Pear Survey*. RNR Research Centre, Wengkhar, Mongar.

Phuntsho, L., et al. (2008). Evaluation of Local Pears as Rootstocks., Bhu. J. RNR. Vol 4. No. 1, 65-70: 2008

Renewable Natural Resources Research Centre. Wengkhar. (2008). National Horticulture Research Program Report, 9th Five Year Plan, July 2002 to June 2008, RNR RC Wengkhar, CoRRB, Ministry of Agriculture.

Renewable Natural Resources Research Centre. Wengkhar. (2007). Annual Report 2006-2007, RNR RC Wengkhar, CoRRB, Ministry of Agriculture.

Local mandarin germplasm evaluation and selection

Sonam Gyeltshen, Kinley Tshering, Khampa, Gyeltshen Tshering,
Thinley Penjor & Yuchi Tomiyasu

ABSTRACT

The cultivation of citrus in Bhutan is mainly dominated by mandarin although some amount of lime, lemons, pummelos and citron do exist. The objectives of germplasm collection are to preserve the indigenous mandarin cultivars, identify superior lines with better horticultural characteristics and good yield potential and to serve as disease free germplasm source.

Subsequent to the detection of Citrus Huanglongbing (HLB) disease in the country in 2000, RDC Wengkhar (then the RNR RC Wengkhar) carried out a major survey. Based on the survey of HLB incidence, germplasm (scion woods and seeds) were collected from 57 locations covering all major citrus growing areas, where the HLB was found to be negative. The scion woods were grafted on to Wengkhar Tshalu Rhato 1 rootstock and seedlings were raised from seeds. The disease free germplasm block was established at Wengkhar in 2002. The germplasm block consists of both the grafted as well as the seedling origin.

The fruit quality analysis was started only after full bearing in 2008 although they started bearing fruits from 2006 and some lines were analyzed in 2007. The evaluation aimed at screening the superior lines from the nationwide collections for mid altitude areas in the country and at the same time to maintain repository of national citrus collection in the centre.

Among the 57local mandarin lines evaluated, Lines 23-24, 26, 27-28, 33-34, 39-40, 41-42, 45-46, 47-48, 49-50, 51-52, 59, 69-70, 71-72 and 75-76 were found promising in terms of fruit quality characteristics. More so, the line 47-48 was found the most promising in terms of tree growth and agronomic performance.

KEY WORDS: Citrus Huanglongbing (HLB), local mandarin lines, germplasm and performance

INTRODUCTION

In Bhutan, the cultivation of citrus is mainly dominated by mandarin (*Citrus reticulata* Blanco), although some amount of lime, lemons, pummelos and citron do exist. Plantations are found mainly in humid sub-tropical areas in the foothills at elevation ranging from 300 to 1500 m (RNRRC-East, 2006). Compared to other fruit crops such as apple and Arecanut, citrus occupies the maximum area and cultivated on commercial scale followed by apples and arecanut. In total there are around 1,831,312 citrus trees, of which 983,407 are fruit bearing trees. Over 70% of the crop is grown in the five Dzongkhags of Samdrupjongkhar, Chukha, Sarpang, Tsirang and Samtse (in decreasing order), while the Dzongkhags of Punakha, Wangdue, Trongsa, Mongar, Tashiyangtse and Trashigang grow on a much smaller scale (DoA, 2004).

Unfortunately, citrus in most these Dzongkhags are infected with a destructive disease of citrus called Huanglongbing (HLB) disease (Tomiyasu *et al*., 2000; NPPC, 2002) caused by a phloem-restricted, non-cultured, Gram-negative bacterium, *Candidatus liberibacter asiaticus*. With the detection of this devastating disease in Bhutan since 2000, the area under citrus, the production level, yield and the number of citrus trees have been declining. The disease is transmitted by the Asian Psyllid (*Diaphorina citrii*) that is found in abundance, especially in all major citrus growing areas.

Following the detection of citrus Huanglongbing disease in major citrus growing areas in the country, RDC Wengkhar with the technical expertise of the Horticulture Expert felt the need to maintain disease free germplasm block at the Centre to serve as the progeny and mother block in future.

In 2000 major survey was done in all the citrus growing regions to collect seeds and scion wood from disease free trees for the purpose of maintaining germplasm collections. The germplasm collection block consists of both the grafted as well as of the seedling origin has been developed at the RDC-Wengkhar.

The trees in the germplasm collection block started bearing fruits from 2006, but fruit quality assessment was done in 2008 when trees have attained full bearing stage. However, fruit quality assessment for some lines was done in 2007 also. Through such collection and evaluation, it

is expected to screen the superior lines targeted for growing in the mid altitude environment in the country, and at the same time, to further develop the germplasm collection block as the repository of citrus collection in future.

MATERIALS AND METHODS

Survey and collection of germplasm

First selection of disease free orchards was identified in the farmers' field. More than 200 samples of seeds and scion wood of the selected local mandarins were collected from 57 farmer's orchards that represent all the major citrus growing Dzongkhags in the country. Scion woods were grafted on to USDA *Trifoliate orange* rootstock. Seedling origin collections were also established to compare the tree growth and vigour between grafted and seedling origin plants.

Establishment of germplasm block and fruit analysis

Fig 1: Local mandarin germplasm block

The germplasm block was established and maintained as the local citrus germplasm block at the centre. The germplasm block consists of trees that are grafted as well as of the seedling origin. The trees in the collection started bearing fruits from 2006, but the fruit quality analysis for 57 samples was carried out only from 2007. The fruit quality analysis during 2007 resulted in discarding 19 samples/lines which were inferior in quality; Only 38 lines of local citrus collection were selected for analysis in 2008. The scion woods collected from more than 57 orchards were grafted on to Wengkhar Tshalu Rhato 1 rootstock (formerly USDA Trifoliate). In addition to fruit quality

assessment, tree growth, tree form, fruit shape and fruit taste were also evaluated. The cumulative ranking was done to assess the overall merit and the ranking was done with respect to fruit quality parameters, tree growth, tree forms, fruit shape and fruit taste. The tree growth was classified as Very good (3), Good (2) and Satisfactory (1); tree forms was classified as Very good (3), Good (2) and Satisfactory (1); fruit shape was classified as Uniform (2) and Non-uniform (1), and fruit taste was classified as Very sweet (4), Sweet (3), Average (2) and Sour (1). The list of germplasm collection is shown in Table 1.

Tab. 1: The list of local mandarin germplasm as established in 2002

Lines	Farmer's Name	Location	Dzongkhag	Lines	Farmer's Name	Location	Dzongkhag
	Sonam						
23-24	Wangchuk	Pemagatshel	Pemagatshel	120-121	Khan Nath Nepal	Dorokha	Samtse
27-28	Thinley	Shumar	Pemagatshel	128-129	Suk Bahadur Rai	Samtse	Samtse
26	C. Wangdi	Kengkhar	Mongar	312-133	Nar Bahadur Rai	Samtse	Samtse
29	Rinchen Dorji	Kengkhar	Mongar	124-125	Dil Bahadur Gurung	Sarpang	Sarpang
41-42	Pema Tenzin	Kengkhar	Mongar	85-86	Tshering Wangda	Chukha	Chukha
	Chorten						
43-44	Tshering	Kengkhar	Mongar	110-111	Wangchuk	Chukha	Chukha
47-48	Shacha	Kengkhar	Mongar	33-34	Bhudiman Alley	Dagana	Dagana
49-50	Sonam Tashi	Kengkhar	Mongar	37-38	Santa Bahadur Rai	Dagana	Dagana
51-52	Thinley	Kengkhar	Mongar	71-72	Gopal Singh	Dagana	Dagana
53-54	P. Wangdi	Kengkhar	Mongar	89-90	Bhudiman Alley	Dagana	Dagana
57-58	Sonam Choden	Kengkhar	Mongar	91-92	Naku Goshi	Dagana	Dagana
59	Rinchen Dorji	Kengkhar	Mongar	105	Pema Tshering	Dagana	Dagana
30	S. Gyeltshen	Narang	Mongar	106-107	Kharkaman Ghaley	Dagana	Dagana
60	S. Gyeltshen	Narang	Mongar	114-115	Bhudiman Alley	Dagana	Dagana
63-64	Vabjay	Narang	Mongar	126-127	Kal Bahadur Mongar	Dagana	Dagana
69-70	Sonam Tashi	Narang	Mongar	130-131	Kal Bahadur Tamang	Dagana	Dagana
75-76	Sonam Tashi	Narang	Mongar	35-36	D.P. Rai	Tsirang	Tsirang
83-84	Dechen	Saling	Mongar	61-62	Sukpal Subha	Tsirang	Tsirang
39-40	Norbu	Yadi	Mongar	65-66	I. L. Prafuly	Tsirang	Tsirang

77-78	Norbu	Yadi	Mongar	81-82	I. L. Prafuly	Tsirang	Tsirang
55-56	Anim Tshomo	S/jongkhar	S/jongkhar	87-88	D.P. Rai	Tsirang	Tsirang
112-113	Pema Rinzin	S/jongkhar	S/jongkhar	101-102	Suk Bahadur Rai	Tsirang	Tsirang
31-32	Santa Bahadur Rai	Samtse	Samtse	67-68	Thangbi	Trongsa	Trongsa
79-80	Birkha Bahadur Gurung	Dorokha	Samtse	122-123	Kinzang Choden	Trongsa	Trongsa
96	Bir Bahadur Tiwari	Dorokha	Samtse	116-117	Mamey Doya	Dorokha	Samtse
99	Tula Ram Adhikari	Samtse	Samtse				
103-104	Dup Tshering Lepcha	Dorokha	Samtse	73-74	Darchung	Thempey	
118-119	Govinda Prasad	Samtse	Samtse				

To measure the acid content of randomly selected fruits of different mandarin lines, juice was extracted from the fruit samples. The acidity was determined by titrating juice with 0.01 N sodium hydroxide solutions. Phenolphthalein was used as an indicator for the titration. The acidity was calculated using the formula cited below:

% Acid content (Citric acid) = Volume of 0.01 N NaOH used for titration X Titre value.

Total Soluble Solids (TSS) in the fruit was determined by using *Hand Refractometer*, expressed as ^{o}Brix. Similarly, the fruit size (diameter and length) was measured with the help of Digital Vernier caliper.

Data Analysis

The data was compiled in MS Excel Sheet and analyzed in One Way ANOVA (Randomized Block) on GenStat 10.1 (PC/Windows) Copyright 2007, Lawes Agricultural Trust (Rothamsted Experimental Station), Registered to: University of Reading. The Cumulative ranking of the 14 selected superior lines with respect to tree growth, tree forms, fruit shape and fruit taste was done using Pivot table in MS Excel.

RESULTS AND DISCUSSION

Since one of the main objectives of this activity is to select superior local mandarin lines with good horticultural characteristics and yield; evaluation and selection of superior lines from 2008 to 2010 will be discussed. Based on the evaluation results of each year, fruit quality rating has been done and selection of superior lines made. At the same time, the fruit characteristics, tree growth and vigor as observed in 2008 were also used as a guide for selecting the superior lines. The evaluation results for 2008 had been used as baseline for selecting the superior lines in the successive years.

Local mandarin germplasm evaluation and selection in 2008

Based on the fruit quality analysis, fruit characteristics and tree growth and vigor, 19 superior lines were selected in 2008 (Table 2). These superior lines have been evaluated further in 2009 and the results are discussed. In 2008, there was no major incidence of insect-pests and disease except leaf miner and powdery mildew on new shoots during flushing period.

Tab. 2: Superior lines of Local mandarin germplasm selected in 2008

Sl. No.	Tree No.	Farmer's Name	Locations	Sl. No.	Tree No.	Farmer's Name	Locations
1	23-24	Sonam Wangchuk	Pemagatshel	20	59	Rinchen Dorji	Kengkhar
2	26	Choiten Wangda	Kengkhar	21	61-62	Sukpal Subha	Tsirang
3	27-28	Thinley	Shumar	22	63-64	Vabjay	Narang
4	29	Rinchen Dorji	Kengkhar	23	65-66	I. L. Prafuly	Tsirang
5	30	Sonam Gyeltshen	Narang	24	67-68	Thangbi	Trongsa
6	31-32	Santa Bdr Rai	Samtse	25	69-70	Sonam Tashi	Narang
7	33-34	Bhudiman Alley	Dagana	26	71-72	Gopal Singh	Dagana
8	35-36	D. P. Rai	Tsirang	27	73-74	Darchunng	Thempey
9	37-38	Santa Bdr Rai	Dagana	28	75-76	Sonam Tashi	Narang
10	39-40	Norbu	Yadi	29	77-78	Norbu	Yadi
11	41-42	Pema Tenzin	Kengkhar	30	79-80	Birkha Bdr. Gurung	Dorokha
12	43-44	Choiten Tshering	Kengkhar	31	81-82	I. L. Prafuly	Tsirang
13	45-46	Sadrung	Sadrung	32	83-84	Dechen	Saling
14	47-48	Shacha	Kengkhar	33	85-86	Tshering Wangda	Chukha
15	49-50	Sonam Tashi	Kengkhar	34	89-90	Bhudiman Alley	Dagana
16	51-52	Thinley	Kengkhar	35	106-107	Kharkaman Ghaley	Dagana
17	53-54	Pema Wangdi	Kengkhar	36	112-113	Pema Rinzin	S/jongkhar
18	55-56	Anim Tshomo	S/jongkhar	37	114-115	Bhudiman Alley	Dagana
19	57-58	Sonam Choden	Kengkhar	38	120-121	Khan Nath Nepal	Dorokha

Evaluation & Selection of Local mandarin lines in 2009

The superior lines selected in 2008 were further evaluated in 2009. From the evaluation results, it was observed that there was a significant difference in fruit weight of different local mandarin lines (P=>0.001) with highest fruit weight observed in Lines 30 (221.4 gm) and lowest fruit weight in Lines 79-80 (56 gm). On the other hand, there was no significant difference among other quality parameters of different lines. However, for further selection, cumulative variety ranking matrix of different lines was done based on the evaluation results.

From the cumulative ranking, it was observed that Lines 29 (Rank 35) and 30 (Rank 35) were the highest in rank whereas the Line 73-74 (19) was the lowest in the ranking. Therefore, based on the cumulative ranking, 17 lines have been identified as the superior lines based on the 2009 evaluation results (Table 3).

Tab. 3: Superior lines of Local mandarin selected in 2009

Sl. No.	Local lines	Farmer's Name	Locations
1.	23-24	Sonam Wangchuk	Pemagatshel
2.	26	Choiten Wangda	Kengkhar
3.	27-28	Thinley	Shumar
4.	29	Rinchen Dorji	Kengkhar
5.	30	Sonam Gyeltshen	Narang
6.	33-34	Bhudiman Alley	Dagana
7.	37-38	Santa Bdr Rai	Dagana
8.	39-40	Norbu	Yadi
9.	41-42	Pema Tenzin	Kengkhar
10.	45-46	Sadrung	Sadrung
11.	47-48	Shacha	Kengkhar
12.	49-50	Sonam Tashi	Kengkhar
13.	51-52	Thinley	Kengkhar
14.	59	Rinchen Dorji	Kengkhar
15.	69-70	Sonam Tashi	Narang
16.	71-72	Gopal Singh	Dagana
17.	75-76	Sonam Tashi	Narang

Evaluation and Selection of Local mandarin lines in 2010

The superior lines selected in 2009 were further evaluated in 2010. From the results, it was observed that there was a significant difference in fruit weight of different local mandarin lines (P=>0.001) in all three years. Since the total soluble solids content and fruit weight are the main quality parameters in mandarins, these two parameters of the selected lines for 2010 results were compared with 2008 and 2009 results as presented in Figure 2 and 3.

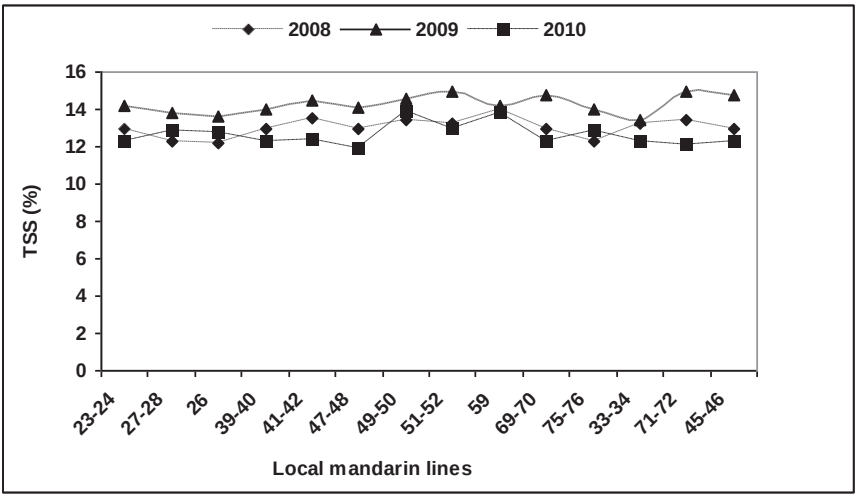

Fig. 2: ***TSS content of Selected Local mandarin lines***

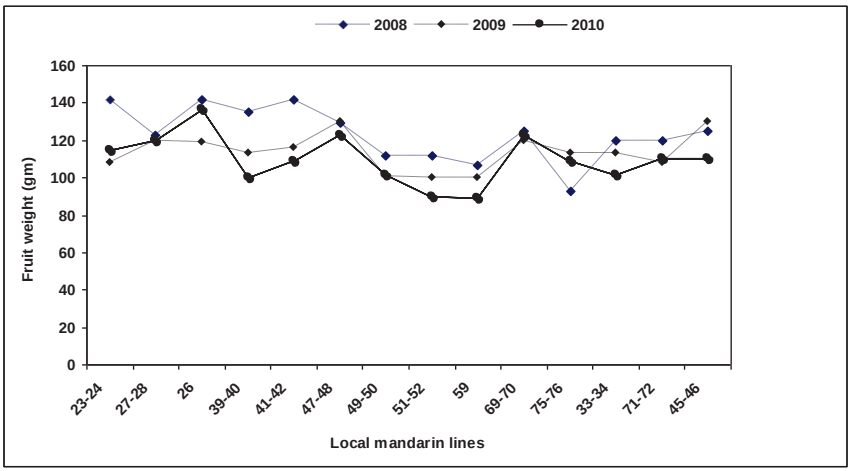

Fig. 3*: Fruit weight of Selected Local mandarin lines*

It was observed that fruit quality varies from year to year. In 2008, the highest fruit weight was observed in Lines 23-24, 26 and 41-42 (142 gm) and lowest fruit weight in Line 75-76 (93 gm). However, highest fruit weight was observed in Lines 45-46 and 47-48 and lowest in Line 49-50 in 2009 as compared to highest fruit weight in Line 26 and lowest in Line 59 in 2010. The fruit weights of all the lines differ significantly from year to year. Similarly, the total soluble solid content of the selected lines for 2010 were also compared to mean results of 2008 and 2009 which, indicated that TSS also varies from year to year. The annual variation in vital fruit quality parameters such as TSS and fruit weight may be attributed to the variation in annual temperature and rainfall which directly affects the TSS content and weight of fruit More so, the annual variation in fruit quality may be due to the variation in inherent nutrient status of the soil and soil moisture content because the soil nutrient status varies greatly from terrace to terrace in a counter terrace system (Fig 2 and 3).

