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Effect of different rates and combinations of Farm Yard Manure and inorganic fertilizers on chilli (*Capsicum annum*) yield

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ABSTRACT

Chilli is an important crop in Bhutan and it is grown widely throughout the country. However, the quantity produced is not adequate to meet the demands of the people and therefore a substantial amount is imported from India. Chilli yield of about 6 to 8 t ac^{-1} in Bhutan is lower than the yield of 7 to 10 t ac^{-1} reported from elsewhere outside the country. Beside the limited land area available for growing chillies, inadequate plant nutrients supply is also a crucial yield limiting factor. Farm Yard Manure (FYM) is the main external source of plant nutrients but the amount of nutrients supplied through FYM is not adequate to meet the demand of the crop. Since chilli is an important crop in Bhutan it is important to study how best the crop yield could be improved both in quantity and quality. This study conducted over four (2001-2004) consecutive years had the objectives to examine the effect of different rates of FYM and inorganic fertilizers on the quality and quantity of chilli, to examine the effect of the integrated use of organic and inorganic fertilizers on chilli yield and to formulate an optimum nutrient requirement for chilli under the Bhutanese condition. The study conducted on station at the National Soil Services Centre had four treatments with three replicates. The study showed higher yields of 5.7 to 6.3 t ac^{-1} with higher rates of FYM and NPK combinations. The weight and the number of fruits per plant also were higher with higher rates of NPK and FYM. However, the number of rejected fruits was higher with higher rates of NPK and FYM. The economic analysis indicated higher marginal rate of return with integrated use of organic and inorganic fertilizers.

KEY WORDS: Chilli, Organic, Inorganic, Yield, Integrated, Plant nutrient.

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INTRODUCTION

Chilli is one of the most important vegetables constituting the Bhutanese diet. Although it is grown widely throughout Bhutan, a substantial amount is still imported from India to fulfill the requirements of the Bhutanese people. Field experiences suggest low soil fertility status to be an important constraint in chilli production.

Chilli is the favoured cash crop for most farmers as the potential returns per unit area are high which can be achieved in one season. Until recent times, chillies were mainly grown on kitchen garden scale with little or no external nutrient input. Some farmers in places like Punakha and Paro have now started growing chillies on a commercial scale. Chilli yield in Bhutan has been quoted to be about 6 to 8 t ac^{-1} (Bajo, 1998) and elsewhere chilli yields of 7 to 10 t ac^{-1} have been mentioned. One of the major factors limiting the yields of chillies in Bhutan would be the availability of plant nutrients as FYM is the main external source of nutrients for chillies. About 1195 kg ac^{-1} of FYM is applied in chillies annually and this amount of FYM supplies about 19 kg N ac^{-1}, 4 kg P ac^{-1}, 24 kg K ac^{-1}, 28 kg Ca ac^{-1} and 4 kg Mg ac^{-1} (RNRRC Bajo and SSF & PNM, 2001). Nutrients supplied through FYM alone are not sufficient, as the crop nutrient requirements have been quoted to be about 72.8 N kg ac^{-1}, 72.8 P_2O_5 kg ac^{-1}, 80.9 K_2O kg ac^{-1} and 14 S kg ac^{-1} (The chilli pepper company). The objectives of this study:

- To examine the effect of different rates of FYM and inorganic fertilizers on the quality and quantity of chilli fruits;
- To examine the effect of the integrated use of FYM and inorganic fertilizers on the quality and quantity of chilli fruits; and
- To formulate an optimum nutrient requirement for chilli under the Bhutanese condition.

METHOD & MATERIALS

Trials were conducted at the National Soil Services Centre (NSSC) in Simtokha between June and September for four consecutive years i.e. 2001 to 2004. The trial procedures and treatments were same for all the four years. Before the land preparation, the trial area was divided into

three large plots i.e. upper, middle and lower plots and a composite soil sample was collected from each of these plots. Each composite sample had at least five sub-samples and the samples were collected from the depth of 0-20 cm. The samples were analysed at the Soil and Plant Analytical Laboratory (SPAL) for nutrient status. Land preparation was done in May and to get a fine tilth, the land was dug several times manually after the initial ploughing by a tractor. Raised bed method was used to prevent chilli blight. Each plot measuring 150 cm by 200 cm was raised by about 20 cm. In the first week of June, farmyard manure (FYM) and inorganic fertilizers (suphala & urea) were applied and mixed thoroughly with the soil a day or two before planting chilli seedlings. The chilli seedlings from Teobsa were used in all the trials. Seedlings were planted at a distance of 30 cm by 25 cm and each plot therefore accommodated 40 seedlings. Urea top dressings were done around the second week of July after the first weeding. The plants were irrigated intensively, every morning and evening for at least one week after transplanting, till the roots were well established. Weeding was done manually on a regular basis. In the third week of August, leaf samples were collected and analysed for nutrients contents. The farmyard manure used in the trial was also analysed for nutrient contents.

Each year, fruits were harvested sequentially. First harvesting was carried out in the third week of August, second in the first week of September, third towards the end of September and the final harvest was done in the third week of October. During each harvest, the harvested fruits were separated into two categories i.e. one category consisting of saleable fruits and the other category consisting of fruits which were likely to be rejected by buyers. Weights and counts of fruits in each of the two categories were recorded.

After the harvest, soil samples were collected from each of the sixteen treatments and analysed for nutrient status. Plant samples selected randomly in each treatment plots were cut above the ground and analysed for plant nutrient status. Genstat 5 Second Edition for Windows and Excel were used to analyse the trial data and produce graphs.

Trial design and treatment

The design of the experiment was a 4x4 factorial involving four rates of FYM and four rates of NPK in a randomised complete block design with three replications. Each replication had 16 factorial treatment combinations of FYM and inorganic fertilizers.

i. FYM application rate at four levels:
FYM_1 - 0 tonnes ac^{-1}
FYM_2 - 2 tonnes ac^{-1}
FYM_3 - 4 tonnes ac^{-1}
FYM_4 - 8 tonnes ac^{-1}

ii. Uniform NPK rate at four levels:
$(NPK)_1$ - 0 – 0 - 0 kg ac^{-1}
$(NPK)_2$ - 8 -6 - 6 kg ac^{-1} (2 kg N ac^{-1} to be applied as top dressing)
$(NPK)_3$ - 16 -8 - 8 kg ac^{-1} (8 kg N ac^{-1} to be applied as top dressing)
$(NPK)_4$ - 24 - 18 - 18 kg ac^{-1} (6 kg N ac^{-1} to be applied as top dressing)

Table 1. The 4 x 4 factorial treatment combinations of four FYM rates and four NPK levels:

FYM rates	NPK1	NPK2	NPK3	NPK4
FYM1	FYM1, NPK1	FYM1, NPK2	FYM1, NPK3	FYM1, NPK4
FYM2	FYM2, NPK1	FYM2, NPK2	FYM2, NPK3	FYM2, NPK4
FYM3	FYM3, NPK1	FYM3, NPK2	FYM3, NPK3	FYM3, NPK4
FYM4	FYM4, NPK1	FYM4, NPK2	FYM4, NPK3	FYM4, NPK4

RESULTS AND DISCUSSIONS

Pre-trial soil fertility status

The soil fertility status at the start of the trial before fertilizer application is shown in Table 2. The soil pH of 5.6 was slightly on the lower side but suitable for growing chillies. Chillies are known to grow well under a wide range of soil pH from 5.5 to 7.5.