Therefore, based on the cumulative ranking matrix, 14 superior lines were identified and selected (Table 4). The Cumulative ranking of 14 selected lines was also done with respect to the tree growth, tree forms, fruit shape and fruit taste, which clearly indicates the superiority of the selected collections (Table 5). The mean results of the fruit quality parameters of the selected superior lines are shown in the Table 6.

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Tab. 4: Superior lines of Local mandarin selected in 2010

Sl. No.	Local lines	Farmer's Name	Locations
1.	23-24	Sonam Wangchuk	Pemagatshel
2.	26	Choiten Wangda	Kengkhar
3.	27-28	Thinley	Shumar
4.	33-34	Bhudiman Alley	Dagana
5.	39-40	Norbu	Yadi
6.	41-42	Pema Tenzin	Kengkhar
7.	45-46	Sadrung	Sadrung
8.	47-48	Shacha	Kengkhar
9.	49-50	Sonam Tashi	Kengkhar
10.	51-52	Thinley	Kengkhar
11.	59	Rinchen Dorji	Kengkhar
12.	69-70	Sonam Tashi	Narang
13.	71-72	Gopal Singh	Dagana
14.	75-76	Sonam Tashi	Narang

Tab. 5: Cumulative ranking matrix of selected lines based on Tree growth, tree forms, fruit shape and fruit taste (2010)

Local lines	Tree growth	Tree forms	Fruit shape	Fruit taste	Total
23-24	1	1	2	2	6
26	1	1	2	2	6
27-28	1	1	2	1	5
33-34	1	1	2	2	6
39-40	1	1	2	3	7
41-42	1	1	2	2	6
45-46	1	1	1	2	5
47-48	3	2	2	3	10
49-50	1	1	2	2	6
51-52	3	1	2	4	10
59	1	1	2	3	7
69-70	2	2	2	3	9
71-72	2	2	2	1	7
75-76	1	1	2	2	6

Tab. 6: Mean result of fruit quality parameters of the selected superior lines as observed in 2010

Lines selected in 2009	Fruit Wt. (gm)	Fruit Ht. (cm)	Fruit Dia. (cm)	Pulp Wt. (gm)	Juice Wt. (gm)	Rind Thickness (Cm)	Seed Content	TSS (%)	Acidity (%)
23-24	114.8	5.2	5.8	42.5	34.8	0.26	16	12.3	3.2
26	136.8	4.9	5.7	30.5	25.0	0.24	12	12.8	3.5
27-28	119.5	5.4	6.2	37.3	29.5	0.27	17	12.9	3.5
33-34	101.2	4.7	6.1	33.8	37.3	0.31	15	12.3	3.6
39-40	101.2	5.2	6.2	33.8	38.5	0.34	16	12.3	3.6
41-42	108.8	5.0	6.4	36.8	49.5	0.33	16	12.4	3.2
45-46	110	5.1	6.5	34.0	41.3	0.36	17	12.3	4.4
47-48	122.5	5.3	6.3	30.5	45.5	0.39	13	12.0	2.5
49-50	101.8	5.2	6.0	32.3	34.3	0.29	18	13.9	2.7
51-52	90	4.9	5.9	35.0	30.3	0.27	15	13.0	3.4
59	88.8	4.5	5.7	27.5	31.5	0.20	13	13.8	2.9
69-70	122.5	5.3	6.5	53.8	35.3	0.28	17	12.3	3.2
71-72	110	4.9	6.4	31.3	37.8	0.24	16	12.1	3.4
75-76	108.8	4.8	5.8	33.8	38.5	0.24	16	12.9	2.5
p value	<0.001	0.011	0.073	0.005	<.001	<.001	0.303 (n.s)	<.001	*
lsd	24.17	0.5483	0.6677	11.346	11.281	0.07524	6.32	0.9105	*
cv %	15.9	7.8	7.8	23.6	22.4	18.7	30.5	5.1	*

**Not determined by Statistical package; n.s, Not significant at 0.001%*

Tab.7: Cumulative ranking matrix of selected lines based on fruit quality parameters (2010)

Lines	Fruit wt.	Fruit Ht.	Fruit Dia.	Pulp Wt.	Juice content	Rind thickness	Seed content	TSS	Acidity	Total
23-24	7	3	4	7	5	3	3	2	4	38
26	9	2	4	5	3	4	4	3	4	38
27-28	7	3	5	6	4	3	2	3	4	37
33-34	6	2	5	5	6	2	3	2	3	34
39-40	6	3	5	5	6	2	3	2	3	35
41-42	6	2	5	6	8	2	3	2	4	38
45-46	7	3	5	5	7	1	2	2	2	34
47-48	8	3	5	5	8	1	4	1	5	40
49-50	6	3	4	5	5	3	2	5	5	38
51-52	5	2	4	5	4	3	3	3	4	33
59	4	1	4	4	5	5	4	5	5	37
69-70	8	3	5	9	5	3	2	2	4	41
75-76	6	2	4	5	6	4	3	3	5	38
71-72	7	2	5	5	6	4	3	2	4	38

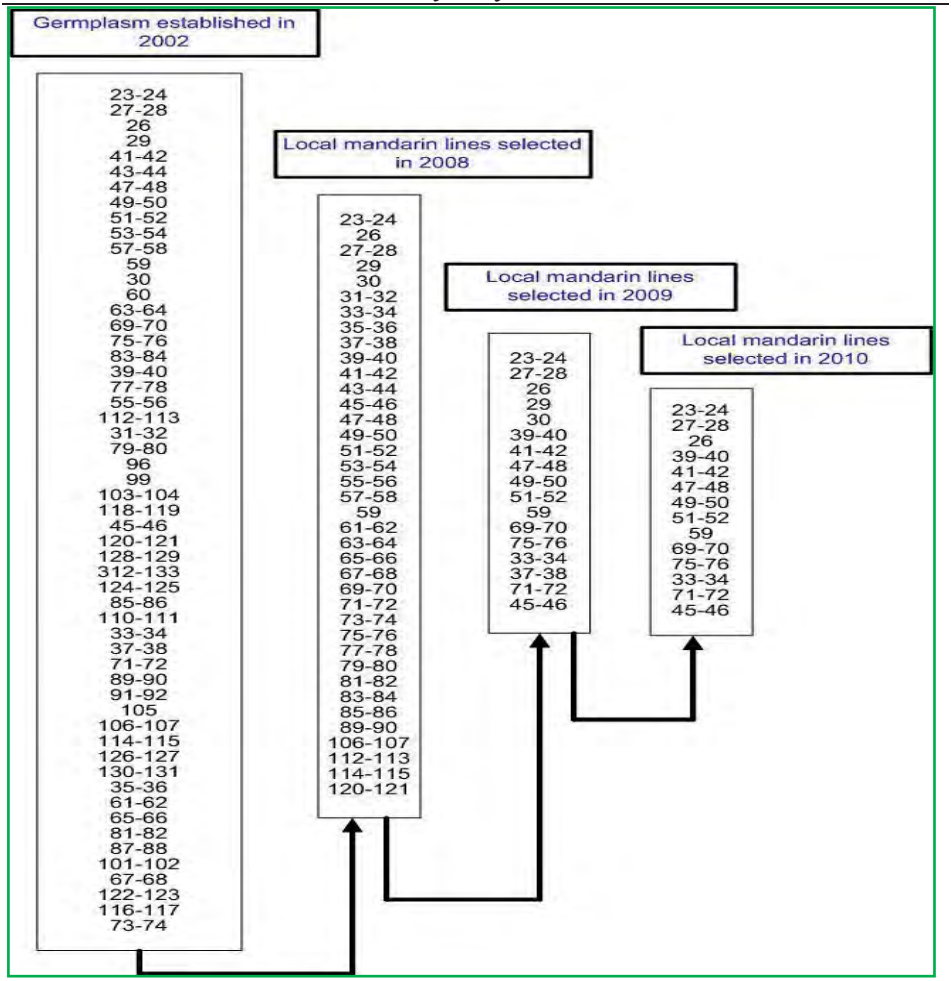

Fig 4: Year-wise germplasm evaluation and selection of superior lines

Based on the Cumulative ranking matrix of fruit quality parameters, tree growth, tree forms, fruit shape and fruit taste of 14 superior lines of citrus collection selected in 2010, the Lines 69-70, 47-48, 23-24, 26, 41-42, 49-50, 71-72 and 75-76 were found potential (Table 7). The year wise evaluation and selection of superior lines of local mandarins are shown in Figure 4 below.

CONCLUSION

Germplasm collection and evaluation of local mandarin lines collected from all major mandarin growing areas of the country not only helps in conservation of mandarin germplasm but also ensure sustainable utilization of mandarin diversity. Moreover, the subsequent evaluation and selection of superior lines would help in expanding the mandarin cultivation by grafting on to the dwarfing and cold tolerant rootstock Wengkhar Tshalu Rhato 1.

The year-wise evaluation and selection of local mandarin lines have resulted in screening of 14 superior lines viz. 23-24, 26, 27-28, 33-34, 39-40, 41-42, 45-46, 47-48, 49-50, 51-52, 59, 69-70, 71-72 and 75-76 respectively. Amongst these superior lines, 69-70, 47-48, 23-24, 26, 41-42, 49-50, 71-72 and 75-76 were found potential and the promotion of line such as 47-48 may help in rehabilitation of mandarin cultivation in the country.

ACKNOWLEDGEMENT

We would like to thank all our horticulture research colleagues both past and present who have worked tirelessly from survey to the establishment of germplasm block at Wengkhar. Our sincere thanks are also due to the administrative support of the successive management of the centre for making this study happen.

REFERENCES

Phuntsho, L. (2010). Horticulture Research Trial Report: a compilation of horticulture research trial reports for 2004-2008, RNR RDC Wengkhar Technical Document No. 46/ Ho/ 2009-2010, RNR RDC, Wengkhar, Mongar, Department of Agriculture, Ministry of Agriculture and Forests.

T. Yuichi. (2004). General Report on Agriculture in Bhutan (March, 2000 - June, 2004). RNR RC-East, Wengkhar, Mongar, Farm Village Development (Pomology).

Renewable Natural Resources Research Centre. Wengkhar. (2008). National Horticulture Research Program Report, 9th Five Year Plan, July

2002 to June 2008, RNR RC Wengkhar, CoRRB, Ministry of Agriculture.

Renewable Natural Resources Research Centre. (2008). Annual Report 2007 - 2008, RNR RC, Wengkhar, Mongar, CoRRB, Ministry of Agriculture.

FORESTRY

CO_2 emission from natural forest, forest plantation and agricultural areas in the Northeast of Thailand

Pongthep Suwanwaree* and Karma Dorji

ABSTRACT

Recently, scientists have focused attention on soil as a major source and sink for atmospheric CO_2. To study the difference in soil respiration rates among tropical ecosystems, field CO_2 efflux measurements were carried out in eleven different ecosystems at Suranaree University of Technology, Sakaerat Environmental Research Station and Sakaerat Silvicultural Research Station, Nakhon Ratchasima province, Thailand. In each ecosystem, a line transect was laid and then plastic chambers with airtight lids were fixed into the soil at 20 m interval. The 24-h soil respiration was measured by soda-lime method once a month from January to April 2010. There was a significant difference ($p<0.01$) in mean soil respiration rates among the ecosystems with highest in dry evergreen forest (DEF), followed by cornfield, sunflower , Acacia auriculiformis, grassland, Dalbergia cochinchinensi, Eucalyptus camaldulensis, Acacia mangium, dry dipterocarp forest (DDF), rubber, Eucalyptus sp. (Eu1) plantation sites with the value of 4.31, 4.20, 3.75, 3.48, 3.35, 3.23, 3.16, 2.85, 2.83, 2.79 and 1.89 g CO_2 m^{-2} d^{-1}, respectively. The significant higher water content and neutral soil pH of cornfield and sunflower soils were the causes of higher soil respiration rates. The significant higher ($p<0.01$) soil organic carbon, total nitrogen and water content of DEF soil had also contributed to its higher respiration rates. The soil water content, pH, carbon and nitrogen were the main factors influencing soil respiration rates of tropical ecosystems.

KEY WORDS: CO_2 emission, Soda-lime method, Soil respiration, Sakaerat Environmental Research Station, Tropical ecosystem

INTRODUCTION

CO_2 is the most important anthropogenic greenhouse gases (GHG) and its annual emissions have grown between 1970 and 2004 by about 80%, from 21 to 38 gigatonnes (Gt), and represented 77% of total anthropogenic GHG emissions in 2004 (IPCC, 2007). The main carbon

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reservoirs are the oceans, soils, the atmosphere, and land plants containing about 38,000, 1,500, 750 and 560 Pg C, respectively (IPCC, 2001).

Recently, scientists have focused attention on soil as a major source and sink for atmospheric CO_2. On a global scale, soil respiration produces 80.4 Pg C y^{-1} with a range of 79.3–81.8 Pg C y^{-1}, accounting for 60–90 percent of total respiration of global terrestrial ecosystems and it is eleven times of current fossil fuel combustion (Peng *et al*., 2009). Tropical soil has highest respiration rates with about twenty fold more than tundra and contributes to the highest CO_2 efflux into the atmosphere (Luo and Zhou, 2006). The soils hold twice as much carbon as the atmosphere with highest in tropical soil (IPCC, 2001). Thus, a small change in soil respiration rates may have a significant effect on the global C balance and therefore on climate change.

Soil respiration includes three biological processes, namely microbial respiration, root respiration and faunal respiration. Soil microflora contributes 99% of the CO_2 efflux through decomposition of organic matter, while the contribution of soil fauna is much less (Rastogi *et al*., 2002). Several studies have shown that factors such as soil texture, temperature, moisture, pH, available C and N content of the soil influence soil CO_2 production and emission. Soil respiration determine whether a specific land-use or management practice causes a system to be C source or a sink and fluctuation in soil CO_2 flux can result in significant changes in global C cycle (Peng *et al*., 2009).

Therefore, detailed information on soil respiration and its controlling factors is critical for constraining the ecosystem C budget and for understanding the response of soils to changing land use and global climate change (Buchmann, 2000), which is still not yet sufficiently understood to incorporate them into global-scale C cycling models (Chapin *et al*., 2009).

Changes in land use almost always lead to a change in vegetation, density of above and belowground biomass, the amount of resources available for soil microbes, the physical and chemical characteristics of the soil. Thus it is of primary importance to know the mean respiration rates in each ecosystem and their responses to environmental factors when considering the C cycle.

This study aims to compare soil respiration of different land use types from natural forests, forest plantations to agriculture.

MATERIALS AND METHODS

Sites

Eleven land use types were selected for this study. They are cornfield (C), sunflower (S), grassland (G), 20 years old *Eucalyptus* sp. (Eu1) and rubber (R) plantations from Suranaree University of Technology (SUT), dry evergreen forest (DEF) and dry dipterocarp forest (DDF) from Sakaerat Environmental Research Station (SERS), and 25 years old *Acacia auriculiformis* Cunn. (Aa), *Acacia mangium* Willd (Am), *Dalbergia cochinchinensis* Pierre (Dc) and *Eucalyptus camaldulensis* Dehnh (Eu2) from Sakaerat Silvicultural Research Station (SSRS).

Experimental Design

In each SUT site, two 40 m parallel line transects were laid randomly with 15 m apart and three plastic chambers (15 cm diameter and 15 cm height) were fixed in each line by inserting 5 cm into the soil at 20 m intervals. But in SERS and SSRS sites, only one line transect of 40 m long were laid and three chambers of 20 m apart were fixed in each line. The chambers were fixed few days before the CO_2 measurements by using alkali-absorption method (Duiker and Lal, 2000). Two plastic cups filled with 40 ml of 1M sodium hydroxide were used to determine the amount of CO_2. One was placed in the chamber while the other was closed and kept in the laboratory. The chamber was then covered by a plastic lid with rubber bands wrapped around to ensure proper sealing. After 24 h, the cups were removed from the chambers, closed with lids and transported to the laboratory for titration. Excess NaOH was titrated to pH 8.2 in the presence of excess $BaCl_2$ using 1M HCl and phenolphtalein as an indicator. Soil CO_2 efflux were measured once a month from January to April, 2010. Soil factors like soil pH (1:1 soil: water suspensions), soil organic carbon (Walkley-Black method) and total nitrogen (Kjeldahl method) were also analyzed (Gupta, 2007) using soil samples from only January month. Field air and soil temperatures and soil moistures were also measured during each sampling time.

One-way ANOVA followed by post hoc Duncan test was used to find the differences in soil respiration rates and other parameters among the ecosystems. Pearson Correlation Coefficient was calculated to find the relationship between soil respiration rate and its controlling factors. SPSS version 16 program was used for all of these analyses.

RESULT

Soil respiration rate

The mean soil respiration was significantly different ($p<0.01$) among the ecosystems with highest in dry evergreen forest followed by cornfield and lowest in *Eucalyptus* plantation areas in SUT with 4.31, 4.20 and 1.89 g CO_2 $m^{-2}d^{-1}$, respectively (Figure 1).

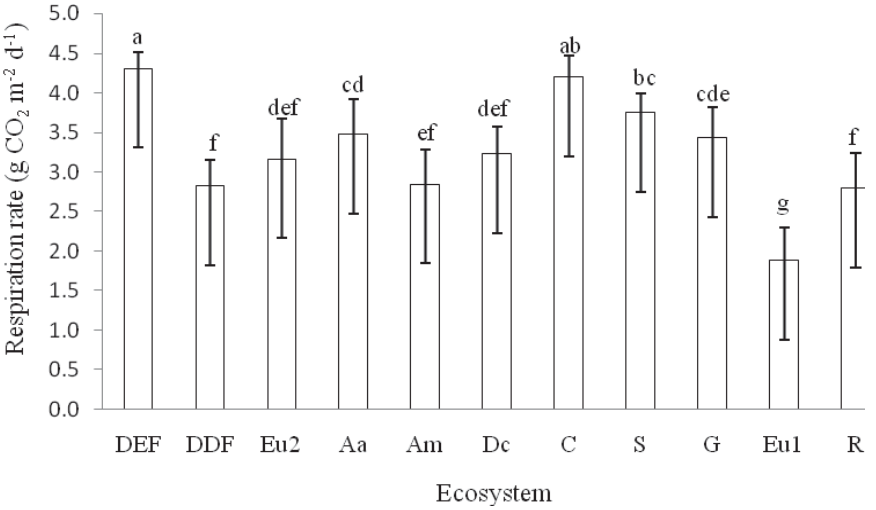

Fig. 1 Mean soil respiration rates of different ecosystems in SUT, SERS and SSRS. Dry evergreen forest (DEF), dry dipterocarp forest (DDF), *Eucalyptus camaldulensis* (Eu2), *Acacia auriculiformis* (Aa), *Acacia mangium* (Am), *Dalbergia cochinchinensis* (Dc), cornfield (C), sunflower (S), grassland (G), *Eucalyptus* sp. (Eu1) and rubber (R). (n = 24 for SUT ecosystems and n = 12 for SERS and SSRS ecosystems).