Total carbon percentage of 1.7% was within the medium range indicating moderate soil organic matter content. Available K of 41 mg kg^{-1} and the available P of 24.7 mg kg^{-1} were both within the moderate ranges of 36-60 mg kg^{-1} and 16-30 mg kg^{-1} respectively recommended for chillies (Hochmuth & Hanlon, 1995). Calcium and Mg were both

low and the response to fertilizers supplying these elements could be expected.

Table 2. Soil nutrient status before the trial

pH	C%	C:N ratio	Av K mg/kg	Av. P mg/kg	Ca me/100g	Mg me/100g
5.55	1.70	12.52	41.20	24.69	4.18	0.52

FYM nutrient content

The mean FYM dry matter nutrient contents are shown in the Table 3. The mean dry matter content of three FYM sub sample was 36%. Mean values for FYM nutrient content of 1.49% N, 0.57% P, 1.70% K, 1.08% Ca and 0.42% Mg are similar to those reported by others in Bhutan, e.g. Lingmutey Chhu FYM survey, RNR-RC Bajo, 2000. They are also comparable to those reported as average values in the literature by Euroconsult, 1989. The average nutrient additions based on the amount of FYM ac^{-1}, the mean dry matter and individual nutrient contents are also given in the table. Nitrogen, potassium and calcium additions from the FYM were quite significant as compared to others like the phosphorus and magnesium.

Table 3. FYM nutrient content and nutrients applied (in kg ac^{-1}) based on the amount of FYM applied.

	N%	P%	K%	Ca%	Mg%	Na%
FYM nutrient content	1.49	0.57	1.70	1.08	0.58	0.42
2 t ac^{-1} FYM	11	4	12	8	4	3
4 t ac^{-1} FYM	22	8	25	16	8	6
8 t ac^{-1} FYM	43	16	49	31	17	12

Note: Mean dry matter of FYM was 36%

Post trial soil fertility status

There was no significant treatment effect on any of the soil variables measured after the crop harvest. The soil fertility status determined based on the mean values of the soil variables after the trial is shown in the figures 1 and 2 below. The percentage of the samples falling within

the different categories or ratings of the soil fertility status is shown in figure 1. About 90% of the samples had pH within the medium range; all the samples had moderate soil organic matter content and low total nitrogen percentage.

Available P of the samples ranged between medium (60%) to high (40%) while the available K was within the very low (30%) to low (70%) range. About 70% of the samples had low calcium and about 50% had very low Mg. The overall soil nutrient status is quite low and in the absence of other production constraints, crops would show good response to higher rates of fertilizers.

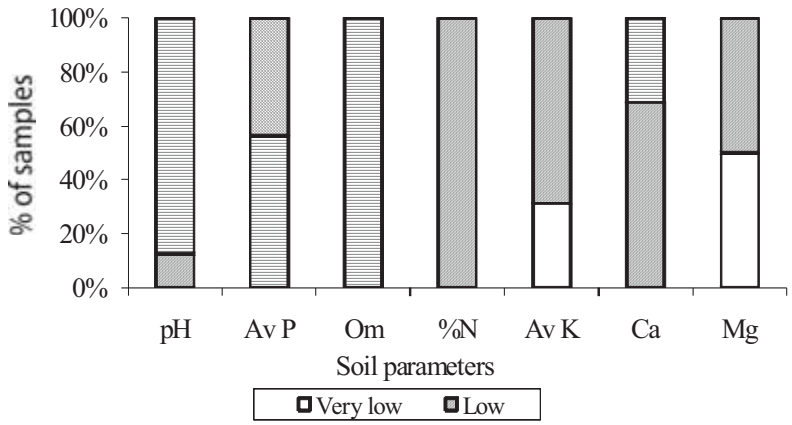

Figure 1. Soil fertility status.

Although the effect of different treatments on the soil nutrient status was not statistically significant, the effect of treatments on available P and K were quite apparent as shown in figure 2 below. Increasing the application rate of NPK upto 16-8-8 kg ac^{-1} in combination with increasing FYM application upto 4 tons ac^{-1} increased the available P and decreased the available K. The farmyard manure is made up of cattle dung and urine with bedding materials like rice straw and other plant materials. The potassium concentration in the rice straw can be quite high and when applying huge quantities of FYM consisting mainly rice straw, a significant amount of potassium would be returned to the soil. Therefore, applying 8 tons ac^{-1} of FYM seems to have immensely improved the soil K status. Soil pH, total carbon and Ca were not affected by the treatments.

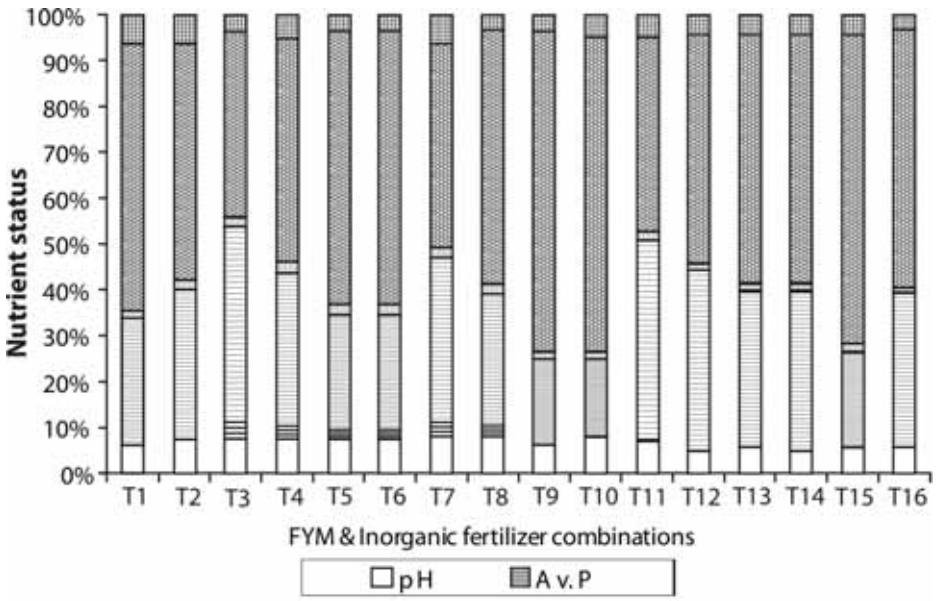

Figure 2. Soil fertility status in relation to the treatments.