Soil physical and chemical factors

The mean soil water content, pH, carbon and nitrogen were significantly different ($p<0.01$, resprectively) among the ecosystems. The mean soil water content was highest in cornfield followed by and sunflower but lowest in Eu1. Soil pH of sunflower and cornfield were neutral while the rest were acid, especially in forest plantation. Mean soil organic carbon was highest in DEF followed by *Dalbergia cochinchinensis* but lowest in rubber plantation with 3.208, 2.174 and 0.698%, respectively. Mean soil total nitrogen was highest in DEF (0.45%) followed by *Acacia mangium* (0.17%) but lowest in rubber plantation (0.16%). Soil water

content, pH, carbon and nitrogen had significant positive correlation with soil respiration (Fig. 2 and Table 1)

Tab. 1: Pearson correlation coefficient of soil respiration rates and its controlling factors.

	Temp.	Water cont	pH	C	N
Soil resp.	-.202**	.728**	.488**	.579**	.582**

**Correlation is significant at p<0.01

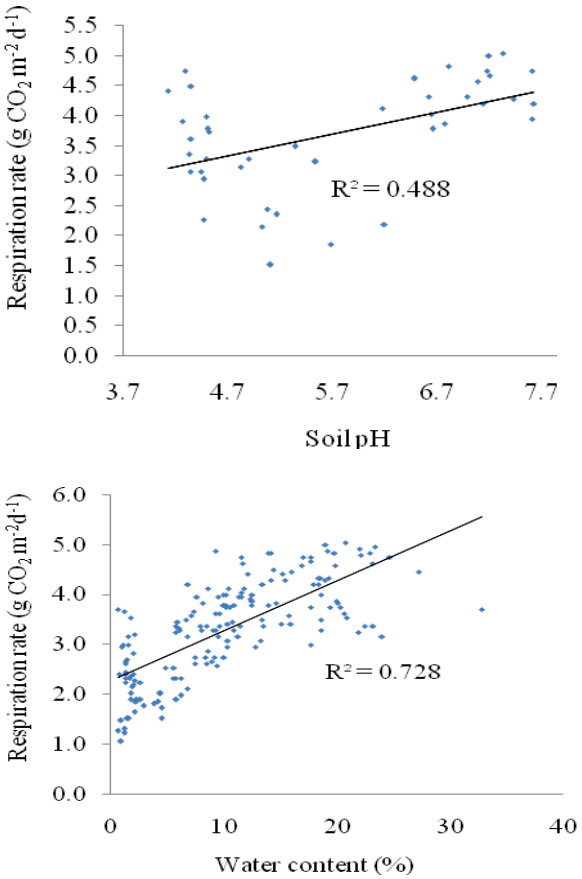

Fig. 2 Relationships of soil respiration rates with soil water content, pH.

DISCUSSION

The CO_2 efflux from sunflower, cornfield and dry evergreen forest were (3.8, 4.2 and 4.3 g CO_2 $m^{-2}d^{-1}$) significantly higher than other ecosystems which agrees with the values of Campos (2006) who obtained the value of corn–potato–corn rotation plot and tropical cloud forest to be 1.58-11.25 and 1.98-8.1 g CO_2 $m^{-2}d^{-1}$ respectively using alkali-absorption method. There were no significant differences in average CO_2 efflux between cornfield and DEF. The high CO_2 efflux of DEF soil was due to its high organic carbon, total nitrogen and water contents. While high CO_2 efflux of cornfield and sunflower, despite its comparatively lower soil carbon and nitrogen contents than DEF, was attributed by their higher water contents from constant field watering and its neutral pH. Miao *et al.* (2004) also found increased in soil respiration rates within the water content limits of 21 to 37% but decreased above that limit. The higher soil CO_2 efflux with higher water content was also supported by many studies (Hashimoto *et al.,* 2004; Miao *et al.*, 2004; Haper *et al*., 2005; Keith *et al.*, 1997; Kosugi *et al*., 2007; Hanpattanakit *et al.*, 2008; Schaefer *et al.*, 2009; Takahashi *et al*., 2009). The plant and microbial activity increases in response to soil water content increase (Lee *et al*., 2002; Luo and Zhou, 2006).

The soil pH was positively correlated with soil respiration ($p<0.01$), which was supported by Reth *et al.*(2005). The acidity of ecosystem soils other than cornfield and sunflower might have lead to lower soil respiration rate. Kemmitta *et al*. (2006) observed reduction in soil respiration with increasing acidity in agricultural soil.

A few soil respiration researches have been done in Thailand before. Both Panuthai *et al*. (2005) and Hashimoto et al. (2004) found higher soil respiration in dry evergreen forest at SERS and tropical monsoon evergreen forests of Kog-Ma Watershed than my observation due to more advance equipment as IRGA and a whole year study period. Adachi *et al*. (2006) also got higher CO_2 efflux than this study in tropical primary and secondary forest with 19.94 and 20.11g $CO_2m^{-2}d^{-1}$ in Malaysian Peninsula. The static chamber method gave lower values compared with dynamic chamber methods (Nay *et al*., 1994). However, Wiriyatangsakul (2004) obtained slightly lower value (3.082 g $CO_2m^{-2}d^{-1}$)in tropical dry evergreen forest in Phanom Sarakarm district in dry season as well.

Iqbal *et al.* (2008) measured soil respiration rates in sesame–peanut rotation site and paddy field in subtropical China and obtained the value of 1.52 and 2.47 g CO_2 $m^{-2}d^{-1}$ much lower than the values of cornfield and sunflower in this study due to its subtropical climatic condition where respiration rate is lower than tropical areas. The higher soil respiration rates in agricultural fields in the present study compared with other ecosystems were supported by Miao *et al.* (2004) and Adachi *et al.* (2006).

The soil temperatures at SUT, SERS and SSRS were negatively correlated with soil respiration rates but found significant at $p<0.05$ only for SERS and SSRS sites which were the same as the results of Hanpattanakit *et al.* (2008) who studied DDF site in Chombung District, Ratchaburi province. But some researchers like Mo *et al.* (2008) observed positive exponential relationship between soil respiration and soil temperature in tropical forest in China. Iqbal *et al.* (2008) also observed soil temperature as an important variable controlling 26–59% of soil CO_2 flux variability. Increasing in soil temperature over the time may cause reduction of soil water and thickness of the soil water films and also the temperature above 35^{o}C may cause protoplasm system to start breaking down (Luo and Zhou, 2006).

The yearly CO_2 efflux was found highest in DEF, cornfield followed by sunflower and lowest in *Eucalyptus* sp. with 4.21, 4.19, 4.11 and 1.84 t C ha^{-1} y^{-1} (Table 2). The values were lower than many other studies like Keith *et al*. (1997) who measured soil respiration of *Eucalyptus pauciflora* in Brindabella Range, Australia for a year using soda lime method and obtained 7.11 t C ha^{-1} y^{-1}. The reason behind is that we measured only during dry season where as they have data for full year.

Tab. 2: Daily and yearly field soil CO_2 efflux from different ecosystems in SUT, SERS and SSRS calculated based on present study from January to April 2010 (n = 24 for SUT ecosystems but n = 12 for SERS and SSRS ecosystems.

Ecosystem	Respiration rate		
	$gCO_2\ m^{-2}\ d^{-1}$	$gC\ m^{-2}\ d^{-1}$	$tC\ ha^{-1}\ y^{-1}$
Cornfield	4.201	1.150	4.105
Sunflower	3.748	1.023	3.663
Grassland	3.351	0.915	3.275
Eucalyptus sp.	1.885	0.515	1.842
Rubber	2.794	0.763	2.730
Dry evergreen forest	4.308	1.176	4.210
Dry dipterocarp forest	2.825	0.771	2.761
Eucalyptus camaldulensis	3.164	0.864	3.092
Acacia auriculiformis	3.479	0.950	3.400
Acacia mangium	2.846	0.777	2.781
Dalbergia cochinchinensis	3.231	0.882	3.157

CONCLUSION

There was a significant difference ($p<0.01$) in mean soil respiration rates among the ecosystems with highest in the DEF and lowest in Eu1. The significant higher ($p<0.01$) soil organic carbon, total nitrogen and water content of DEF soil might contribute to its higher respiration rates. The higher respiration rate of cornfield and sunflower were attributed by it neutral pH and high water contents. However, soil respiration of DEF and cornfield were not significantly different. The mean soil respiration rates of the ecosystems were strongly correlated ($p<0.01$) with soil water, pH, carbon and nitrogen contents.

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REFERENCES

Adachi, M., Bekku, Y. S., Rashidah, W., Okuda, T., and Koizumi, H. (2006). Differences in soil respiration between different tropical ecosystems. Applied Soil Ecology. 34: 258-265.

Buchmann, N. (2000). Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. Soil Biology and Biochemistry. 32: 1625-1635.

Campos, A. (2006). Response of soil surface CO_2-C flux to land use changes in a tropical cloud forest (Mexico). Forest Ecology and Management. 234: 305-312.

Chapin, F. S., McFarland, J., McGuire, A. D., Euskirchen, E. S., Ruess, R. W., and Kielland, N. (2009). The changing global carbon cycle: linking plant-soil carbon dynamics to global consequences. J. Ecology. 97: 840-850.

Duiker, S. W. and Lal, R. (2000). Carbon budget study using CO_2 flux measurements from a no till system in central Ohio. Soil and Tillage Research. 54: 21-30.

Gupta, P. K. (2007). Soil, Plant, Water and Fertilizer Analysis. Jodhpur, India: AGROBIOS.

Hanpattanakit, P., Panuthai, S., and Chidthaisong, A. (2009). Temperature and Moisture Controls of Soil Respiration in a Dry Dipterocarp Forest, Ratchaburi Province. Kasetsart Journal (Natural Science). 43: 650-661.

Harper, C. W., Blair, J. M., Fay, P. A., Knap, A. K., and Carlisle, J. D. (2005). Increased rainfall variability and reduced rainfall amount decreases soil CO_2 flux in a grassland ecosystem. Global Change Biology. 11: 322-334.

Hashimoto, S., Tanaka, N., Suzuki, M., Inoue, A., Takizawa, H., Kosaka, I., et al. (2004). Soil respiration and soil CO_2 concentration in a tropical forest, Thailand. J. Forestry Research. 9: 75-79.

IPCC, (2001). Special report on land use, land-use change and forestry: Part I, global carbon cycle overview. http://www.grida.no/publications/other/ipcc_sr/?src=/climate/ipcc/land_usem/index.htm.

IPCC, (2007). Climate change 2007: The physical science basis. Summary for policymakers. http://news.b bc.co.uk/2/shared/bsp/ hi/pdfs/02_02_0 7_climatereport.pdf.

Iqbal, J., Ronggui, H., Lijun, D., Lan, L., Shan, L., Tao, C., et al. (2008). Differences in soil CO_2 flux between different land use types in mid-subtropical China. Soil Biology and Biochemistry. 40: 2324-2333.

Keith, H., Jacobsen, K. L., and Raison, R. J. (1997). Effects of soil phosphorus availability, temperature and moisture on soil respiration in *Eucalyptus pauciflora* forest. Plant and Soil. 190: 127-141.

Kemmitta, S. J., Wrighta, D., Goulding, K. W. T., and Jonesa, D. L. (2006). pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biology and Biochemistry. 38: 898-911.

Kosugi, Y., Mitani, T., Itoh, M., Noguchi, S., Tani, M., Matsuo, N., et al. (2007). Spatial and temporal variation in soil respiration in a Southeast Asian tropical rainforest. Agricultural and Forest Meteorology. 147: 35-47.

Lee, M.-S., Nakane, K., Nakatsubo, T., Mo,W.-H., and Koizumi, H. (2002). Effects of rainfall events on soil CO_2 flux in a cool temperate deciduous broad-leaved forest. Ecological Research. 17: 401-409.

Luo, Y. and Zhou, X. (2006). **Soil** Respiration and the Environment. Burlington, USA: Elsevier.

Miao, W., Qiu-rong, L., Dong-mei, X., and Bai-li, D. (2004). Effects of soil temperature and soil water content on soil respiration in three forest types in Changbai Mountain. J. Forestry Research. 15: l 13-118.

Nay, S. M., K. G. Mattson, and B. T. Bormann. (1994). Biases of chamber methods for measuring soil CO2 efflux demonstrated with a laboratory apparatus. Ecology. 75: 2460-2463.

Panuthai, S., Junmahasatein, S., and Diloksumpun, S. (2005). Soil CO_2 emissions in the Sakaerat dry evergreen forest and the Maeklong mixed deciduous forest. In: Proceeding on Forest Climate Change "Potential of forest to support Kyoto Protocol". Maruay Garden Hotel.

Peng, S., Piao, S., Wang, T., Sun, J., and Shen, Z. (2009). Temperature sensitivity of soil respiration in different ecosystems in China. Soil Biology and Biochemistry. 41: 1,008-1,014.

Rastogi, M., Singh, S., and Pathak, H. (2002). Emission of carbon dioxide from soil. Current Science. 82: 510-517.

Reth, S., Reichstein, M., and Falge, E. (2005). The effect of soil water content, soil temperature, soil pH-value and the root mass on soil CO_2 efflux-A modified model. Plant and Soil. 268: 21-33.

Schaefer, D. A., Feng, W., and Zou, X. (2009). Plant carbon inputs and environmental factors strongly affect soil respiration in a subtropical forest of southwestern China. Soil Biology and Biochemistry. 41: 1,000-1,007.

Takahashi, M., Haria, K., Limtong, P., Leaungvutivirog, C., Suksawang, S., Panuthai, S., et al. (2009). Soil respiration in different ages of teak plantations in Thailand. JARQ. 43: 337-343.

Wiriyatangsakul, S. (2004). Soil respiration in tropical uplands responded to temperature and moisture changes. Master Thesis, School of Energy and Environment. King Mongkut's University of Technology, Thonburi, Bankok.

Floristic composition and species diversity of the chirpine forest ecosystem, Lobesa, Western Bhutan

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ABSTRACT

A study was conducted to quickly analyze diversity richness and correlation between different diversity richness at species level (trees, shrubs and herbs). The research exercise was done in the pure chirpine forest of College of Natural Resources campus. The numbers of chirpine trees, shrubs and herbs were recorded in the circular sample plot of size 0.05 ha (radius of 12.62 m). SPSS was used to analyze the data. The species richness was calculated through single factor Analysis of Variance (ANOVA). Correlation of coefficients also analyzed to see if there is association and relationship between the different data variables collected.

A total of 2,028 species were recorded from the study area, out of which 56 were chirpine trees, 462 shrubs and 1,510 herbs. The diversity richness on total count of chirpine, shrubs and herbs was significant, F (2, 39) = 36.095, p = .000. It was deduced that chirpine richness was comparatively poor than the shrubs and herbs richness. This means the species richness have significant effect on the biodiversity richness.

KEYWORDS: *Chirpine, Biodiversity richness, Species richness, Correlation, Relationship, Significant effect*

INTRODUCTION

Himalayas are one of the world's sixth largest bioregions. It harbours approximately 8,000 species of flowering plants including 25.30% endemic (Semwal, *et al*. 2010). The diverse altitudinal and climatic conditions which ranges from sub-tropical type (c. 150 m a.s.l.) at the foothills of the southern belts to alpine type (above 4000 m a.s.l.) results Bhutan as one of the biological hot spot in the world (Gillison, 2009).

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The forest diversity is the main livelihood to rural and semi-urban people of Bhutan. Out of Bhutan's physiogeographical area, 80.89% is covered by forest that includes shrubs cover too. Major chunk of forest is covered by broadleaf forest (about 34.30%) and more than 26.50% is conifer forest and out of which 2.50% is of pure chirpine forest (Pelden, 2011, & Dhital, 2000).

Most of the chirpine forest is found in Eastern Bhutan. Once it has become the major source of income for the poor rural communities of the Eastern Bhutan. The farmers used to tap and collect chirpine resin and sell to Rosin and Turpentine Factory in Samdrupjongkhar, earning a good sum of income for the household. This flourishing factory was established in 1974 by the Tashi Group of Campany, that used to collect more than 1,000 metric tonnes (MT) a year. But since 2006, the yield of resin was considerably reduced to 177 MT. Because of low productivity of resin, farmers have switched over to extracting of lemongrass oil, from which farmers used to fetch more than Nu.500 a Kilogramme (Dema, 2007).

The chirpine forest ecosystem is very much essential for the extraction of lemongrass oil. The lemongrass industry provides an off-farm source earning on periodical basis for the farmers of Eastern Dzongkhags that sustain the safety of farmers' livelihood. The industry produced 89.50 MT of lemongrass oil generating a total sale of Nu.43.50 million, from the year 1998 to 2004. Chirpine tree fuel woods are used while heating drums to extract lemongrass oil. Other trees' lops and tops are also used to supplement fuel wood. Besides that, chirpine forest ecosystem furnishes farmers of Eastern Bhutan with construction timbers, resin for sell and cattle bedding (Prommegger, & *et. al*., 2005).

The chirpine trees are also grown available in few forest areas of western-central part of Bhutan, viz, Punakha – Wangduephodrang and Trongsa valleys. The mixed chirpine and broadleaved forest ecosystem within the altitude of 900 to 1800 m a.s.l. also played a vital role in Punakha-Wangduephodrang valley in sustaining and protecting the watershed areas. Beside this, chirpine forest provides the farmer with firewoods, roofing shingles, timbers for construction, and leaf-litters for cultivation of crops and of course the grazing ground for the livestock. Thus, the aspects of sustainable management and silvicultural decision-making for the chirpine forest ecosystem is important to understand by

the forest management planners, field implementers even by the local communities (Kleine, 1996).

The forest especially of College of Natural Resources (CNR) campus, Lobesa, Punakha Dzongkhag is dominated by Pinus roxburghii Sarg (Chir Pine*)* with few other broadleaved trees. With several anthropogenic activities and tremendous biotic pressure in recent years in this region, has interrupted and impacted the pure P. roxburghii forest ecosystem which has resulted in the stock reduction of the cited tree species.

The fast pace of developmental activities occurring in the developing countries like Bhutan might impact the functioning of such vast biodiversity including chirpine forest ecosystems. This emergence made indispensable concern to the effect of diversity on ecosystem process and on ecosystem services within an environmental society (Ram, *et. al*., 2003). Thus, conservation of biodiversity in the Himalayan region has to regard as of great significance to enhance the socio-economic development by means of sustainable farming of the rural livelihood. To such context, many biological scientists have conducted numbers of natural live science studies on various aspects of different forest ecosystems. However, this entailing study describes the diversity richness, correlation between different diversity richness at species level (Chirpine, shrubs and herbs) and the natural effects in the pure chirpine forest ecosystem.