Crop nutrient content

There were no significant treatment effects on nutrient contents of the whole chilli plant and therefore the data analysis has been based on the average nutrient contents of the chilli leaf only. As there is a scarcity of nutrient contents data for chilli in the region, comparisons have been made to the reports from elsewhere. The nutrient contents of the chilli in this trial were within the adequate range reported by Hochmuth, 1994. Nutrient uptake by one ton of fresh fruit in this trial was 3.72 kg N, 0.28 kg P, 4.87 kg K, 1.99 kg Ca and 0.32 kg Mg. According to the data on nutrient uptake in different studies from elsewhere, to produce one ton of fresh fruit, plants need to absorb, on average, 3 – 3.5 kg N, 0.7 – 1 kg P, 5 – 6 kg K, 1.2 kg Ca and 1.2 kg Mg in the case of chilli or bell pepper (Hegde, 1997). In this study, Zn and Fe contents were high with 112.3 mg kg^{-1} and 189.9 mg kg^{-1} respectively. In chillies, an adequate range for Zn between 25 – 80 mg kg^{-1} and Fe between 30 – 150 mg kg^{-1} have been reported. In the absence of any other production constraints, nutrient uptake and dry matter production (fruit yield) are closely related (Hegde, 1989).

In chillies, concentrations of NPK, Ca and Mg are highest in leaf, followed by that in fruit and stem (Hegde, 1988). If chilli plants are not removed from the field after the crop harvest, the nutrients in them are released back into the soil.

Crop yield

Figure 3a shows the chilli yield difference between the treatments. With p=0.05, the effect of different rates of NPK on the yield was significant at 5% significance level. Generally the increasing rate of NPK increased the crop yield. The highest yield of 5.17 t ac^{-1} was obtained with the highest rate of NPK of 24-18-18 kg ac^{-1} and the lowest yield of 3.75 t ac^{-1} was with the lowest NPK rate of 8-6-6 kg ac^{-1} averaged over different rates of FYM (Figure 1, $r^2 = 0.64$). The effect of different rates of FYM or the effect of FYM and NPK combination on the crop yield was not significant. However, the general trend was increasing FYM rates increased crop yield ($r^2 = 0.92$). The highest yield of 4.78 t ac^{-1} was obtained with the highest FYM rate of 8 t ac^{-1} while the lowest yield of 3.89 t ac^{-1} was without the FYM application averaged over different rates of NPK.

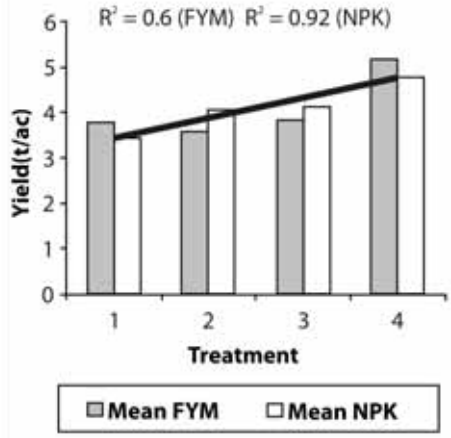

Figure 3a. Crop yield trend as affected by different treatments.

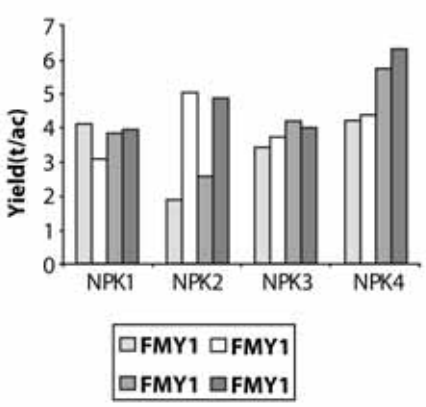

Figure 3b. Crop yield as affected by different FYM and NPK combinations.

Higher yields (5.74 t ac^{-1} to 6.32 t ac^{-1}) were obtained at higher rates of FYM and NPK and with combined use of FYM and fertilizers as shown in Figure 3b and Table 4. Hosmani (1993) and Subbiah et al. (1985) also reported higher yields of chilli, tomato and eggplant with integrated use

of chemical and organic fertilizers than with the use of either of these separately.

Table 4. Yield in t ac^{-1} as affected by the source and amount of nutrients.

FYM rates	NPK1	NPK2	NPK3	NPK4	Treatment average
FYM1	4.13	3.78	3.43	4.23	**3.89**
FYM2	3.10	5.01	3.73	4.37	**4.05**
FYM3	3.85	2.56	4.19	5.74	**4.09**
FYM4	3.92	4.87	4.01	6.32	**4.78**
Treatment average	**3.75**	**4.06**	**3.84**	**5.17**	

The yields obtained in this trial were similar to that quoted for Bhutan (Bajo 1998) but much higher than the yields of 1.6 to 2.4 tones per acre obtained elsewhere (ADC Bulletin # 6, 2001).

Yield and fruit per plant

Figure 4 shows the yield per plant and number of fruits per plant and they have similar trend. Different rates of NPK significantly affected the yield per plant ($p=0.046$, $r^2=0.66$) and fruits per plant ($p= 0.045$, $r^2=0.60$). The highest yield of 256 g per plant was obtained with the highest rate of NPK and the lowest yield of 171 g per plant was with the lowest rate of NPK averaged over FYM rates. The highest number of 22 fruits per plant was also obtained with the highest NPK rate and the lowest of 16 fruits from a plant was obtained with the lowest NPK rate averaged over different rates of FYM. While the effect of different rates of FYM and FYM-NPK combination on the yield per plant and fruit per plant was not significant, the general trend was increasing yield and fruit number per plant with increasing rate of FYM application ($r^2 = 0.97$, $r^2 = 0.92$ respectively) or with increasing rates of FYM and NPK combination. The highest yield of 229 g per plant was with the highest rate of FYM and the lowest yield of 168 g per plant was without FYM application averaged over different rates of NPK. The highest number of 19 fruits per plant was obtained with the highest rate of FYM application and the lowest of 16 fruits per plant was obtained without the FYM application averaged over different NPK rates. The lowest yield of 99 g per plant and the lowest number of 12 fruits per plant were obtained with

the lowest rate of NPK and without FYM application. Both these parameters generally increased with increasing FYM and NPK combinations (Figure 5). The correlation between the yield per plant and fruit per plant is significant at the 0.01 level.