Study area

The study area is situated at an altitude of 1,300 m a.s.l in the CNR Campus, Lobesa, Punakha Dzongkhag. The dominant vegetation consists of Pinus roxburghii stand with a few planted species of Cupressus corneyana, Albizia gamblei and Sapindus

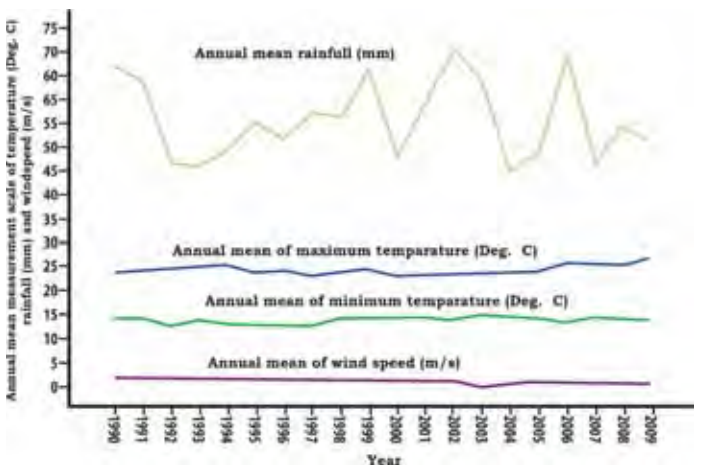

Fig. 1: Weather graph (Annual mean reading)

sp. The shrubs like Berberis, Sapium, Zanthoxylum, Phyllanthus, Desmodium and Indigofera and herbs like, Innula, Artemesia, Stephania, Thalictrum, Cymbopogon, Asparagus, Eupatorium, Biden and Sida are found.

The climate of the campus area is hot and wet summer with an average amount of rainfall and dry windy and cool winter. The annual mean temperature was 26.88°C in 2009 and 14.94°C in 2003. In 1992, annual mean temperature of 12.68°C was recorded. Increasing trend of annual mean temperature has been observed since the past two decades onwards. The maximum annual mean rainfall of 70.82 mm was recorded in 2002 and minimum of 44.77 mm in 2004. From 2004 onwards annual mean rainfall has been decreasing and it was only 50.94 mm in 2009. The wind speed observed was always constant at minimum annual speed of 0.19 m/s (metre second^{-1}) and maximum of 1.80 m/s in 1995 (Fig.1). With all such conditions, Lobesa weather can be termed as very dry windy place with scanty rainfall (Data Source: RNR RDC, Bajo).

MATERIALS AND METHODS

The study was conducted in pure chirpine forest. The 14 sample circular plots were randomly selected with a plot size of 12.62 m radius or 0.05 ha. The enumeration of chirpine trees, shrubs and herbs were done in these sample circular plots. The other plot data like chirpine tree DBH (cm) and its height (m), average slope percentage of the plot area and aspect were recorded.

In this forest all the vegetation layers viz. chirpine, shrubs and herbs were analyzed for species richness and diversity. The crown closure % was measured according to the principle given in Guidelines for Forest Management Inventory Fieldwork, Bhutan (Laumans, 1994).

Species richness was determined as the number of species per unit area calculated using MS excel. Species diversity was calculated by using Shannon-Wiener information index (Shannon and Weaver 1963) as:

$H = -\sum\{Pi * Ln (Pi)\}$ (where $Pi = \frac{ni}{N}$, ni = the number of individuals in species i or the abundance of species i. N = the total number of all individuals, Pi = the relative abundance of each species or proportion and Greater H = higher biodiversity)

Evenness = $\frac{H}{LnS}$ (Where S = no. of sample and Ln = Natural log)

SPSS was used to analyze the data. The species richness was calculated through single factor Analysis of Variance (ANOVA). Correlation of coefficients also analyzed to see if there is association and relationship between the different data variables collected.

RESULT

A total of 2,028 species were recorded from the study area, out of which 56 were chirpine trees, 462 shrubs and 1,510 herbs. The area was dominated by a single dominant tree species P. roxburghii. As per Shannon Index calculation, the overall species evenness was found to be 0.83. At the individual species level, the evenness showed relatively lower diverse. Evenness for chirpine was 0.06 indicating poor species richness; similarly, shrubs evenness was 0.33. On the contrary, the herbs diversity / evenness were relatively higher at 0.77.

Tab. 1: ANOVA for effect of diversity richness on chirpine, shrubs and herbs

Source	df	MS	*F*	*P*
Among Groups	2	.065	36.095*	.000
Within Groups	39	.002		
Total	41			

* $p<.05$

The diversity richness on total count of chirpine, shrubs and herbs was significant, F (2, 39) = 36.095, p = .000.

There was significance of difference (Calculated F = 36.095 ≥ Distribution table F = 3.32, $p<.05$) on the diversity richness of chirpine, shrubs and herbs.

The Post Hoc test like Bonferroni, Gabriel and Games-Howell also predicted that the mean biodiversity richness of chirpine, shrubs and herbs was significant at the .05 level, p = .010, .000 and .002 respectively.

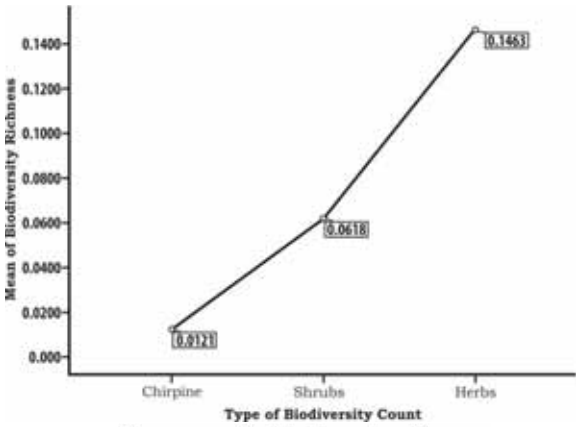

Fig. 2: Mean curve for biodiversity richness

The biodiversity richn-ess mean curve (Fig.2) has found out that when tree mean species richness is low, shrubs mean richness will be medium and herbs mean species richness will be the highest. The enumera-tion was done with a circular sample plot size of 0.05 ha. This mean curve simply predicted that the tree density is very low or poor may be because of heavy biotic factors.

Tab.: 2 Correlations[a] between different biodiversity (chirpine, shrubs and herbs)

		Biodiversity Richness	Species level diversity richness
Biodiversity Richness	Pearson Correlation	1	.715**
	Sig. (2-tailed)	.000	
Species level diversity richness	Pearson Correlation	.715**	1
	Sig. (2-tailed)	.000	

**. Correlation is significant at the 0.01 level (2-tailed)
a. Listwise N = 42.

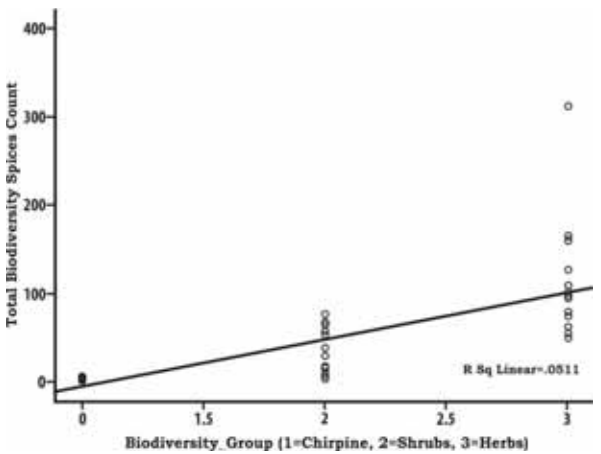

Fig. 3: Correlation trend-line for the effect on biodiversity

There was very strong association (Table 2) between the species level diversity richness within and among the sample plots. It showed significant relation-ship or signi-ficantly correlated at the .01 level between the richness of chirpine trees, shrubs and herbs in the sample plots, $r = .715$, $p = .000$.

The figure 2 indicates that correlation trend-line showing significant effect on biodiversity population by the group of individual species R^2 = .511. It had determined that chirpine richness (1 = chirpine, 2 = shrubs and 3 = herbs) was comparatively poor than the shrubs and herbs richness. Herbs richness was highest among others because the forest had wide open canopy owing to minimum numbers of chirpine population. Wide open canopy allows maximum sunlight to reach the forest floor which favours many herbs to grow.

DISCUSSION

If considered richness of herbs and shrubs in this chirpine forest then it seems the forest is rich in biodiversity (Evenness = 0.83). But if it is to regard at individual species level, the richness of chirpine forest is almost degraded (at an average only 4 chirpine trees in a plot size of .05 ha.). This indicated that CNR chirpine forest is started to degrade. The reason behind is many constructions are coming up within the campus forest area that showed the biodiversity of these forests is under great anthropogenic pressure.

Ram, *et al*. 2003 also stated that there is significant difference in species level diversity richness in chirpine forest mix with broadleaved which means the species richness will have significant effect on the biodiversity richness. That determines there will be a significant difference in plant species richness in different forest types. Rathore (1993) has also noted high species richness and diversity in the chirpine areas. Present study has also showed the richness of biodiversity in the chirpine forest. But Singh, *et, al*. (1994) has stated pure P. roxburghii and high elevation forest had the lowest biodiversity richness.

In contrary, the minimum biotic disturbances promote undergrowth species diversity, possibly by allowing several species to maintain their population in open canopy. The wide open canopy makes accessible for the penetration of skylight to forest floor that favours poor forest to enable each herbs and shrubs species to develop into large population. Poor population of chirpine tree may be because of biotic influence and that may play an important function in metamorphism losing or maintaining the plant biodiversity of the forest.

CONCLUSION

The total numbers of biodiversity species recorded in the study area are 2,028 species of which 56 are chirpine trees, 462 shrubs and 1,510 herbs. This data shows that richness of biodiversity is good but it is poor in term of individual species level. Significant relationship in species richness between individual species level is deduced. The chirpine tree richness found to be weak compare to shrubs and herbs.

It was found more number of chirpine trees in the eastern aspects even the shrubs and herbs were thick. This hold true that chirpine is light demander species. It was observed and noted, the CNR chirpine forest is at the beginning of tree depleting stage. Therefore, it is necessary to replenish the blank area of the campus by planting chirpine tree species or any other species which are suitably grown. Lastly we would like to suggest that the researcher should do thorough study on the biodiversity of the campus and if possible with its economic value. It will be good idea to do further research on chirpine in regards to its biomass, regression between its height and diameter and its growth and distribution setting **aspects** of the sample plot as one of the independent variables.

ACKNOWLEDGEMENT

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REFERENCES

Dema, K. (2007). *Resin tapping dying out.* Retrieved from http://kuenselonline.com/2010/modules.php?name=News&file=article&sid=8428.

Dhital, D. B. (2000). *Forest management planning in Bhutan*. Department of Forestry Services Thimphu, Bhutan. Proceedings of

South Asian Regional Workshop on Planning, Database and Networking for Sustainable Forest Management. Retrieved from http://www.fao.org/docrep/007/ad676e/ad676e15.htm#P1451_129564.

Gillison, A. N. (2009). *Developing a Functional Landscape-Scale Land Cover, Biodiversity, Hydrology Modeling Framework (DrukDIF) for the SLMP areas of Bhutan Phase I*: Rapid Natural Resource Assessment Along Land Cover and Land Use Gradients. Center for Biodiversity Management, Yungaburra, Queensland 4884, Australia. www.cbmglobe.org.

Kleine, M. (1996). *Silvicultural Management of Broad-leaved and Chir Pine Forests in the Punakha and Wangdue - Phodrang Districts of Bhutan: Final Report*. Short-Term Consultancy to the Bhutan-German Integrated Forest Management Project.

Laumans, P. (1994). *Guidelines for Forest Management Inventory Fieldwork*. No.18, FO: DP/BHU/91/002. Department of Forestry, Ministry of Agriculture, RGOB, Thimphu Bhutan.

Pelden, S. (2011). *Forest cover 70.4 percent*. Bhutan land cover assessment 2010. Retrieved from http://www.kuenselonline.com/2010/modules.php?name=News&file=article&sid=19686.

Prommegger, W., Budur, K., Dorji and Chhetri, P. B. (2005). *Lemon grass distillation in Eastern Bhutan – A scenario analysis*. Collaborative Research Study by SFD, RC-Jakar / CORET and RC – Wengkhag.

Ram, J., Kumar, A. & Bhatt, A. B. (2003). *Plant Biodiversity of Uttaranchal, Central Himalayan Forests, India*. G.B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora- 263 643 (Uttaranchal), India. Retrieved October 13, 2010 from http://www.fao.org/DOCREP/ARTICLE/WFC/XII/0756-B2.HTM.

Rathor, S. K. S. (1993). *Resource utilization patterns in a Central Himlayan catchment*. Ph.D. thesis. Kumaun University, Nainital, India.

Semwal, D. P., Uniyal, P. L. & Bhatt, A. B. (2010). *Structure, Composition and Dominance – Diversity Relations in Three Forest Types of a Part of Kedarnath Wildlife Sanctuary, Central Himalaya, India*. Not Sci Biol 2 (3), 128-132.

Shannon, C. E. and Weaver, W. (1963). *The mathematical theory of communication*. University Illinois Press, Urbana.

Singh, S.P., Adhikari, B.S. and Zobel, D.B., 1994. *Biomass productivity, leaf longevity and forest structure in the Central Himalaya*. Ecological Monograph, 64: 401-421.

Forest structure and diversity changes along the altitudinal gradient of Chelela-Woochu series, Paro

Kunzang Choden[1] & Masahiko Ohsawa[2]

ABSTRACT

Floristic composition and structural changes of dominant tree species were studied along the altitudinal gradients from the Chelela ridge top (3685 m.a.s.l) to Woochu at the valley bottom (2315 m.a.s.l.) A total of nine (9) vegetation plots were set up along the altitudes from 3685 m to 2315 m with an altitude interval of 200 m. The floristic composition of the study area consists of fourteen (14) families with twenty-three (23) tree species. The altitudinal series were grouped into six forest zones based on floristic similarity; 1, Rhododendron thomsonii scrub forest (3685 m); 2, Abies densa forest (3485 m); 3, Abies densa- Larix griffithii forest (3270 m); 4, Tsuga dumosa forest (3155 m); 5, Picea spinulosa forest (3120 m); 6, Pinus wallichiana (P. excelsa) forest (2315 m - 2940 m). The highest basal area recorded was 135.8 m^2/ha at an altitude of 3155 m (plot 4). The maximum height ranges from 4 m (Betula utilis) at 3685 m (plot 1) to 35.7 m (Tsuga dumosa) at 3155 m (plot 4) in 9 study plots. The maximum diameter at breast height varies from 10 cm (Rhododendron thomsonii) at 3685 m (plot 1) to 117.5 cm (Abies densa) at 3485 m (plot 2). The organic carbon and Nitrogen (N) are in the increasing trend with an increase in altitude while pH decreases. High C: N ratio were inversely related with Ca: Mg ratio thus indicating high organic matter content and high leaching at higher altitudes brought about by low temperatures, low pH and high precipitation. Two types of forest structure were observed and although no significant impact of forest usage on the forest structure was observed, it was seen that, the extraction of Pinus wallichiana (Blue pine) for construction purposes as beams could create an impact on the forest structure. Pinus wallichiana forest showed irregularities in the diameter class distribution. The multiple layered Pinus wallichiana forest is clear indications of anthropogenic interferences. Thus proper management and conservation

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plans differentiated for each altitudinal zones are necessary for overall sustainable use.

***KEY WORDS**:* Bhutan, forest zones, forest structure, soil properties anthropogenic interferences

INTRODUCTION

Forest plays major roles in the sustainable development of mountain regions (Sati, 2006). About 69% of Bhutan's population lives in rural areas and 79% of the rural populace depends on natural resources for their livelihood. Forests provide food, wood, fodder, fuel wood, leaf-litter and other non-wood forest products (Norbu, 2003). With increase in population of 3%, (Population and housing Census 2005), urbanization and rapid economic developmental activities, the pressure on forest to meet the demands of people is increasing. Bhutan is one of the highest fuel wood consumers in the world with per capita annual fuel wood consumption of 1.92 m^3 (NEC, 2004) and meets about 98% of the domestic energy needs (Hocking &Wangdi, 2000).

Therefore it is necessary to understand the functions of the forest ecosystem and dynamics of forest regenerations under different forest use system. The general aim of the study is to understand the types, structure and regeneration dynamics of forest ecosystem along the altitudinal gradient and studying the impact of forest use on forest structure. The study findings can provide management options for forest resource managers for sustainable use of the forest resources.

Research Goals and Objectives:

The general goal of the research is to study the structure, regeneration dynamics of forest ecosystems and contribute towards sustainable management of the forest resources.

The specific objectives are as follows:

1. To study forest zonation in terms of species composition, species population structure and species performance along the altitudinal gradient of Chelela-Woochu series.
2. To understand human use of natural resources
3. To study the impacts of forest use on the structure and regeneration pattern of the main tree species.

MATERIALS AND METHODS

Study site

The study area is located in Luni geog under Paro district. The area extends from Pa chhu in the east to Chelela in the west and Paro-Haa highway passes through the study area. Geographically the area falls between 27° 22' 6" and 27° 24'1. 3" N and between 91° 20' 40.1" and 91° 25" 51.1" E. The vegetation plots stretched from an altitude of about 2315 m near the valley bottom (Woochu village) to c. 3685 m on the ridge top (Chelela). The socio-economic survey includes two villages (chiwogs) namely Woochu and Jewphu of the lower settlement area.

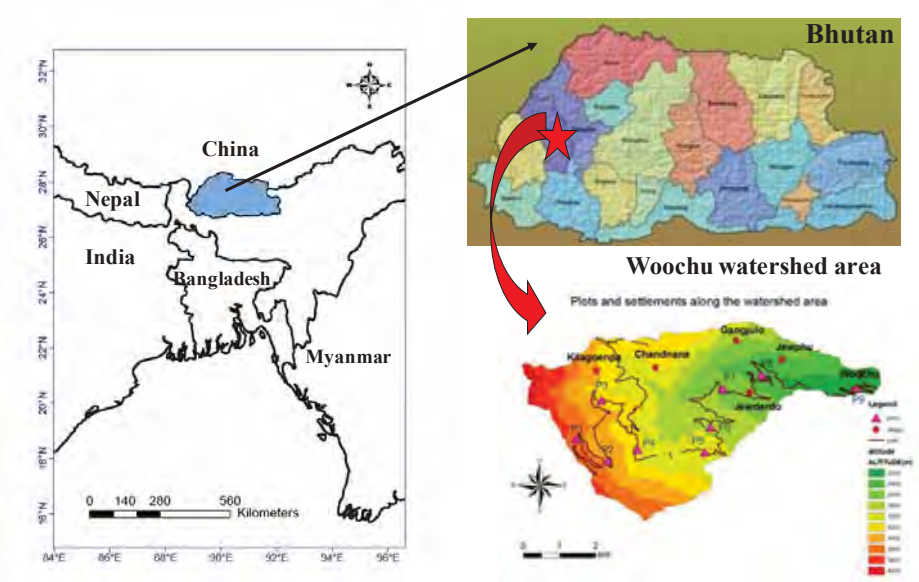


Fig.1 Location of Bhutan Himalaya and the study area (Woochu sub catchment, Paro, Bhutan)

Field survey and tree inventory

The fieldwork was conducted during September- October 2008. A total of 9 vegetation plots measuring 20 x 20 m were set up along the altitudinal gradient from 3685 m at Chelela top comprising of alpine vegetation to 2315 m at the valley bottom each with an interval of c. 200 m. All the species above 3 cm DBH (diameter at breast height) were measured for diameter and height. The DBH was measured to determine the basal area and also for DBH class distribution. On the ground, the herbs were measured for its height (cm) of the tallest of each species and their coverage (%).The seedlings in each plot were counted and their height was recorded. The soil moisture and soil hardness were also measured.