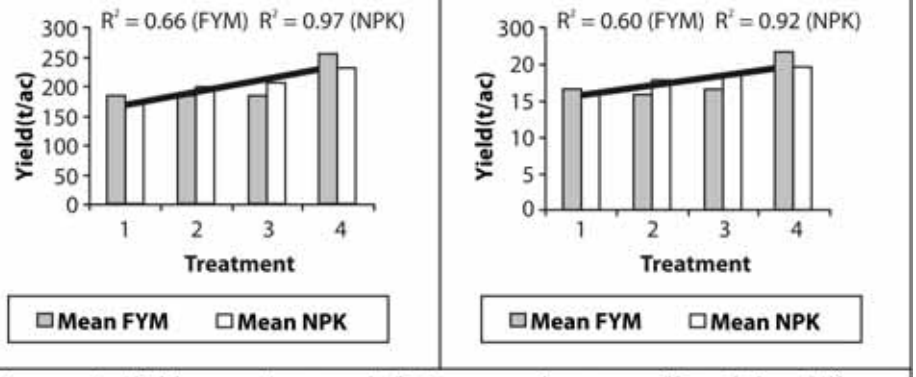

Figure 4. Yield per plant and fruits per plant as affected by different treatments.

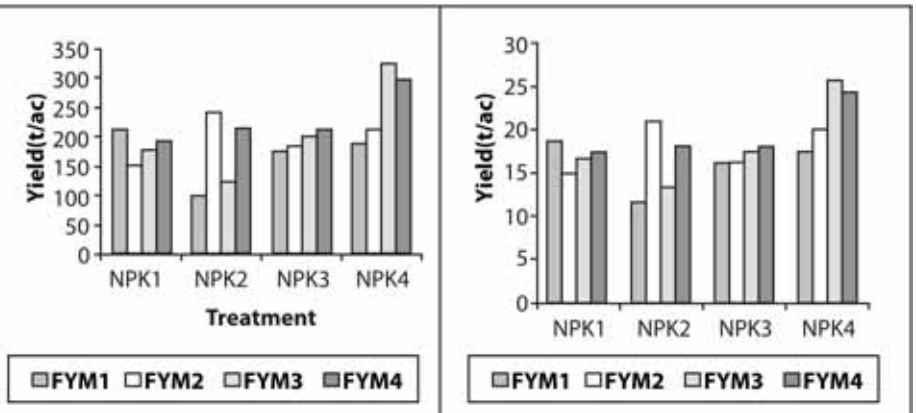

Figure 5. Yield per plant and fruits per plant as affected by differentFYM and NPK combinations.

Rejects

The effect of different rates of NPK on the number of rejected fruits was significant at 5% significance level. Generally the number of rejected fruits increased with increasing NPK rates. The highest number of 664

rejected fruits in an acre was with the highest NPK rate while the lowest number of 293 was without NPK application averaged over different FYM rates. The effect of different rates of FYM and FYM-NPK interaction on the number of rejected fruits was not significant. However, the number of rejected fruits generally increased with increasing FYM application (Figure 6). The number of rejected fruits per acre was high (i.e. 529) with the highest FYM application while the lowest number of rejected fruits (i.e. 416) was with the lowest FYM rate of 2 t ac^{-1}. The correlation between the number of plants and the number of rejected fruits is significant at the 0.01 level. Over fertilization especially with nitrogen fertilizers, promotes excessive vegetative growth and creates conducive microclimate for pest and diseases, which reduces the quantity, quality and the market value of the fruit.

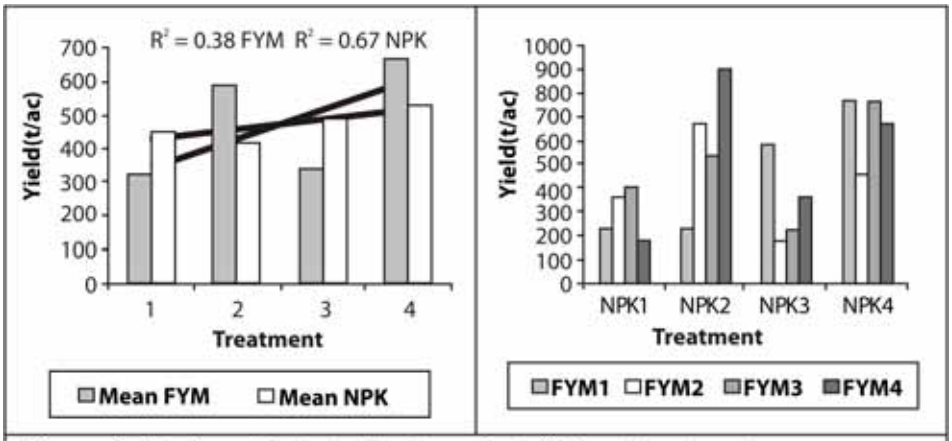

Figure 6. Number of rejected fruits against different treatments.

Economic analysis

The marginal rate of return (MRR = the profit for every additional Nu. 1 spent) was better when using FYM and NPK together than when using them separately. The marginal rate of returns were greater when applying 2 tons ac^{-1} FYM along with 8-6-6 kg ac^{-1}, 16-8-8 kg ac^{-1} and 24-18-18 kg ac^{-1} NPK. Applying 4 and 8 tons ac^{-1} of FYM along with the three rates of NPK was not very economical, however, the value cost ratio (VCR) which is a ratio of the crop value divided by the cost of the inputs used, were all greater than 2. The rule of the thumb is that the VCR should be equal to or greater than 2 (in our part of the region) for the farmers to see the fertilizer application as profitable.