Meteorological data collection

Environmental factors such as air temperature, relative humidity, soil moisture content and soil hardness were all measured. Soil hardness was measured by using Yamanaka's soil hardness meter (push cone). Soil moisture content was measured by hydro- sense (CD 620 +CS 620) instrument having 12 cm and 20 cm probes. The measurements were conducted during the field visit in September-October 2008. At least 5 readings for soil moisture and soil hardness were taken within the plot and an average of those values was taken as the final value of factor. Several meteorological data loggers (HOBO, ONSET) measuring air temperature, relative humidity and rainfall set at hourly recording were installed by Wang Watershed Management Project (WWMP) and later handed over to RDC-Yusipang along the altitudinal series of Woochu sub-catchment area. The climatic data were used from these data loggers.

Data analysis

Tree inventory data analysis

In every tree inventory measurements of plots, species basal area (BA) was calculated using the DBH of the trees whose diameter were greater than 3 cm and subsequently the basal areas in percent (RBA) was calculated. DBH classes and height classes were analyzed using the DBH and height datas of each species. Species dominance was analyzed using the dominance analysis (Ohsawa 1984). The RBA of each species was used as abundance of species. In a community dominated by single species, its relative dominance may be stated as 100%. If two species share the dominance the relative dominance may be stated as 50% and if

there are three dominants then the relative dominance is stated as 33.3% and so on.

Species diversity index (H) was calculated using the Shannon & Wienner equation. The vegetation data were analyzed using Microsoft excel (Office 2003) and PC-ORD version 5 program. Cluster analysis was performed using the distance measure of Sorensen (Bray-Curtis) to determine the forest types.

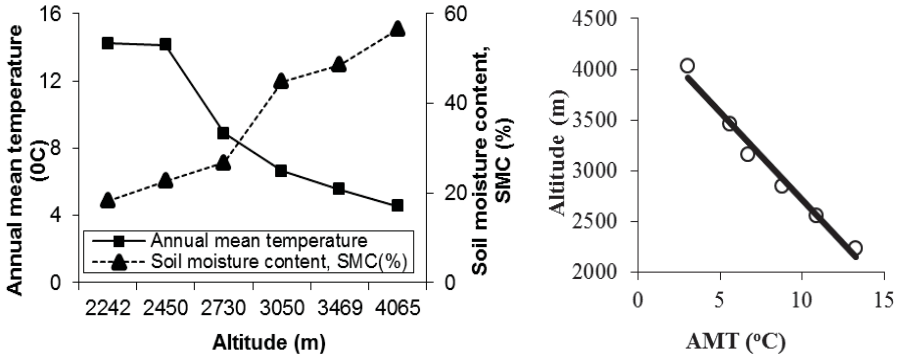
Meteorological data analysis

All the data loggers were downloaded and the climate data were processed to means and totals of daily, monthly and yearly to determine various thermal indices. Lapse rate was calculated for each month for different altitudes. The warmth index was calculated using Kira's method; WI=$\sum$ (t- 5) (for months in which t > 5^{o} C) where t = monthly mean temperatures above 5^{o}C (5^{o}C as threshold temperature for plant growth).

RESULT

Altitudinal climate and vegetation change

The major environmental factors governing the forest distribution along the altitudinal series were temperature and moisture conditions. The annual mean temperature at Woochu 2242 m and Chelela 4040 m were 13.2 ^{o}C and 3^{o}C. The warmest and coldest months mean temperatures were 19.8^{o}C and 5.3^{o}C at 2242 m and 9.4^{o}C and –5.1^{o}C at 4040 m. In the mid altitudes at 2565 m, 2848 m, 3165 m, 3466 m the annual mean temperatures were 10.9, 8.7, 6.7, 5.6 ^{o}C (2005-2008). In addition to the climatological data from data loggers, soil moisture contents were measured using hydro sense instruments during the field survey (September-October, 2008). The soil moisture content increases along the altitude from 18.2 % at 2315 m to 56.5% at 3685 m measured using hydro sense having 12 cm and 20 cm probes (average value of two probes).



(A) (B)

Fig. 2. A. Annual mean temperature and soil moisture content along the altitudinal series
B. Lapse rate of temperature along the altitudinal gradient

The forest vegetation change at different altitudes was found to be a function of temperature and soil moisture content. The annual mean temperature decreases with increasing altitude it was seen that both temperature and soil moisture content are highly significant environmental factors. Temperature is negatively correlated (r=0.98) and soil moisture content is positively correlated (r= 0.85) with altitude (Fig. 2 A)

The annual mean temperature at the valley bottom (2242 m) was 13.2^{o}C and the temperature declines at the lapse rate of 0.57^{0}C / 100 m upslope to 3^{o}C at the Chelela ridge top (4040 m.a.s.l) (Fig.2 B)

Tab. 1: Climatic conditions along the altitudinal gradient of the study area

Stations	Altitude(m)	AMT(^{0}C)	CMT(^{0}C)	WMT(^{0}C)	WI(^{0}C)	PPT(mm)
Woochu	2242	13.2	5.3	19.8	147.0	693.18
Jewphu	2565	10.9	2.8	17.8	75.6	2413.3
Zemnashi	2848	8.7	0.5	15.8	55.7	
NRDCL	3165	6.7	-1	13.3	38.0	
Kilagonpa	3466	5.6	-2.1	13.2	30.6	
Chelela	4040	3	-5.1	9.4	12.4	6368.9

Note: AMT= Annual mean temperature, CMT=Coldest month's mean temperature, WMT=Warmest month's mean temperature, WI=Warmth Index

Thermal indices like warmth index was calculated which indicates available heat energy during the growing season. The warmth index was highest in the *Pinus excelsa* (Blue pine) forest zone at an altitude of 2242 m and then it goes on decreasing with increase in altitude and is the lowest at an altitude of 4040 m in *Rhododendron thomsonii* forest (Table1).

The soil hardness and soil moisture content was studied along the altitudinal gradient (Fig.3). The soil moisture content was increasing with increasing altitude. The standard deviation was also increasing with increasing altitude. Soil moisture content was positively correlated with altitude. The soil hardness showed a decreasing trend with increasing altitude. The standard deviation was decreasing with increasing altitude. The soil hardness was negatively correlated with altitude. Higher soil hardness at lower altitudes could be attributed to the fact that the lower altitudes forests are more accessible. Therefore this accessibility increases the human interference to the natural ecosystem. Continuous trampling by both human and livestock could result in increased soil hardness.

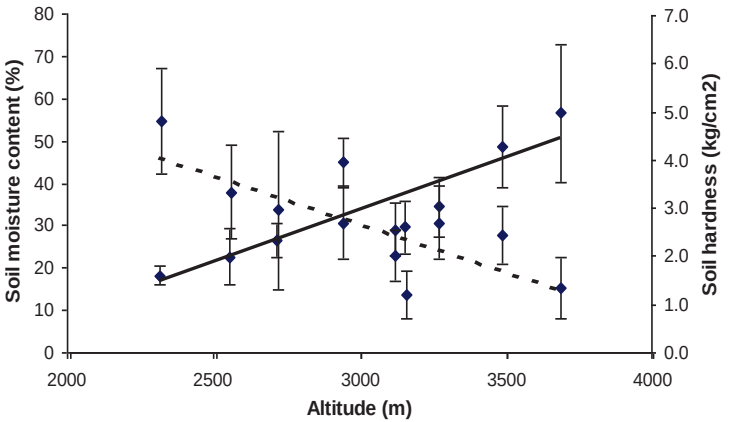


Fig.3. Soil moisture and hardness along the altitudinal gradient of Woochu watershed area

Floristic composition and forest zones along the altitudinal gradient of the study area

All the forest tree species were classified into three major life forms of coniferous, evergreen broad-leaved and deciduous broad-leaved trees (Fig. 4). Along the whole of the altitudinal series a total of 23 species representing 14 families were recorded. These species were composed of

5 conifer trees, 9 evergreen broadleaved trees and 9 deciduous broad-leaved trees (Fig.4). The conifer trees composed of Pinaceae (*Abies densa, Picea spinulosa, Larix griffithii, Pinus wallichiana, Tsuga dumosa).*

The evergreen broad-leaved trees consisted of Ericaceae (*Rhododendron thomsonii, R.arboreum, R.falconeri, R.barbatum, Pieris formosa*), Fagaceae (*Quercus semecarpifolia*), Celastraceae *(Euonymus* sp), Aquifoliaceae (*Ilex crenata*), Oleaceae (*Osmanthus suavis*).

The deciduous broad-leaved trees consisted of Betulaceae (*Betula utilis*), Aceraceae (*Acer caudatum*), Rosaceae (*Sorbus macrophylla*), Hydrangeaceae (*Hydrangea heteromalla),* Caprifoliacea (*Viburnum nervosum*), Symplocaceae (*Symplocos paniculata*), Coriariaceae (*Coriaria nepalensis*), Elaeagnaceae (*Elaeagnus parviflora*) and Fagaceae (*Quercus griffithii*).

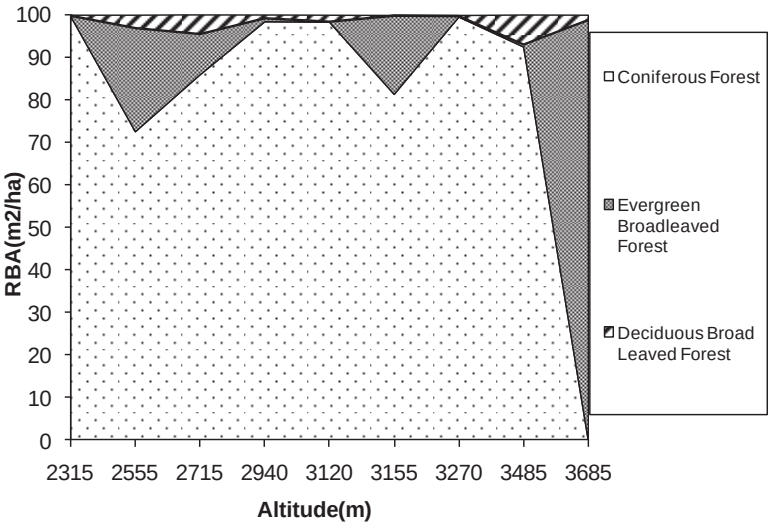


Fig. 4. Altitudinal distribution of three major life- forms of the study area

From the floristic composition we can see that the most dominant life form along the altitude is conifer trees (Fig.4). From the total relative basal area of all the life forms along the altitudinal series it was found

that conifer trees has the highest percentage share of 88.8% followed by evergreen broad-leaved trees of 9.7% and deciduous broad-leaved trees of 1.5%. (Fig.4)

Tab. 2: Plot details

Plot Details and list of dominant tree species classified according to life form

Plots	P1	P2	P3	P4	P5	P6	P7	P8	P9
Altitude(m)	3685	3485	3270	3155	3120	2940	2715	2555	2315
SMC(%)	56.5	48.6	34.4	29.6	29.1	44.8	26.7	22.6	18.2
BA(m^2/ha)	17.9	85.2	55.8	114.8	65.1	41.5	50.3	31.3	13.5
Max DBH(cm)	10.0	117.5	67.0	115.0	70.0	110.0	54.0	43.5	26.5
Max Height(m)	4.0	28.2	35.4	35.7	24.8	27.4	34.1	22.2	13.5
Stem Density	290	44	45	85	36	29	79	40	23
Species richness	3	6	5	5	4	5	7	9	1
Shannon's H(bit)	0.4	0.3	1.0	0.6	0.6	0.7	0.5	0.9	0.0
CONIFEROUS TREES	**RBA**	**RBA**	**RBA**	**RBA**	**RBA**	**RBA**	**RBA**	**RBA**	**RBA**
Abies densa		**96.5**	**58.2**	**2.3**					
Picea spinulosa			**19.6**		**77.3**	**45.1**		**0.8**	
Larix griffithii			**21.8**						
Pinus excelsa			**0.3**		**21.2**	**52.2**	**88.8**	**95.4**	**100**
Tsuga dumosa				**77.5**					
EVERGREEN BROAD-LEAVED TREES									
Rhododendron thomsoi	**99.0**	**0.5**							
Rhododendron falconeri		**0.0**							
Rhododendron barbatum			**0.1**	**0.0**					
Pieris formosa				**20.2**	**0**		**4.9**	**0.4**	
Rhododendron arboreum				**0.1**		**0.1**			
Quercus semecarpifolia						**1.3**		**2.6**	
Euonymus sp							**0.5**	**0.3**	
Ilex crenata							**0.1**		
Osmanthus suavis							**0.5**		
DECIDUOUS BROAD-LEAVED TREES									
Betula utilis	**0.7**	**0.2**							
Acer caudatum		**2.6**							
Sorbus macrophylla	**0.3**	**0.2**							
Hydrangea heteromalla					**1.5**				
Viburnum nervosum						**1.3**			
Symplocus paniculata							**5.2**		
Elaeagnus parviflora								**0.5**	
Quercus griffithii								**0.2**	
Total RBA	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Forest zonations along the altitudinal gradient

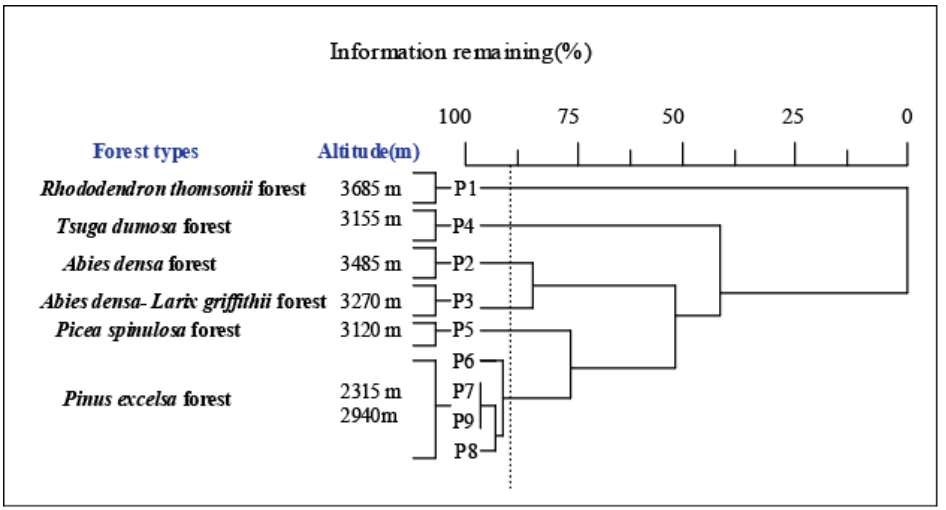


Fig. 5 (A) Dendrogram showing different forest types

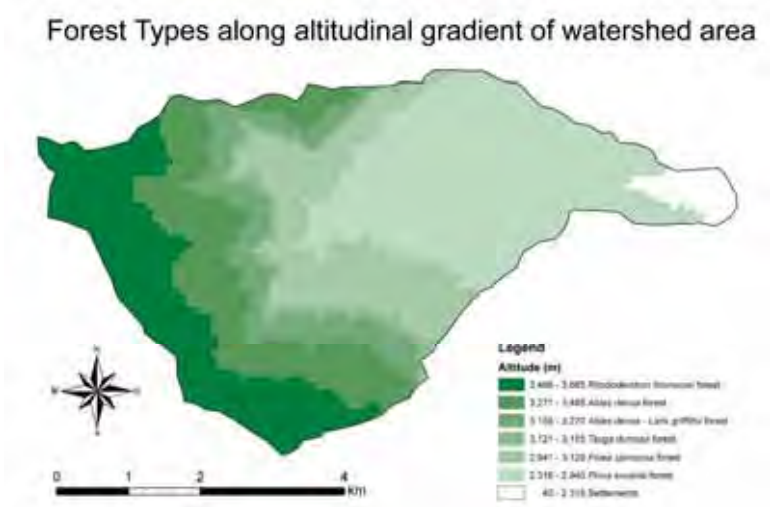


Fig. 5 (B) Map showing forest zonation along the altitudinal gradient

The dominance (relative basal area) of all the tree species in each plot was subjected to cluster analysis for classifying forest zones by species similarity index using dendrogram. Six forest zones were classified by the cluster analysis with an arbitrary similarity threshold at 90% (Fig.5 (A).

The above map shows the forest zonation along the altitudinal gradient of Woochu watershed area, which has been classified using cluster analysis based on species dominance Fig.5 (B).

Forest structural features along the altitudinal gradient of the study area

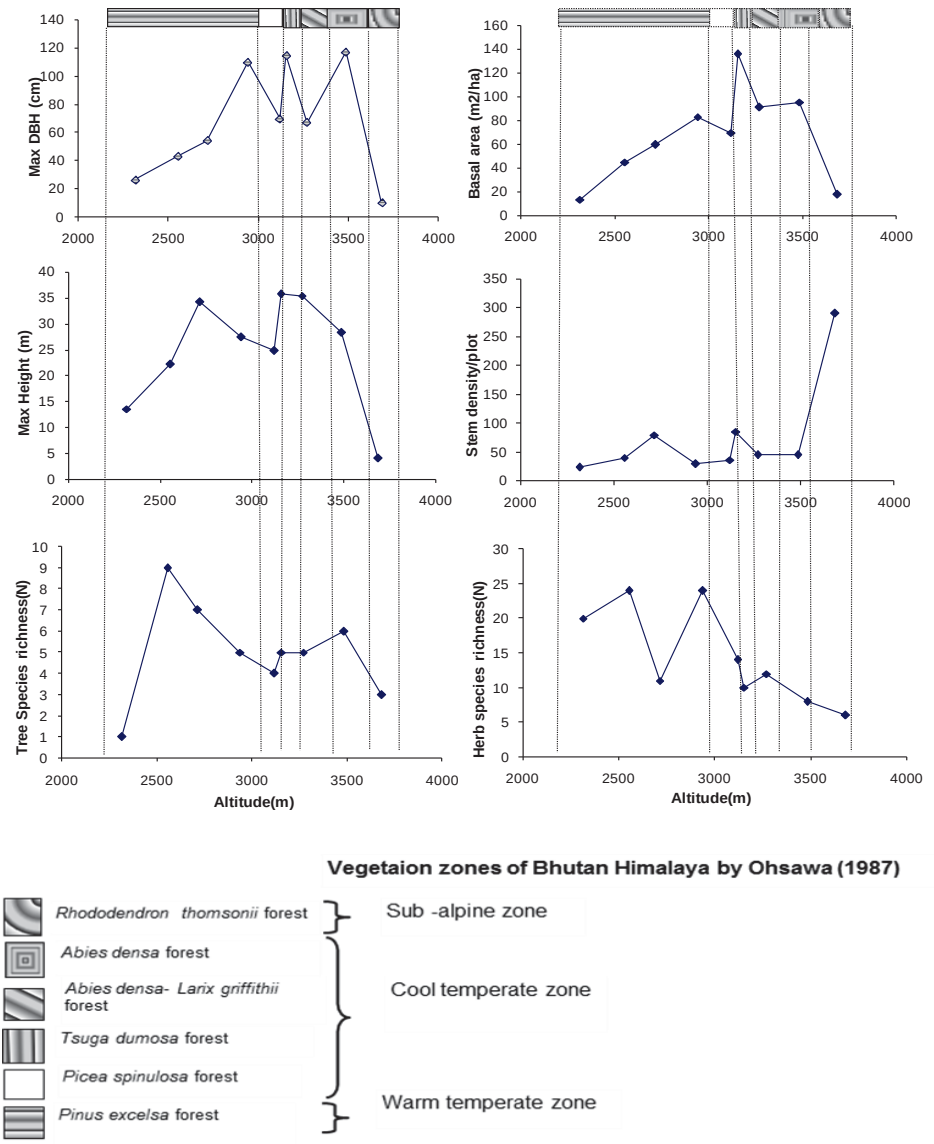


Fig. 6. Forest structural features along the altitudinal gradient (2315 – 3685 m). Pattern scale indicates six forest zones classified through cluster analysis

Structural organization of forest communities along the altitudinal gradients was described based on six (6) features (maximum diameter at breast height, maximum height, species richness, basal area, species diversity and stem density). The forest structural features are illustrated in the figures (Fig.6). The maximum tree height was 35.7 m (*Tsuga dumosa*) at an altitude (3155 m) and the lowest was 4 m of (*Betula utilis)* at an altitude of 3685 m in the fir forest. The maximum tree height overall exhibited a decreasing pattern, first a steep increase (from 13.5m to 34.1m) up to 2715m altitude, then a gradual decrease (from 34.1m to 24.8m) up to 3120m altitude. Then again an increase and then nearly flat and a sharp decline (35.4m to 4m) at an altitude of 3685m.The maximum diameter at breast height (DBH) fluctuated greatly from 10m to 117.5 m. The maximum DBH recorded was 117.5m at an altitude of 3485m. The basal area (BA) was the lowest at the highest and lowest altitude. The maximum BA was 135.8m^2/ha at the altitude of 3155m, where the climatic conditions are favorable and growth of trees is luxuriant (Fig.6).