Table 4. Economic analysis (MRR = marginal rate of return calculation)

Treatment	MRR%	Profit Nu. for every Nu. 1 spent
FYM (0 t ac^{-1}) + NPK (24-18-18 kg ac^{-1})	1705	17.05
FYM (4 t ac^{-1}) + NPK (0 kg ac^{-1})	2392	23.92
FYM (2 t ac^{-1}) + NPK (8-6-6 kg ac^{-1})	6765	67.65
FYM (2 t ac^{-1}) + NPK (16-8-8 kg ac^{-1})	2249	22.49
FYM (2 t ac^{-1}) + NPK (24-18-18 kg ac^{-1})	10344	103.4

CONCLUSION

This study conducted over a period of four years, clearly showed the response of chilli to different rates and combinations of FYM and inorganic fertilizers. The quantity of fruit increased while the quality of fruit tended to decrease with increasing NPK and FYM applications. The highest crop yield of 5.17 t ac^{-1} was obtained with the highest rate of 24-18-18 kg ac^{-1} NPK and the lowest yield of 3.75 t ac^{-1} was with the lowest NPK rate of 8-6-6 kg ac^{-1} while the highest yield of 4.78 t ac^{-1} and the lowest yield of 3.89 t ac^{-1} were obtained with the highest FYM rate of 8 t ac^{-1} and without FYM respectively. Both the yield per plant and fruit per plant also increased with increasing NPK rates. The highest yield of 256 g per plant and the highest number of 22 fruits per plant were obtained with the highest NPK rate while the lowest yield per plant of 171 g and the lowest fruit number per plant of 16 fruits were with the lowest NPK rate.

The highest yield per plant of 229 g and the highest number of fruits per plant of 19 fruits were obtained with the highest FYM application while the lowest yield of 168 g per plant and the lowest numbers of fruits per plant of 16 fruits were obtained without FYM. The highest number of 664 and 529 rejected fruits per acre were with the highest NPK and FYM rates while the lowest number of 293 and 416 rejected fruits per acre were without NPK and with the lowest FYM rate respectively.

Although the effect of NPK and FYM combination on the quantity and quality of the fruits was not statistically significant, the general trend was increasing fruit quantity and decreasing quality with increasing NPK and FYM rates together. As shown by many studies conducted by

other, this study also demonstrated the benefit of the integrated use of organic and inorganic sources of plant nutrients. The yields were higher with integrated use of inorganic and FYM than with the use of either of these separately.

Even in Bhutan, the integrated nutrient use has assumed great significance in recent years in vegetable production. The results of a large number of experiments on manures and fertilizers conducted in several countries reveal that neither chemical fertilizers alone, nor organic sources used exclusively, can sustain the productivity of soils under highly intensive cropping systems (Singh and Yadav 1992). Keeping in focus, the findings of this study and that of others from elsewhere, our farmers who have been using FYM for centuries and in recent times the inorganic fertilizers, should be advised and encouraged to use them correctly to meet the nutrient demands of crops

The economic analysis indicated higher MRR with FYM and inorganic fertilizer used together. The best MRR of 10344% was from using 2 tons ac^{-1} FYM along with 24–18–18 kg ac^{-1} NPK. Using 4 and 8 tons ac^{-1} FYM along with any of the three NPK rates was not very economical, however, as indicated by the VCR, applying FYM and inorganic fertilizers together and in greater amounts than normally used by the farmers is still profitable.

Given the fact that there is a scarcity of fertilizer recommendation guides for chillies, the integrated use of FYM and inorganic fertilizers tried in this study can be used as a reliable guide for our condition.

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Performance of Rice under System of Rice Intensification (SRI) at CNR, Lobesa

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ABSTRACT

Rice production trials using SRI methods in a few locations in Bhutan have shown positive effects. This paper reports the performance of four rice varieties: IR 64, Bajo Maap 2, and Khangma Maap, and a local variety Nyabja using SRI practices conducted at Lobesa. IR 64 showed the highest yield performance of 10.1 t/ha followed closely by Nyabja and Bajo Maap 2 with 9.7 t/ha each. For all varieties tested, the SRI method out yielded the conventional methods. Statistical analysis showed significant differences ($p<0.01$) in yield among the cultivars and the two methods tested. Input cost for seed, fertilizer and water reduced with SRI methods. These results show that SRI techniques may provide an efficient option for farmers to enhance their rice production.

KEY WORDS: Lobesa, conventional methods, SRI, yields, varieties, methods.

INTRODUCTION

In Bhutan, one of the major constraints for achieving nation-wide food security is the current low rice yield. In 2006 the average national rice production was 2.7 t/ha (MoA, 2006), which is far less than the global yield of 3.8 t/ha (Uphoff, 2004). There have been several measures undertaken to raise rice productivity in Bhutan, for instances, like introduction of high-yielding varieties and certain technological developments. Yet, enhancing rice production still remains a priority goal of the Ministry of Agriculture (Ghimiray et al., 2008).

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The System of Rice Intensification (SRI) which is reported to be an efficient, economical and environmentally sound method of growing rice has received attention of the global farming community (Goud, 2008; Mishra et al., 2006; Stoop et al., 2002). SRI method shows promise for overcoming low yields. Studies in a number of countries like China and India have shown significant increases in rice yield, with substantial savings of seeds (80-90%), water (25-50%), and cost (10-20%) compared to conventional methods (Uphoff, 2004).

SRI is not a technology, but a set of simple ideas and principles that help produce more productive and robust plants from any rice genotype by providing an improved growing environment (Uphoff, 2005). Reduced use of seedlings, remarkable plant and root growth, profuse tillering from a single seedling, and bumper harvest for the farmers are important features of this method. Further, in the face of declining resources (land, water and labour), deteriorating soil health, increasing environmental concerns, and increasing cost of cultivation, SRI may mitigate or could overcome those resource constraints that confront the rice sector in the 21st century (Uphoff, 2004). Therefore, SRI method is considered a resource-conserving technique of rice production that is good for farmers, consumers, and the environment.

In Bhutan, following successful SRI tests in the preceding two years, both at farmer's field and in research centres, the method is gaining acceptance (Lhendup et al., 2008; Ghimiray and Thinley, 2008; Lhendup, 2008; Chhetri, 2007 & Lhendup, 2007). Further, it was felt necessary to carry out evaluation tests to affirm the performance of rice varieties at different localities using SRI method. Therefore, the study was undertaken to verify the performance of four different varieties: three improved rice varieties (IR 64, Bajo Maap 2 and Khangma Maap) and a local variety (Nyabja) at the College of Natural Resources (CNR) farm using SRI practices.

MATERIALS AND METHODS

Study site

The study was carried out in the 2008 season at the CNR farm, located at an altitude of 1450 masl. The site falls under typical dry valley along the Punatsangchu in west Bhutan with potential evapotranspiration ratio of 1.2. The relative air humidity is 75.5%. The site receives mean annual

precipitation of 883 mm. The annual mean temperature is 17.70C (maximum of 23.20C and 9.90C minimum). The site has sandy clay loam soil and vegetation consists of dry habitat shrubs and mostly chirpine tree species (Wangda and Ohsawa, 2006).

Experimental Design

A modified complete randomized block design was used for the study with three replications using two methods (SRI and conventional) and four varieties (treatments). The four rice varieties tested using both SRI methods and conventional methods are IR 64, Bajo Maap 2, Khangma Maap and Nyabja. Treatments with SRI methods were assigned plots randomly and were separated by 50 cm wide spacing from each other. Similarly, treatments with conventional methods were randomly laid and were separated by 50 cm wide spacing. A bund was constructed within the field to separate the treatments with SRI methods and those with conventional methods to minimize cross-plot effects. The size of each plot was 6 m x 3.5 m.

Nursery and Seedling Transplanting

Nursery was established using pre-soaked incubated seeds on third week of May 2008. The rate of seeds used was 3.0 kg per acre (7.4 kg per ha). For SRI methods, transplanting of three leaf stage seedlings was done singly and at shallow depth (2-3 cm) without removing soil attached to the seedling roots into well-puddled and moist soil following a square pattern of 25 x 25 cm spacing (plant to plant and row to row). Three leaf stage seedlings were attained and transplanted at about 15-18 days after seeding in the nursery. For conventional methods, transplanting of older seedlings of 40-45 days in flooded field was done randomly at close spacing of less than 15 cm and in bunches of two to four seedlings.

Application of Organic Matter

Application of farm yard manures at the rate of 2 t/ha was done in both the SRI and conventional plots during land preparation. To be consistent with the conventional plots, no inorganic fertilizers were applied.

Water Management

After transplanting the SRI plots were left moist but without flooding for at least 12-14 days. This was then followed by alternate wetting and drying (AWD) until the end of the vegetative stage. This was done by

irrigating the field for three to six days and then draining the field to dry out for a similar number of days. The field was completely left dry for about two to three weeks before harvest. In conventional plots however the field was kept flooded until two to three weeks before harvest.

Weeding

A total of three weedings were done on SRI plots using a rotary weeder, small locally- made tools followed by hand weeding. The first weeding was carried out between 16-18 days after transplantation and the subsequent weedings were done at an interval of two to three weeks.

Application of Herbicide

The pre-emergence selective herbicide (Butachlor) was not applied for this study although practiced by farmers in this locality.

Data analysis

Data analysis was done using SPSS. Analysis of variance was performed to determine the significance of difference between methods and within treatments besides simple statistical test.

RESULTS AND DISCUSSION

Results

Yield (t/ha)

Table 1 presents the mean yield and yield difference of four rice varieties evaluated using SRI and conventional methods. The average yield performance was better on SRI plots than conventional plots for all varieties. Among the cultivars, better yield response to SRI methods was observed with all four varieties against the conventional methods (Figure 1).

Yield performance of IR 64 was the highest with 10.1 t/ha followed closely by Nyabja and Bajo Maap 2 varieties with 9.7 t/ha each (Table 1). Increase in yield using SRI methods compared to conventional methods from the same varieties was 14%, 31% and 14% respectively. Mean yields comparison of four varieties applying two methods showed a significance of difference ($p<0.01$) between methods and varieties

(Table 1). However, interaction between variety and method is not significant (p=0.12).

Table 1. Mean yield and yield difference of four rice varieties tested using SRI and conventional methods.

Method	Spacing	Rice variety and yield (t/ha)			
		IR 64	Bajo Maap 2	Khangma Maap	Nyabja
SRI method	25 x 25 cm	10.1	9.7	5.2	9.7
Conventional method	Farmers random	8.7	6.7	3.7	8.3
% difference (yield)		14	31	29	14
		F pr			LSD
Method and variety interaction		0.115 ns			
Methods		0.000**			0.68
Varieties		0.000**			0.48

** Significance difference at 1% level; ns non significance; CV% = 4.2

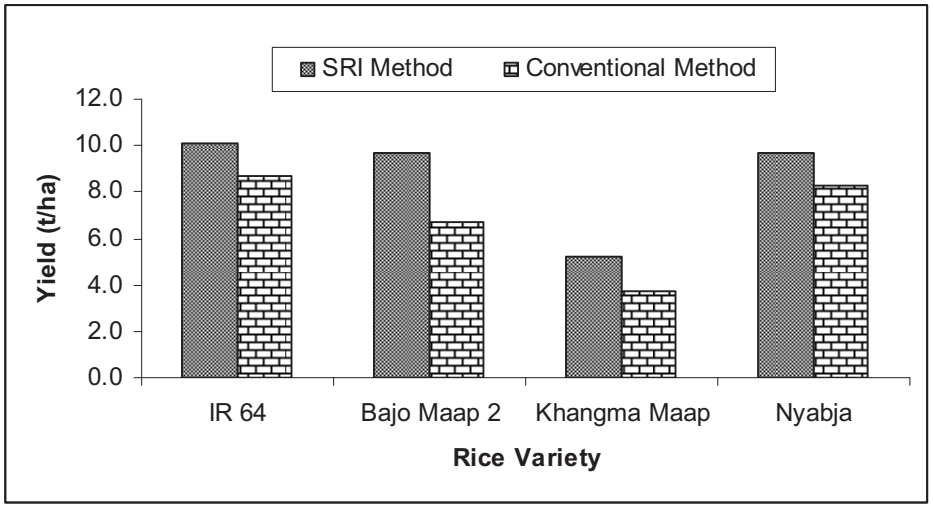

Figure 1. Yields of four rice varieties evaluated using SRI and conventional methods.

Number of Fertile Tillers

Table 2 presents the result for number of fertile tillers, a main yield-contributing parameter of the four rice varieties evaluated at the CNR

farm. In all four varieties tested, the average number of fertile (effective) tillers per hill was higher in the SRI plots than in conventional plots. Among the varieties, the highest number of productive tillers was found with Nyabja (38), followed by IR 64 (30). This was an increase of 53% and 33% compared to same-variety results with conventional methods. The number of tillers for Bajo Maap 2 is close to IR 64. However, the number of tillers for Khangma Maap was lower.