At low altitudes, the BA value was relatively low but fluctuated greatly. The species diversity was seen to be minimum at the highest altitude and lowest altitude (Fig.6). Similarly species richness was also seen to be lower at the highest and lowest altitude. The stem density showed a little different pattern as compared to other features. The stem density was highest at the highest altitude because the highest altitude plot was a *Rhododendron thomsonii* forest and had lots of sprouts, which increased the stem density at this altitude (Fig.6).

Regeneration and ground vegetation types in the sampling plots

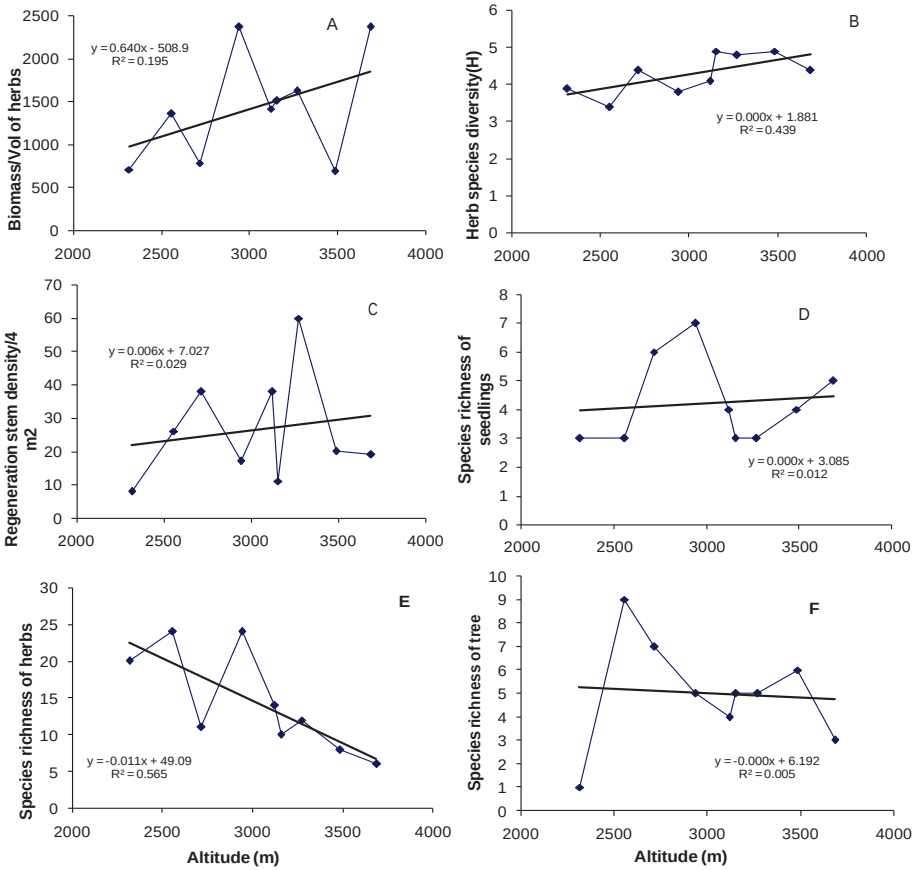


Fig. 7. Forest tree regenerations and ground vegetation features

The floristic compositions of the regenerating tree species and ground vegetation under different plots were studied. The volume of herbs or (ground vegetation) biomass was decreasing with decreasing altitude (Fig.7 A). The herb species diversity (H) was more or less the same along the altitude.

Seedling densities per $4m^2$ plots and species richness of seedlings were increasing with increasing altitude. Species richness of herbs and species richness of trees on the other hand were seen to be decreasing with increasing altitude (Fig.7 E, F). Species richness of trees and herbs were comparatively higher at lower altitudes than higher altitudes. But in the case of trees although the species richness increased it was more of secondary species and less of canopy species.

Chemical soil properties

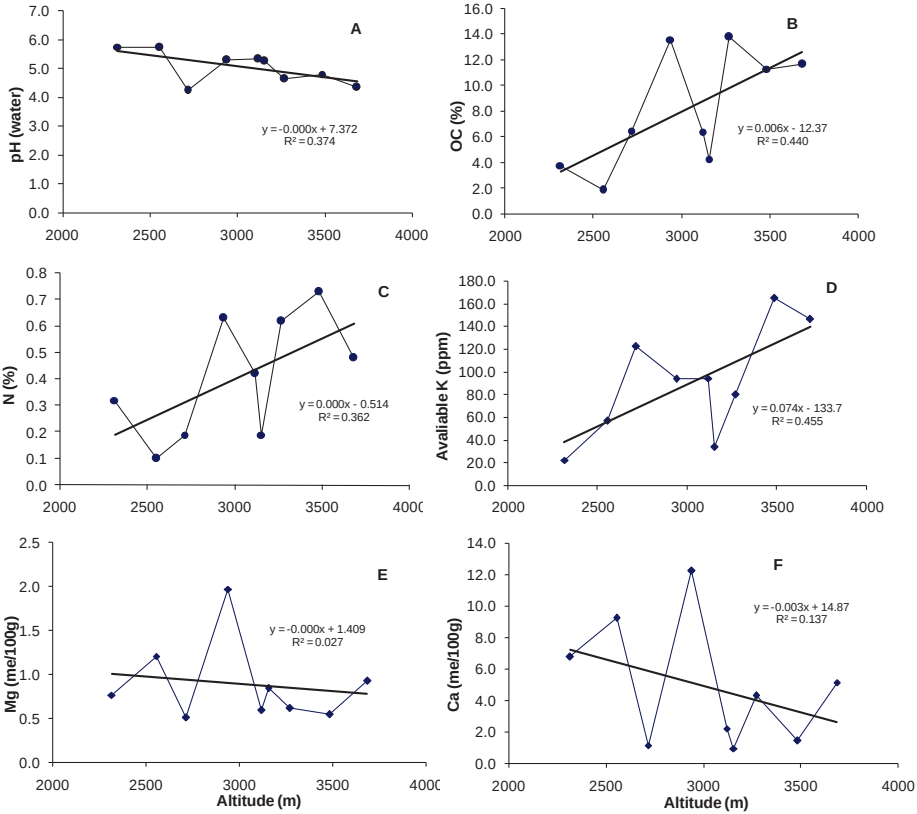


Fig. 8 Soil properties along the altitudinal gradient

Soil plays an important role in species composition of different forest types and in growth and development of vegetation. The soil pH decreases with increase in altitude. The lower pine forests had higher pH as compared to higher conifer forests (Fig.8 A). The organic carbon content (OC) and total nitrogen (N) on the other hand showed an increasing trend with increasing altitude with the highest of 13.8% at an altitude of 3270 m (Fig.8 B & C). The organic carbon content (OC) ranged from 1.9% to 13.8%. The total nitrogen value ranged from 0.1% at an altitude of 2555 m to 0.7% at an altitude of 3485 m (Fig.8 B & C). The exchangeable Ca and Mg showed a decreasing trend with an increasing altitude but the exchangeable K showed an increasing trend with increasing altitude (Fig. 8 E & F).

DISCUSSION

Altitudinal climate change

The annual mean temperature at the valley bottom (2242 m) was 13.2^{0}C and the temperature declines at the lapse rate of 0.57^{0}C /100 m upslope to 3^{o}C at the Chelela ridge top (3685 m.a.s.l). The observed lapse rate is almost equivalent to findings of Eguchi (1987) with 0.54^{o}C lapse rate in much wider areas around Bhutan Himalaya and relatively lower compared to Dochula-Bajo series (0.62) of Wangda & Ohsawa (2006)

Altitudinal forest zones and species performance

Altitudinal zonation of vegetation is one of the most striking gradational patterns of vegetation and much information has been accumulated on its local patterns (Ohsawa, 1984). Several studies have analyzed the altitudinal and latitudinal forest zones in relation to climate of the Himalayas and the surrounding mountains (Saxena and Singh, 1984; Sargent, 1985; Ohsawa, 1987, 1992; Singh and Singh, 1992; Wangda and Ohsawa, 2006). The distribution of tree population at altitudinal range is a result of interactions between physical and biological factors over time. Additionally other physical factors like slope, latitude, aspect, rainfall, humidity had played role in determining forest composition (Grytnes and Vetaas, 2002). In this study the forest zones were classified based on their dominance along the altitudinal series. Six forest zones were classified based on their dominance namely 1.*Rhododendron thomsonii* forest 2. *Abies densa* forest 3. *Abies densa- Larix griffithii* forest 4. *Tsuga dumosa* forest 5. *Picea spinulosa forest* 6. *Pinus excelsa* forest. *Rhododendron thomsonii* forest at an altitude of 3685 m coincides with the sub-alpine zone by Ohsawa (1987) from 3500-4000 m. *Abies densa, Abies densa-Larix griffithii*, *Tsuga dumosa* and *Picea spinulosa* forest from (3120m-3485m) to the cool-temperate zone by Ohsawa (1987). Lastly the *Pinus excelsa* forest from (2315m-2940m) corresponds to warm-temperate zone by Ohsawa (1987) .The forests dominated by *Pinus wallichaina (Syn. Pinus excelsa)* developed mainly in dry valleys and their distribution ranged from 2400-3500 m. Therefore this species is also extra zonal in its distribution (Ohsawa, 1987).

Structural and Environmental features along the altitudinal gradient

Structural features like the maximum diameter at breast height (DBH), maximum height, species richness, species diversity, basal area were studied along the altitudinal gradient (Fig. 6). All these features had

similar trend i.e. lowest at the highest and lowest altitude and highest at the mid altitudes. The maximum DBH, maximum height, species richness, species diversity and basal area were lowest at the highest altitude because of the unfavorable environmental conditions like low temperature (Table 1) .At lower altitudes; the basal area value was relatively low but fluctuated greatly due to heterogeneity of community structure, tree size, individual dispersion etc. The species diversity was seen to be minimum at the highest altitude and lowest altitude. This can be attributed to the fact that there is climatic stress at the higher altitudes. At the lower altitude, the less diversity could be because of human intervention, trampling effects and drought stress. The diversity was highest at the mid altitude where the temperature was favorable and the plot was far away from human interference.

At the lowest altitude these structural features were low because of human intervention. Owing to the proximity and easy accessibility to the farmers in the villages near these forests, the forests were under constant disturbances. On the other hand, species richness of herbs increased with decreasing altitude and was highest in plot number 8 (2555 m) where there was maximum human intervention (Fig.7). It is speculated that in disturbed forest there is more canopy opening and hence more light to enhance growth of herbs in the disturbed forests near the village settlements. Similar kind of findings was reported in Annapurna Conservation Area, Nepal where species richness of herbs increased with more human intervention. The combination of high light levels, naturally nutrient poor soils and disturbances and stress caused by current level of livestock grazing probably creates almost optimal conditions for high herbal species diversity in forests near the villages (Christensen, 2009). Great diversity of the open canopy in the lower forest may be the result of invasion by new species in the resultant canopy gaps (Hobbs and Hueneke, 1998).

Apart from structural features other variables like the chemical soil properties were also recorded. Soil chemistry is well known to be very important for the species composition in various forest types (Clark et al. 1999). Soil properties vary with altitude and topographic positions (Whittaker et al.1968). The organic carbon, nitrogen and Potassium increased with altitude and lower *Pinus excelsa* forest had lower nutrients as compared to other forest types. The low organic carbon and nitrogen content in the lower altitudes could be because the temperature is high and rainfall is less at the lower altitudes which results in higher

decomposition rate and more human intervention in the form of leaf litter collection in Blue Pine forest in the lower altitudes. According to Tang. (2005) organic carbon, nitrogen, exchangeable potassium increased with increasing altitude and attributed lower nutrient contents at lower altitudes to human disturbances.

Forest ecosystem dynamics

Rhododendron thomsonii forest at an altitude of 3685 m, which falls within the sub-alpine zone, had low temperature and high precipitation (Table.1). This results in slower decomposition rate and hence thick layer of litter remains un-decomposed. Therefore the nutrient contents in soil in this zone are higher (Fig.9). Human activities of surface litter collection are minimum because of its inaccessibility or far distance away from the settlements. The cool- temperate zone, which comprises of mixed conifer forest (*Abies densa* forest, *Abies- Larix griffithii* forest, *Tsuga dumosa* forest and *Picea spinulosa* forest) the temperature and rainfall is both moderate (Table.1). The human impact level is moderate therefore the nutrient content is moderate as compared to other forest types. At the lower most warm-temperate forest of *Pinus wallichiana* the temperature is high and precipitation is low (Table.1) which results in higher decomposition rate of litter. Moreover there is more human intervention at these altitudes because of its easy accessibility. Large number of stumps of *Pinus wallichiana*, *Quercus semecarpifolia* and *Pieris formosa* were recorded in this *Pinus wallichiana* forest zone. Farmers also collected pine needles from this forest zone to use as cattle bedding and to use it as organic farmyard manure after decomposition. Therefore there was reduced surface litter depth in this zone (Fig.9). Forest composition, structural features, soil conditions and nutrients changes along the altitudinal gradient of Woochu watershed area. This change is being brought about by both physical factors (temperature and moisture) and biotic factors (human intervention in the form of timber, fuel wood and litter collection). This interaction between the physical and biotic factors constitutes the forest ecosystem.

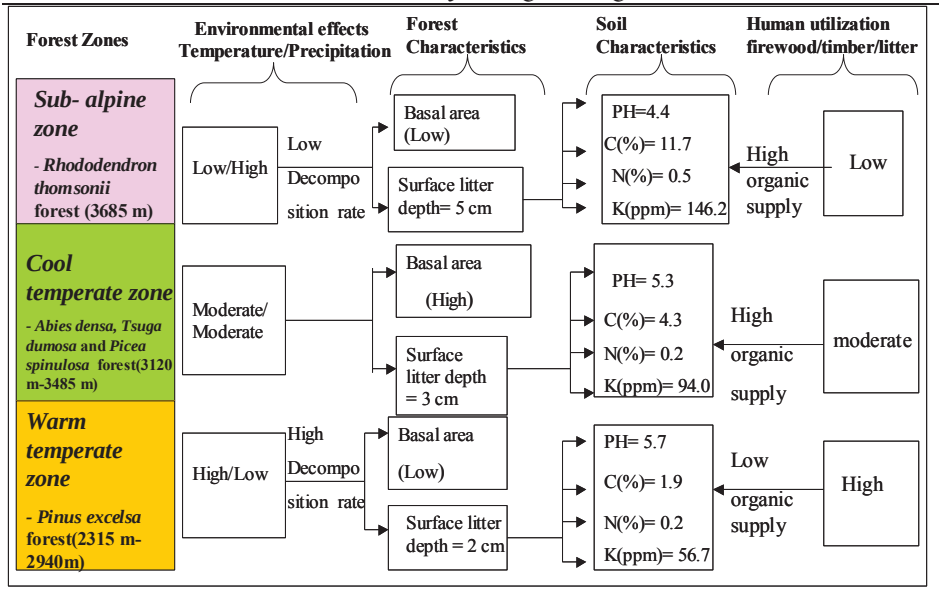


Fig.9. Forest ecosystem dynamics along the altitudinal gradient in different forest zone

CONCLUSION

Pinus wallichiana zone (Blue Pine zone) is heavily utilized by the human for timber, firewood and litter collections and making the pine zone fragile and vulnerable to degradation (Choden, 2010). The basal area (biomass) was also low in this forest type and the soil nutrients were also relatively low. Multi-modal size class distribution of *Pinus wallichiana* forest indicates sporadic regeneration pattern. Regenerating this forest would be difficult once degraded because of less soil nutrient stock and It is speculated that intense utilization of these zones, which have low biomass and soil nutrients, could result in further degradation of the forest if proper future management options and plans are not implemented.

Thus, proper management and conservation plans differentiated for each altitudinal zones are necessary for overall sustainable use.

REFERENCES

Choden, K. (2010). Forest Structural Change and Human Utilization of Natural Resources along the Altitudinal Gradient of Woochu Watershed Area (Master's Thesis). University of Tokyo, Japan.

Christensen, M., Clausen, J.H. (2009). Forest biodiversity gradients and human impact in Annapurna

Clark, D.B., Palmar, M.W., Clark, D.A. (1999). Edaphic factors and the landscape- scale distribution of tropical rain trees. Ecology 80: 2662-2675

Eguchi, T. (1987). Synoptic analysis of temperature, precipitation and moisture conditions in the Bhutan Himalaya. In: Ohsawa, M. (ed), Life Zone Ecology of Bhutan Himalaya II, Published by Chiba University, Japan, pp. 209-247

Grytnes, J.A & Vetaas, O.R. (2002). Species richness and altitude: A composition between null models and interpolated plant species richness along the Himalayan altitudinal gradient, Nepal. Am Nat 159: 294-304

Hobbs, R.J. & Huenneke, L.F.(1998). Disturbance, diversity and invasion: Implications for conservation. Conservation Biology 6(3): 324-337

Hocking, D & Wangdi, J. (2000). Review of literature in Bhutan on Agro forestry. Ministry of Agriculture, Royal Government of Bhutan.

Ministry of Home and Cultural Affairs, (2005). Results of Population and Housing Census. Office of Census Commissioner, Ministry of Home and Cultural Affairs, Thimphu, Bhutan.