Inputs

Average seeds use was 3.0 kg per acre in SRI methods as compared to 22.5 kg seeds per acre for conventional methods. Use of inorganic fertilizer was nil in SRI methods and irrigation water use was reduced through AWD compared to flooding practiced in conventional methods.

Table 2. Yield-contributing parameters for four rice varieties at CNR farm (average of three replications).

Sl. No.	Parameters	Rice variety			
		IR 64	Bajo Maap 2	Khangma Maap	Nyabja
1	**Fertile tillers/hill**				
	SRI method	30	28	19	38
	Conventional method	20	19	13	18
2	**Plant height (cm)**				
	SRI method	91	115	150	110
	Conventional method	90	110	155	108
3	**Number of hills/6m^2**				
		96	96	96	96
	SRI method	110	125	100	118
	Conventional method				

Discussion

The average yield performance was better on SRI plots than conventional plots for all varieties. This is supported by the absence of significance of difference in interaction between variety and method. The significant ($p<0.01$) increase in yield with SRI methods for all varieties is mainly attributed to the increased number of productive tillers. Transplanting of young seedlings singly seems to preserve the potential within plants for profuse tillering and root growth. According to Stoop (2005), the extensive root development under SRI translates into accelerated vegetative development phase and an increased nutrient uptake capacity leading to an extended, more effective, grain filling phase (Stoop, 2005).

The increase in yield for all varieties also indicates that all cultivars respond positively to SRI methods as reported by Uphoff (2004). According to Uphoff (2004) SRI practices improve the growing environment of plant by managing soil, water and nutrients differently so that more productive phenotypes having much larger root systems can result from any rice genotype. Alternate wetting and drying processes seem to facilitate root growth by accessing both adequate water and air. Further, application of FYM in SRI methods not only seems to improve the soil structure but also enhances the number and diversity of useful soil organisms in the field.

Yields obtained for IR 64 and Bajo Maap 2 varieties with SRI methods reported are higher than average yields obtained for IR 64 (6-8 t/ha) and 6-7 t/ha for Bajo Maap 2 at RC station (Ghimiray et al., 2008). The recent trial at Bajo using SRI method also showed an average yield of 8.56 t/ha for IR 64, an increase in yield by 15-20% compared to conventional practice in Bajo on station (Ghimire and Thinley, 2008). The yield for Khangma maap, although recommended for higher altitude, using SRI methods is comparable or slightly higher than the observed yield of 5.12 t/ha at the Khangma Research station (ibid). Study done by Lhendup et al. (2008) at Khangma research station reported higher yield for this variety using SRI methods. Yield increase for these varieties may be because of the favorable soil and climatic conditions. According to Anthofer (2004), highest yield for any variety can be expected under favorable environmental conditions with SRI methods. Such conditions are met where soil fertility is higher, rainfall is

sufficient and well distributed, and crop management is sufficiently good (ibid).

Additionally, with SRI methods there is a saving of inputs like seed, water, and fertilizer. Use of seed reduced by about 86% (considering average use of 22.5 kg seeds per acre for conventional methods against a maximum use of 3.0 kg seed per acre with SRI methods). This could be attributed to transplanting of young seedlings singly in wider spacing which reduces plants' competition for nutrients, water and sunlight. This result can be interpolated to reduction of cost for the purchase of about 20 kg of seeds per acre. Likewise there was no need to use inorganic fertilizer.

CONCLUSION

The results showed positive effect of SRI methods on the yield and yield-contributing parameters assessed in this study. The higher yield obtained for IR 64 and Bajo Maap 2 varieties with SRI methods compared to the conventional practice in RC Bajo indicates a significant impact of SRI methods on rice productivity. Even local variety like Nyabja seems to perform equally better using SRI methods. Further, seed use was reduced by about 86% compared to the conventional methods. Use of additional inputs like inorganic fertilizer was not necessary thereby saving cost. Thus there is a potential for subsistence farmers in the country to eventually achieve higher yields with reduced costs using SRI techniques. This will provide farmers with the opportunity to supplement their incomes through the sale of excess products. Hence, the adoption of SRI techniques by farmers could be encouraged to contribute to higher rice production and ensure household food security. However, further studies using SRI methods are required to understand the cost benefit analysis, effective crop establishment methods and straw yield.

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Morphological and molecular characterization of *Colletotrichum capsici*

Kinlay Tshering[1]

ABSTRACTS

The taxonomy and diversity of Colletotrichum capsici was validated by morphological characters (conidia shape & size, colony characteristics, appressoria size) and molecular analysis using ISSR (Intersimple sequence repeats) markers with the objectives to adopt ISSR technique to assess the level of genetic variation among C. capsici isolates, to determine if the genetic variation among isolates corresponded with variation in morphological traits, and to determine if differentiation among C. capsici isolates based on morphological or ISSR variation was related to the host from which the isolates were collected. Morphological characters were quite variable within the species. Molecular analysis revealed that there was a genetic diversity with Thai isolates from ***Capsicum*** *annuum being more similar than the other isolates from Queensland collected from other hosts like sugar apple, soy bean, etc. The phylogenetic groupings of C. capsici based on ISSR polymorphism did not appear to be congruent with morphological pattern and the rate of growth of isolates in culture. Further molecular analysis with a larger samples from a range of countries are required to determine if the clustering based on geographic origin and host plant is occurring. A high degree of molecular variability indicated that ISSR markers can be well utilized for the intraspecific characterization of C. capsici.*

KEYWORDS: *Colletotrichum capsici,* ISSR, Morphology, Molecular variability, *Capsicum annuum*

INTRODUCTION

Many problems exist in providing a workable taxonomy of species in the genus *Colletotrichum.* Traditional methods of identifying or defining species of *Colletotrichum* have relied primarily on host ranges and

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differences in morphological features such as colony colour, size and shape of conidia and appressoria, optimal temperature for growth, growth rate, presence or absence of setae, vegetative compatibility, benomyl sensitivity and existence of the teleomorph, *Glomerella* (Sutton 1992, Smith and Black 1990). However, due to environmental influences on the stability of morphological traits, the existence of intermediate forms, the similarities in morphology between some *Colletotricum* species, the overlapping of host ranges and the extensive variability shown in culture by some isolates, it has been very difficult to separate many *Colletotrichum* species by traditional taxonomical methods. In addition, the traditional methods have not been satisfactory for differentiating sub-species of *Colletotrichum*.