National Environment Commission, (2000). *State of the Environment 2001.* National Environment Commission Secretariat, Thimphu, Bhutan.
Norbu, L. 2003. Nature conservation and biodiversity for poverty reduction-case of Bhutan. FAO Corporate Document Repository

Ohsawa, M. (1984). Differentiation of vegetation zones and species strategies in the sub alpine region of Mt. Fuji. Vegetatio 57, 15-52.

Ohsawa, M. (ed.) (1987). Life Zone Ecology of Bhutan Himalaya. Laboratory of Ecology, Chiba University, Tokyo, Japan.

Sargent, C., Sargent, O., and R. Parsell. (1985). The forests of Bhutan: a vital resource for the Himalaya. Journal of Tropical Ecology. 1:265-286
Sati, V.P. 2006. Forest resource management in mountain regions: A case for the Pindar basin in Uttranchal Himalaya. Lyonia, Volume 11(1), Pages (75-84)

Saxena, A.K., Singh, J.S., (1984).Tree population structure of certain Himalayan forest associations and implications concerning their future composition. Vegetatio 58: 61-69

Singh, J.S., Singh, S.P., (1992). Forest of the Himalaya. Structure, Functioning and impact of man. Fine arts Press, Delhi.

Tang, C.Q. (2005). Forest vegetation as related to climate and soil conditions at varying altitudes on a humid subtropical mountain, Mount EMI, Sichuan, China. Ecol Res 21: 174-180

Wangda, P & Ohsawa, M. (2006). Structure and regeneration dynamics of dominant tree species along the altitudinal gradient in dry valley slopes of Bhutan Himalaya. Forest Ecology and Management 230: 130-150.

Whittaker, R.H., Boul, S.W., Niering, W.A., Havens, Y.H. (1968). A soil and vegetation pattern in the Santa Catalina Mountains, Arizona. Soil Science 105: 440-450

Thamnocalamus spathiflorus, a temperate bamboo flowering and regeneration along Yotongla and Pelela Pass

P. Wangda[1], K. Tenzin, D. Gyaltshen, K. Rabgay, D. K. Ghemiray & T. Norbu

ABSTRACT

Mass bamboo flowering was observed since 2008 in Bhutan. The three gregarious flowering of bamboos in Bhutan were identified as Borinda grossa*,* Thamnocalamus spathiflorus *and* Yushania microphylla *a temperate bamboo species along Yotongla, Pelela and Dochula. The first incidence of gregarious flowering of* Borinda grossa *was recorded in 2005 at Sakteng followed by* Thamnocalamus spathiflorus *flowering in 2008 along Pelela and Yotongla and finally* Yushania microphylla *in 2011 at Lawala. Specifically, the present study focused on the flowering and regeneration of T.* spathiflorus *and was observed and studied intensively since 2008. The study found profuse regeneration after flowering and it takes c. 2 years before regeneration takes place. The highest regeneration was recorded along the stream with a regeneration density of 122 seedlings in 1 m^2 plot and the lowest regeneration of 28 seedlings on the ridge. On average, the regeneration density was found 65 seedlings per 1 m^2. This clearly showed profuse regeneration takes place after flowering. Micro topography also plays an important role in regeneration of the bamboo. It is found important to monitor and observed the bamboo flowering phenomena as all three bamboos were important resource for making several bamboo items and also as forage for the cattle. The population of rodents was observed increasing. However, the increased in rodent population will not affect the agriculture fields since fields are located relatively far from the high altitude forest.*

KEY WORDS: Flowering, profuse regeneration, temperate forest, across Bhutan, Pelela, Yotongla

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INTRODUCTION

Bamboos are known for their long flowering cycles, usually occurring once in their life time. They flower gregariously over the whole area producing huge amount of seeds followed by mass dying of the whole clumps. Some bamboos are noticed to flower sporadically over large areas, but their dying is not as severe as those of gregarious flowering.

Bamboo flowering presents an opportunity and challenges to foresters, conservationist and farmers. Large quantities of seeds produced after flowering were either washed down by the rain water or eaten by rodents (Ahlawat, 2002). The viability of seeds of many bamboos remains for 3-4 weeks. Therefore, it is an opportunity as well as a challenge to the foresters and conservationists to collect seeds at the right time for multiplication and conservation of the flowered species. Bamboo seedlings raised through seeds give an opportunity to obtain variations among the populations. The bamboos that are used for cultivation from vegetative propagation are raised from a single propagule which is one clone of that species. If that particular species is prone to disease, then the whole clone will also become susceptible to that disease.

Bamboo flowering is also a challenge to the farmers because it is reported in many countries that bamboo flowering is usually accompanied with food-scarcity. Bamboo flowering is noticed to make rats highly fertile, their population increases at an alarming rate which results in rats destroying the standing crops.

In Bhutan, bamboo flowering was observed in many locations across the country particularly along the highway passes of Pelela and Yotongla in the northern parts (Personnel observation 2008). Specifically, the bamboo flowering was observed and reported to the Department of Forests vide No.RBRPD/23/2008/30 dated August 18, 2008 from the Royal Botanical and Recreational Parks Division.

Hence, the present study was initiated with the general objective to document bamboo flowering and to report the regeneration status to the relevant stakeholders. Specifically, the study aims at the following three main objectives:

1. To identify the flowering bamboo along Dochula (Thimphu), Pelela (Wangude) and Yotongla (Bumthang)

2. Documenting the regeneration status of flowering bamboo by establishing monitoring plots and
3. Finally documenting the life cycle of flowering bamboo

MATERIALS AND METHODS

Study site

The bamboo flowering was observed throughout Bhutan mainly northern part (inner mountains) covering east to west Bhutan Himalaya. The present study was conducted along Yotongla pass at 3290 m a.s.l. under Bumthang-Trongsa Dzongkhag. The visual observations were also conducted along Pelela (3400 m a.s.l.) and Dochula (3100 m a.s.l.). The field survey was conducted during late September 2010.

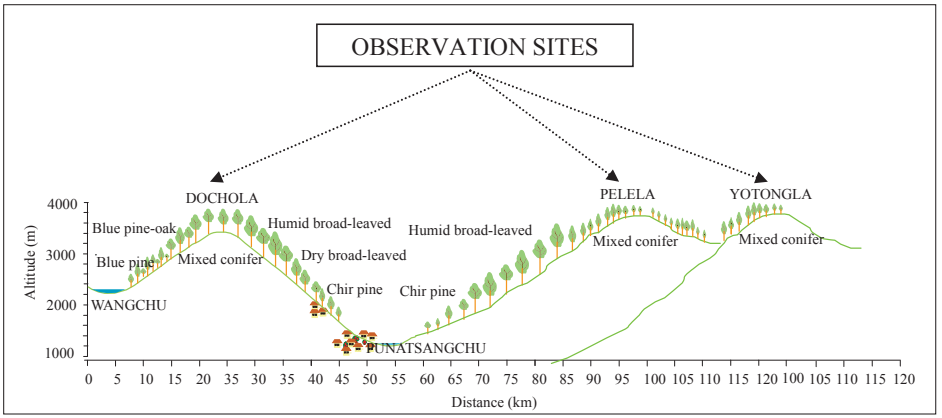

Fig. 1. Thematic cross-section of the study sites along three passes where bamboo flowerings observed.

Bamboo regeneration survey (plot lay-out)

Bamboo regeneration study was conducted by establishing six plots in the bamboo flowering sites. Three plots were set before Yotongla (towards Trongsa) and three plots were set after Pelela pass (towards Bumthang). Regeneration survey includes counting of bamboo seedlings in each plot (1m by 1m plot size) taking into consideration of micro-topography. Along the Trongsa side of Yotongla, the stream side plot was not able to establish owing to limited time and also similar conditions prevailed in both sides of Yotongla.

Bamboo regeneration data analysis

The regeneration data was processed using Microsoft Excel. Graphs are drawn using excel while some figures were drawn using Illustrator and Photoshop.

Flora of Bhutan Volume 3, Part 2, *Grasses of Bhutan* by H.J. Noltie is used for identification of the flowering bamboo.

In addition Stapleton C.M.A. 1994a is also referred for identification and related literature review.

RESULT

Gregarious flowering of bamboo species

The study found gregarious flowering of three temperate bamboos (*Borinda grossa, Thamnocalamus spathiflorus* and *Yushania microphylla*). The species selected for present study was *Thamnocalamus spathiflorus* (Trinius) Munro subsp. *Spathiflorus,* a temperate bamboo belongs to the subtribe Thamnocalaminae under subfamily Bambusoideae. It is also called *hum* in Dzongkha and *rato nigalo* in Nepali and can grow up to 5 m. These bamboos are found above 2800 to 3660 m a.s.l. and are usually grown under the canopy of mixed conifers mainly *Abies densa* forest or in the gaps or roadsides of the passes browsed by yaks. *Thamnocalamus* and *Borinda* have sympodial growth behavior and are grown as a single clump (Fig. 2A, 5C). *Yushania* has a monopodial growth behavior and are found as thickets spreading over large areas (Fig. 6A).

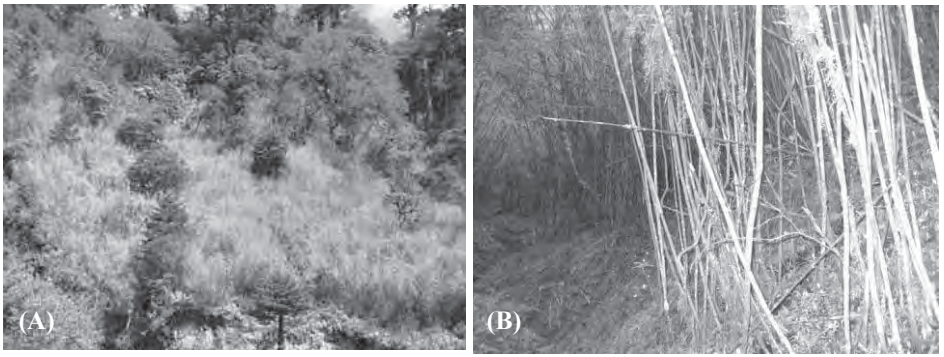

Fig. 2. Mass dying of bamboo at Yotongla (A) in the open area and under sporadic *Abies densa* young stand, (B) Dead clumps of *Thamnocalamus spathiflorus* along the small stream at Yotongla (2010).

First gregarious flowering of *Borinda grossa* was reported sometime in 2005 from Sakteng. In 2008, *Thamnocalamus spathiflorus* was flowering around Pelela, Yotongla and Gedu top followed by mass flowering of *Yushania microphylla* at Lawala in June 2011. These bamboos were found as an important forage resource for the yaks and cattle of higher altitude besides being used as resource for making matt, baskets and fencing.

Regeneration status of *Thamnocalamus spathiflorus*

The regeneration status of *Thamnocalamus spathiflorus* was monitored since first reporting in September 2008 along Pelela and Yotongla when it first started flowering. The monitoring was continued in the following year and finally the regeneration was assessed in September 2010 after two years by laying sample plots at Yotongla pass.

The study found profuse regeneration of *T. spathiflorus* under the dead clump of the study site. Regeneration was found higher along the stream compared to ridge or slopes (Tab. 1, Fig. 3). The highest regeneration was found in plot 1 along the small stream towards Bumthang fro Yotongla with a regeneration density of 122 seedlings in 1 m^2 plot and the lowest was found in plot 2 along the ridge with a regeneration density of 28 seedlings in 1 m^2 plot (Tab. 1, Fig. 3).

On average, regeneration of *T. spathiflorus* was found significantly high with 65 bamboo seedlings per 1 m^2.

Tab. 1. Plot details of the *Thamnocalamus spathiflorus* regeneration of the study site at Yotongla (September 2010)

Bamboo regeneration around Yotongla 3290 m a.s.l.		
Plot location	**Micro-topography**	**Regeneration density/1m^2**
Plot1 Bumthang side	Stream side	122
Plot2 Bumthang side	Ridge side	28
Plot3 Bumthnag side	Stream side	121
Plot4 Trongsa side	Ridge side	40
Plot5 Trongsa side	Ridge side	50
Plot6 Trongsa side	Ridge side	31

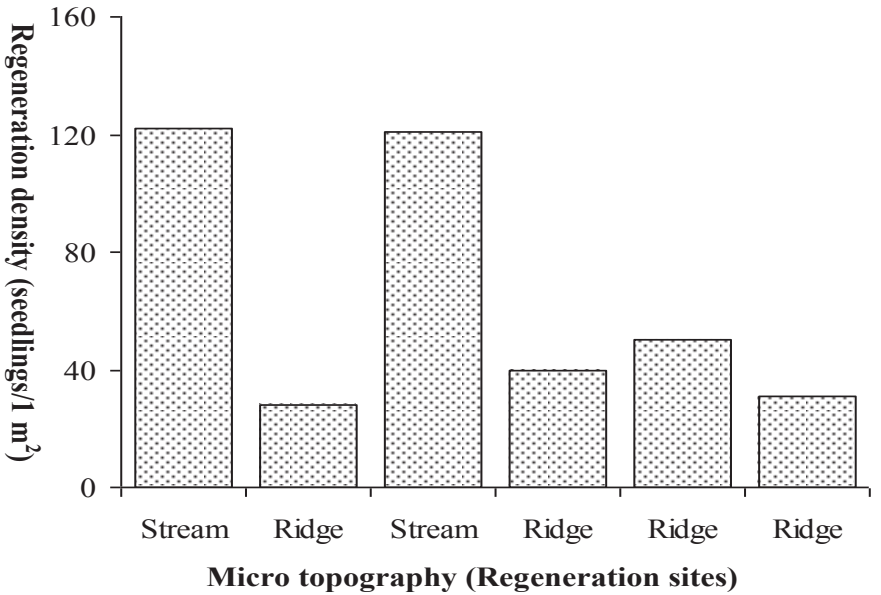

Fig. 3. *Thamnocalamus spathiflorus* regeneration in different topography (Micro site).

Life cycle of *Thamnocalamus spathiflorus*

The study found that it takes c. 2 years to establish seedling after flowering. The flowering of *T. spathiflorus* was observed in September 2008 and dying of mass flowered bamboo continues in 2009. The site was again visited in September 2010 and profuse regeneration was observed (Fig. 4).

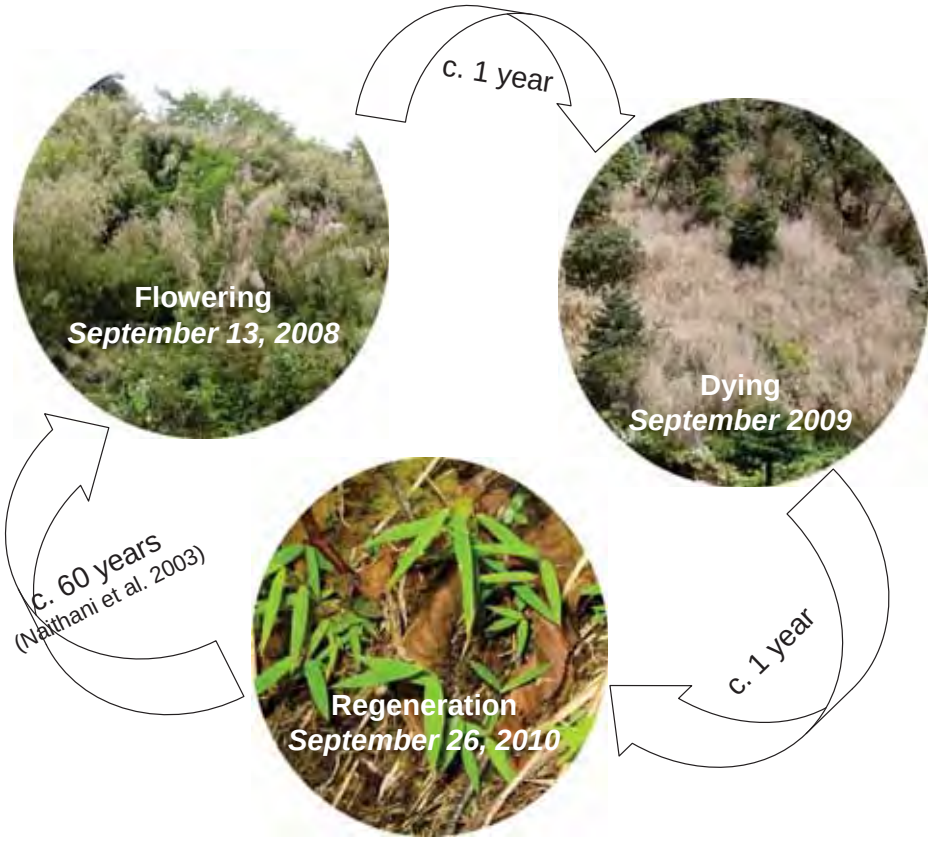

Fig. 4. Life cycle of Thamnocalamus spathiflorus (2008-2010) from flowering stage, seeding stage to seedling stage at Yotongla.

Management of bamboo after flowering

Bamboo species of culturally and economically important should be managed properly to rehabilitate the mass dying bamboos after gregarious flowering. Seeds should be collected after flowering and sown in the seed beds or in poly pots in a nursery. Each individual seeds will germinate into an individual bamboo seedling. Once the seed germinates, the seedlings can further tiller into several seedlings for multiplication. It takes about 2 to 3 years to raise the seedling for out planting into the field. After transplanting in the field it takes about 5-6 years before it becomes ready for harvesting (Fig.5C).

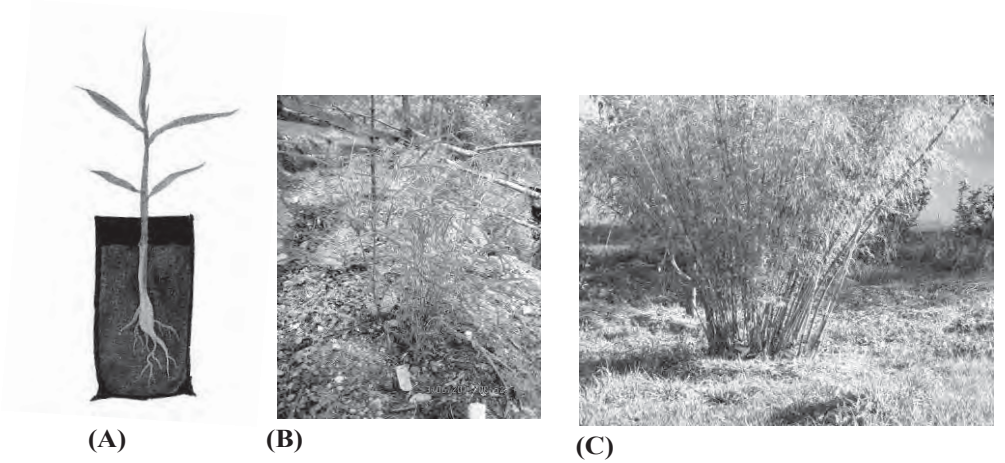

Fig. 5. Bamboo plantation raised from seed (A) seedling in a poly pot, (B) seedlings planted in the field after two years and (C) matured bamboo clump. (*Photo source: Dorji Gyaltshen & Tshewang Norbu RDC-Yusipang*)

Gregarious flowering of *Yushania microphylla*

Recently in June 2011, gregarious flowering of *Yushania microphylla* was observed at Lawala and in the wet-land of Phobjikha (Fig. 6). Lawala being the grazing ridge for yaks and cattle and wetland at the valley of Phobjikha being the habitat for black necked crane cause a serious concern for their habitats. It is thus important and also opportunity for the researchers to monitor and record the flowering phenomena of Yushania in these habitats.

Fig. 6. (A) Gregarious flowering of *Yushania microphylla* at Lawala. (B) Close up of *Yushania microphylla* flowering. (*Photo: Kezang Tshering, RDC-Yusipang*).

DISCUSSION AND CONCLUSION

On the basis of flowering cycle, bamboo flowerings can be categorized into three major groups such as annual flowering, sporadic flowering or irregular flowering and gregarious flowering (Brandis 1899). It is a natural phenomena occurring once in its life time. Bamboo flowers and dies up whole plant leaving behind seeds for regeneration. In case of *Thamnocalamus spathiflorus*, the life cycle takes c.60 years after flowering (Naithani et al. 2003). The study also found that it takes c. 2 years for the bamboo seeds to regenerate after flowering (Seed formation, maturing and germinate).

The gregarious flowerings of three important bamboo species in Bhutan were found as *Thamnocalamus spathiflorus, Borinda grossa* and *Yushania microphylla* respectively.

It is recommended to monitor and observe bamboo flowering by collecting seeds for germination and plantation in the field.

Finally, there is no need to worry seeing bamboo flowering and dying. It is a natural phenomena or process that takes place once in its lifetime. The bamboo regeneration takes about 2-3 years before it establishes profusely under the death bamboo clumps. At the higher altitudes, rodents may not affect the agricultural fields as the agricultural fields are

located far from the forest. However, population explosion of rodents at the higher altitudes of Pelela, Yotongla and Dochula may affect the seed banks in the natural forest. It is highly recommended that nearby farmers should take care of fire out breaks in the bamboo flowered sites. Such wild fire may destroy the fallen seeds of bamboo on the ground except buried seeds in the forest ground.

REFERENCES

Bhattacharya, S., Ghosh, J.S., Das, M. & Pal, A. (2009). Morphological and molecular characterization of *Thamnocalamus spathiflorus* subsp. *Spathiflorus* at population level. Plant System Evolution. 282: 13-20.

Brandis, D. (1899). Biological notes on Indian Bamboos. Indian Forester 25: 1-25.

Naithani, H.B, Pal M & Lepcha STS, (2002). Gregarious flowering of *Thamnocalamus spathiflorus* and *T. falcorneri*, bamboos from Uttaranchal, India. Indian Forester 129:517-526.

Stapleton, C.M.A. (1994a). Bamboos of Bhutan. London: Royal Botanic Gardens, Kew.

Stapleton, C.M.A. (1994). The bamboos of Nepal and Bhutan Part II. Edinburgh Journal of Botany. 51: 275-295.

LIVESTOCK

Suitable time period for chemoprophylactic medication using ivermectin in naturally infected yaks with hypodermosis or warbles in Laya.

Phuntsho Wangdi

ABSTRACT

A trial designed to determine a suitable time period for chemoprophylactic medication using ivermectin at 1ml/50 kgs bw was conducted in 3 yak rearing locations of Laya vide Ngarithang, Tsharijathang and Omtsa, comprising of 4 herds each, naturally infected with Hypoderma species. After medication, study revealed hypodermosis infection rates as 21.52%, 25.00% and 26.27% for August (Ngarithang), September (Tsharijathang) and October (Omtsha) treatments in Control Group. While in Treatment Group, there was drastic reduction of infection rate to 2.35% in August and 0.00% in both September and October treatments, indicating infection with hypoderma species was successfully controlled by chemoprophylactic, medication, at Tshrijathang and Omtsha yak herds.

Study also revealed percent efficacy of ivermectine as 89.08% in Ngarithang (August) and 100% in Tsharijathang (September) and Omtsa (October) in Treatment Group, demonstrating that chemoprophylactic medication is most effective in September and October months in Laya The study thus showed that the most suitable time period for chemoprophylactic medication using ivermectin is in September and October months.

KEYWORDS: Chemoprophylactic, Hypoderma species, Hypodermosis, ivermectin.

INTRODUCTION

Hypodermsis or warble fly infection is a subcutaneous myiasis caused by larvae of *Hypoderma* species that has spread throughout the northern hemisphere, infecting both domestic and wild ruminants (Zumpt 1965, and Li et al 2004). Although causative arthropod parasites is not known in Bhutanese yaks, Otranto and his colleges (2006) reported that hypodermosis in yak rearing locations of China is caused by three

species of warble flies vide *Hypoderma bovis, H.lineatum and H.sinense*. The rate of warbles myiasis in yaks pastured at Tibetan Plateau is reported as 70% to 80% and approaches to 100% in some areas (Xia et al 1998 and Yin et al 2003). Like in Tibetan yaks, hypodermosis is emerging as an important arthropod disease in yaks of Bhutan with warbles prevalence ranging from 15.50% to 95.60% (Wangdi 2010).

Pathological effect of hypodermosis is mainly caused by larvae migration through oesophagus *(H.lineatum)* or spinal cord *(H.bovis)* to the back of animals (Ma et al 2003). In the process
hypodermosis greatly impairs livestock production by inducing mechanical damage to internal organs and skin, down regulating immue system and sometimes mortalities (Scholl 1993, Tarry 1998 and Boulard 2002). Then Otranto and his colleagues (2005) reported that over the past years prophylactic treatment of cattle with ivermectin had greatly decreased the spread of hypodermosis and had even eradicated the disease from many European countries. But prophylactic treatment had caused problems if epidemiology and life cycle of warbles is not known in an infected area. Eosinophilic oesophagitis and paralysis of hind legs were reported in ivermectin treated cattle due to death of migratory larvae when they are in oesophages and in spinal cord (Khan et al 1997 and Hendrickx et al 1993). The present paper thus describes studies designed to determine the suitable time period for chemoprophylactic treatment of yaks naturally infected with hypodermosis at varying time of the year in order to avoid post prophylactic medication problems while introducing the new control schemes.

MATERIALS AND METHODS

Study sites

The study sites were located in summer grazing regions of Laya under Gasa, where high hypodermosis prevalence is reported. The sites were selected based on similar climatic conditions and yak herding practices vide Ngarithang, Tsharijathang and Omtsha with confirmed clinical warbles from February to June months (Wangdi 2007).

Animals

In a total of 9, 13 and 10 yak herds of Ngarithang, Tsharijathang and Omtsha, 4 yak herds each were randomly selected. Yaks of all ages in the herds were selected. These yak herds are grazed on pasture through

the period of warble fly activity, maximizing the likelihood of acquiring natural *Hypoderma i*nfection.

Parasitological techniques

The clinical hypodermosis infections in selected herdes were assessed using palpation techniques described by Handrickx and his colleagues in 1993. The clinical examination consist of palpation of warble swellings in the skin and subcutaneous tissues, observation of breathing holes with or without larvae and resorbing or healed breathing holes. The parasitological examination techniques were applied to find out the clinical infection with *Hypodema* species in yak herds before and after chemoprophylactic medication.

Experiential design

In each study location, 4 yak herds were randomly selected and allocated to Control and Treatment Groups. Total of 12 yak herds were selected and grouped into 6 yak herds each, comprising of 316 and 341 yaks respectively in control and Treatment Groups. The herds in Treatment Groups were medicated with ivermectin at dose rate of 200mg/kg (1 ml/50 kg. BW), supplied as Ivocip, Ivermectin injection 1% w/v by Cipla, India, while Control Groups were dewormed with albendozole at 7.5 mg/kg live weigh. Ivermectin medication in treatment groups were carried out in August, September and October months respectively in locations vide Nagarithang (Lo.1), Tsharijathang (Lo.2) and Omtsha (Lo.3). From February to June 2010, post assessment of clinical hypodermosis infection were initiated in both Treatment and Control Groups.

Analysis of data

The findings were analyzed using, ”Executive Decision Making System using Excel,” through course work recommended by Dr,Galet Shmueli, Professor of Statistics, visiting Smith School of Business, University of Maryland, USA (2009) to Rigsum IT&M, Thimphu and estimated the hypodermosis infection rates between Control and Treatment Groups. The percent efficacy of ivermectin was determined by the formula recommended in World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) guidelines for evaluating the efficacy of anthlmintics in ruminents (Powers et al. 1992), where infection rate in Control Group-Infection rate in Treatment Group ÷ Infection rate in Control Group x100=percent efficacy of ivermectin.

RESULT

The preliminary study from February to May 2009, showed a variation in hypodermosis infection rates within the groups with 16.6 % (Lo.1), 22.15% (Lo.2) and 23.10% (Lo.3) in Control Group. While in Treatment Group, infection rates were revealed as 16.42% (Lo.1), 25.81% (Lo.2) and 20.82% (Lo.3). But the overall infection rates were estimated at 61.39% and 63.05% for Control Group and Treatment Group before ivermectin medication. After ivermectin medication, no adverse clinical sign was observed in treated animals just after administration and 2 days later.

Tab. 1: Hypodermosis infection rates in naturally infected yaks in both Control and Treatment Groups, before and after chemoprophylactic medication using ivermectin at 1 ml/50kgs body weight at Laya.

Locations:	Control Group			Treatment Group			Treatment
before treatment	Total yaks	Total Infet.	% infet	Total yaks	Total infet	% infet	time/period
Lo.1 (4 herds)	73	51	16.14%	81	56	16.42%	August 09
Lo.2 (4 herds)	110	70	22.15%	129	88	25.81%	September 09
Lo.3 (4 herds)	133	73	23.10%	131	71	20.82%	October 09
Total	316	194	61.39%	341	215	63.05%	
After treatment							Assessment period
Lo.1 (4 herds)	73	68	21.52%	81	8	2.35%	February
Lo.2 (4 herds)	110	79	25.00%	129	0	0.00%	To
Lo.3 (4 herds)	133	83	26.27%	131	0	0.00%	June 2010
Total	316	230	72.78%	341	8	2.35%	

N. B Lo. 1 = Location 1 (Ngarithang), Lo. 2 = Location 2 (Tsharijathang), Lo. 3 = Location 3 (Omtsha), Infet = infected with clinical hypodermosis, % inft = Infection rate.

The study also showed that after chemoprophylactic medication with ivermectin within the first weeks of August, September and October months of 2009, infection rates were 21.52% (Lo.1), 25.00% (Lo.2) and 26.27% (Lo.3) in Control Group. But in Treatment Group, only Lo.1 (August treatment) showed the infection rate of 2.35%, while Lo.2 (September Treatment) and Lo.3 (October treatment) no clinical hypodermosis were reported in all the animals. The overall infection rates were estimated at 72.78% and 2.35% for Control and Treatment Groups in 2010.

Before the treatment, study results revealed that 194 and 215 yaks were clinically infected with warbles in Control Group and Treatment Group. But after treatment, 230 yaks in Control Group and only 8 yaks in Treatment Group were infected. The present study results thus indicated that chemoprophylactic medication using ivermectin was effective in reducing infection rate from 63.05% to 2.35% in Treatment Group. The results also showed percent efficacy of ivermectin as 89.08% for August treatment in Ngarithang (Lo.1), and 100% for both September and October treatment in Tsharijathang (Lo.2) and Omtsha (Lo.3) yak herding locations.

Tab. 2: Total yaks infected, hypodermosis infection rate and percentage efficacy of ivermectin prophylactically treated at 1 ml/50kgs body weight in both Control and Treatment groups.

Locations	Control Group		Treatment Group		Percentage efficacy of ivermectin
	Total yaks	% infection	Total yaks	% infection	
Ngarithang (Lo. 1)	68 yaks	21.52%	8 yaks	2.35%	89.08%
Tsharijathang (Lo. 2)	79 yaks	25.00%	0 yaks	0.00%	100%
Omtsha (Lo. 3)	83 yaks	26.27%	0 yaks	0,00%	100%
Total	230 yaks	72.78%	8 yaks	2.35%	97.00%

N. B Lo. 1 = Location 1, Lo. 2 = Location 2 and Lo. 3 = Location 3.

DISCUSSION

The present study was initiated in summer yak rearing locations of Laya vide. Ngarithang (Lo.1), Tshrijathang (Lo.2) and Omtsha (Lo.3) which are proven to be naturally infected with hypodermosis and where no control programme was initiated before (Wangdi 2007). Control activities were not implemented because life cycle and epidemiology of hypodermosis was not studied under yak rearing conditions of Laya. But when compared to hypodermosis myiasis rate of 70% to 80% in northern and in particularly to Tibetan, China (Li et al 2004 and Guan et al 2005), infection rate in Laya is reported as moderate with 40.13% in 2007.

Current preliminary study results initiated from February to June 2009, showed an overall hypodermosis infection rate of 61.39% and 63.05% in both Control and Treatment Groups. The results thus demonstrated an increased in rates before chemoprophylactic treatment of ivermectin at 1

ml./50 kg live weight (0.2mg/kg) per animal compared to 2007. After chemoprophylactic medication, hypodermosis rates were estimated at 21.52% in location 1 of Ngarithnang (August treatment), 25.00% in location 2 of Tsharigathang (September treatment) and 26.27% in location 3 of Omtsha (October treatment) in Control Group. While in Treatment Group, there was drastic reduction of infection rate to 2.35% in location 1 at Ngarithang (August treatment) and to 0.00% in both location 2 of Tsharijathang (September treatment) and location 3 of Omtsa (October treatment).

Inspect of chemoprophylactic medication, hypodermosis was still prevalent as 2.35% in location 1 of August medication under Treatment Group. This could be due to reinfection of 8 yaks by warble flies in the last week of August when efficacy of ivermectin could have reduced to lower blood level in treated yaks (Losson et al 2008). But no infection was reported for September treatment at Tsharijathang and October treatment of Omtsa in 2010. Thus findings obviously indicated that the most suitable time period for chemoprophylactic medication of Laya yak herds is September and October months. The time period various from one region to another and in Tibetan areas, October were reported to be the best month for prophylactic treatment of hypodermosis (Li et al 2004). But in northern China optimum time was found in late October to November (Ma et al 2003). While in Switzerland prophylactic treatment period is from 15th October to 30th November (Kihm 1999). Between December and March of the following year, no treatment must be givens as dead larvae can cause signs of paralysis in the spinal cord.

Several kinds of organophosphate were used for control of hypodermosis in cattle and yaks of China (Lai et al 1992). But in the present study ivermectin was used because of wide spectrum of activity against nematodes and arthropod parasites in man and animals. Ma and his colleagues (2003) demonstrated that ivermectin at dose rate of 0.2mg/kg body weight was able to kill all first stage larvae and no warbles were found on back of treated yaks. Similarly the present study also revealed that percent efficacy of ivermectin as 80.95% in August treatment and 100% each for both September and October treatment. Although ivermectin is very effective for prophylactic medication, it should only be applied in September and October months to avoid post prophylactic treatment problems.

CONCLUSION

Chemoprophylactic medication using ivermectin at 1 ml/50 kg.BW (0.2mg/kg) has proven to be highly effective with 80.95% to 100% efficacy against infection of first stage larvae of *Hypoderma* species in Laya yaks. The present study also revealed that September and October is the most suitable time period for chemoprophylactic medication. Like in Laya, all yak rearing Dzongkhags have similar climate and yak herding practices and prophylactic treatment of hypodermosis shall be implemented in September and October months only. Chemoprophylactic medication should be implemented not just to reduce or control parasitic intensity, but also to avoid the risk of spreading hypodermosis to other livestocks of Bhutan.

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REFERENCES

Boulard, C. (2002). Durably controlling bovine hypodermosis. Veterinary Research, 33:455-464.

Guan, G. Luo, J. Ma, M. Yang, D. Wang, Y. Gao, J. Sun, H. Liu, Z. Liu, A. Dang, Z. Boulard, C. and Yin, H. (2005). Sero-epidemiological surveillance of hypodermosis in yaks and cattle in north China by ELISA. Veterinary Parasitology 129:133-137.

Handrickx, M.O. Anderson,L. Boulard, C. Smith, D.G and Weatherley, A.J. (1993). Efficacy of doramectin against warble fly larvae (*Hypoderma bovis*).Veterinary Parasitology 49:75-84.

Khan,M.Q. Afzal, M. and Kham, M.I. (1997). Efficacy of iverrmectin against cattle warbles in Pakistan. Acta Parasitology 42 (3):173-175.

Kihm,U.(1999).Warble infection in Switzerland. In Animal and Public Health reports 3:15-16.

Lai, D. L. Yang, D. C, Sun, Y. J. and He, S. M. (1992). Control of bovine hypodermosis in Aba district, Sichuan Province. China Yak 1: 39-42.

Li,W. Nasu, T.Ma,Y. Zhu, X. Wanng. W. Song, R. and Mokimura, S. (2004). Migration of warble fly larvae in the yaks and optimum timing of ivermectin treatment. Journal of Veterinary Medicines Sciences 66:891-892.

Ma, M. Guan, G, Lu, B.Liu, A. Liu, Z. Chang,Z.Li, F. Chang, F.Luo, J.Lu, W.Zhang,Q. Yuan, G.Yim, H. and Boulard, C.(2003). Efficacy of different dosages of ivermectin injectable against the *Hypoderma* species in yaks. Veterinary Parasitology 117:147-151.

Powers, K.G. Wood, I.B. Eckert, J. Gibson, T. and Smith H.J.(1982). World Association for the advancement of Veterinary Parasitology (WAAVF) guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine and ovine). Veterinary Parasitology 10:265-284.

Scholl,P.J.(1993). Biology and control of cattle grubs. Annual Review of Entomology 39:53-70.

Torry, D.W (1986). Progress in warble eradication. Parasitology today 2:111-116.

Tarry, D.W (1998). Biology, economic effects and early efforts to eradicate *Hypoderma.* In:Boulard,C. Sol.J.Pithan,K.O Brien, D.wabster, K. and Sampimon, O.C (Eds). Improvement in the control methods for Warble Fly in livestock. Commission of the European Communities, Brussels pp.13-17.

Wangdi, P. (2007). Prevalence of hypodermosis or warbles in yak population of Laya. In: Technical Report on gid disease in 1 to 3 years old yaks, hypodermosis in yaks, taeniasis in yak watch dogs and farmers training in gid disease control techniques pp 21-28.

Wangdi, P.(2010). Summary of warbles or hypodermosis prevalence in yak rearing Dzogkhags of Bhutan. In: Progress reports for diagnosis and control of parasitic disease in the Country for 2009-2010. Parasitology section of NCAH,Serbithang, Thimphu pp.26-37.

Xia, W.X. Tan, S.K.Yu.Z.M. And Song, S.H. (1998). Investigation and treatment of "three flies of livestock" in Qiqihar Heilongjiang province. Chinese Journal of Veterinary Science Techolology 18 (2):40-43.

Yin, H. Ma,M.Yuan, G. Huang, S. Liu, Z. Luo, J. and Guan, G. (2003).Hypodermosis in China. Journal of Animal Veterinary Advances 2:199-183.

Zumpt, F.(1965). Myiasis in Man and Animals in the old world. Butterworths Press, London. pp. 217- 229.