Defining the taxonomy of *C. capsici* has been difficult because *C. capsici* is one of five *Colletotrichum* species which causes anthracnose on chilli pepper and its morphological characteristics are very similar to some other *Colletotrichum* species like *C. dematium* and *C. truncatum*. Pring *et al.* (1995) mentioned that there was a number of *Colletotrichum* species which were genetically different from *C. capsici* but morphologically very similar. They include *C. circinans* (Sutton 1992), *C. truncatum* (Holiday 1980) and *C. dematium*. Furthermore, *C. capsici* has been reported from a wide range of different hosts, including cotton, peppers, tomatoes, legumes, weeds and flowers, where several other *Colletotrichum* species also exist. Thus, molecular techniques to study taxonomy may provide alternative methods and are important tools in solving the problems of species delimitation (MacLean *et al.,* 1993).

Studies of the diversity and molecular relationships among *Colletotrichum* species have employed a range of molecular techniques including, inter simple sequence repeats (ISSR), random amplified microsatellites (RAM), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and amplified restriction fragment length polymorphism (AFLP) fingerprinting; and sequencing of the rDNA ITS, D1/D2 regions and beta-tubulin gene (Sharma *et al.* 2005, Lu *et al.* 2004, O'Neill *et al.* 1997, Bernstein *et al.* 1995, Fabre *et al.* 1995). Lu *et al.* (2004) observed that ISSR and RAPD studies produced broadly similar dendrograms however, the ISSR-PCR dendrogram was somewhat more compact overall, with similarity values ranging between 44–90% as opposed to 18–85% for the RAPD tree.

ISSR analysis usually detected a higher level of polymorphism than that detected with RFLP or RAPD analyses (Godwin *et al.* 1997). Nagaoka and Ogihara (1997) also reported that the amount of polymorphism detected with ISSR analysis was greater than that with RAPD analysis and similar to that of RFLP markers. The characteristic features of ISSR markers, i.e. polymorphism, generation of information without the need for sequence data and ease of handling, was reported to be very attractive for its application to the evaluation and analysis of genetic diversity as well as to the construction of PCR-based genetic diversity maps of *Colletotrichum* species (Blair *et al*. 1999). Thus, ISSR is used in taxonomic studies in combination with morphological characterisation and assessment of host ranges with the following objectives: (i) to adopt the ISSR technique to assess the level of genetic variation among *Colletotrichum capsici* isolates, (ii) to determine if the genetic variation among isolates corresponded with variation in morphological traits, and (iii) to determine if differentiation among *C. capsici* isolates based on morphological or ISSR variation was related to the host from which the isolates were collected.

MATERIALS AND METHODS

Collection of isolates

C. capsici isolates were obtained from the Queensland Department of Primary Industries culture collection, the Thailand Department of Agriculture culture collection (DoAC) and the Kasetsart University, Thailand collection (Table 1). A *C. gloeosporioides* culture, isolated from chilli, from the Thai DoAC culture collection was included as an out group.

Table 1. Fourteen *Colletotrichum* isolates used in the study

Isolate no.	Species	Host	Source
BRIP 25478	*C. capsici*	Annona squamosa (Sugar apple)	DPI Queensland
BRIP 26974	*C. capsici*	*Capsicum frutescens* (Chilli peppers)	DPI Queensland
BRIP 28371	*C. capsici*	*Capsicum annuum* (Chilli peppers)	DPI Queensland
BRIP 25584	*C. capsici*	*Manettia luteo-rubra* (Cinchonineae, Rubiaceae)	DPI Queensland
BRIP 4739	*C. capsici*	*Glycine max* (Soybeans)	DPI Queensland
F1-2E	*C. capsici*	*Capsicum annuum* (Chilli peppers)	Doncha-em, W. Thailand
F2-1E	*C. capsici*	*Capsicum annuum* (Chilli peppers)	Ratchaburi, W. Thailand
F3-1A	*C. capsici*	*Capsicum annuum* (Chilli peppers)	Kanchanaburi, W. Thailand
F4-2C	*C. capsici*	*Capsicum annuum* (Chilli peppers)	Kanchanaburi, W. Thailand
F5-5A	*C. capsici*	*Capsicum annuum* (Chilli peppers)	Kanchanaburi, W. Thailand
F6-5A	*C. capsici*	*Capsicum annuum* (Chilli peppers)	Suphanburi, W. Thailand
F8-3B	*C. capsici*	*Capsicum annuum* (Chilli peppers)	Suphanburi, W. Thailand
DoAC 1511	*C. capsici*	*Capsicum annuum* (Chilli peppers)	DoAC, Thailand
DoAC 1508	*C. gloeosp-orioides.*	*Capsicum annuum* (Chilli peppers)	DoAC, Thailand

Single spore isolation and maintenance of cultures

Mono-conidial cultures were obtained by single spore isolation. Potato dextrose agar (PDA) was inoculated with mycelial discs (5 mm) from the initial inoculum vials. The inoculated plates were incubated for 7 days at 28^{o}C under a white fluorescent 12 hour light/dark cycle after which time the spores were harvested in wet condition. Ten ml of sterile distilled water was added to the surface of the cultures, then they were gently scraped and agitated using a bent glass rod, then the spore suspension was filtered through four-folds of cheesecloth to remove mycelium or other debris. One ml of each spore suspension was spread on the surface of water agar and the plates were incubated at a 45^{o} angle at room temperature (25^{o}C). After 24 hours single spores were picked out with the aid of a dissecting microscope and they were transferred to PDA (one spore per plate). The single spore cultures were incubated at 28^{o}C for 7 days and the colony morphology was compared with the initial cultures. All subsequent sub-culturing was made from the single spore cultures.

Morphological examination

i) Colony characteristics - PDA was inoculated with mycelial discs (5 mm diameter) taken from the growing edge of pure cultures. Cultures were grown in 12 hour light/dark cycle at 28^{o}C. Colony diameter was recorded daily (12 replicates, two measurement per replicate) for 1 week. The radial growth of each isolate was determined by measuring the diameter of each isolate after 7 days growth and the growth rate was calculated as the 7-day average of mean daily growth (mm per day). Colony color was determined by examining cultures against a white background after 7 days growth and at the same time, the colony edge and presence/absence of acervuli and setae were noted.

ii) Appressoria characteristics - Appressoria were studied using a slide culture technique modified from Hawksworth (1974). Slide cultures were made of each isolate by placing a drop of cooled, molten water agar (23 g of agar per litre) on a sterilized slide and transferring a mycelial tip or a drop of conidial suspension harvested from 14-day-old mono-conidial cultures onto the agar. A sterile coverslip was placed over the seeded water agar, and the slide culture was held in a 9 cm diameter Petri dish that served as a moisture chamber for 6-8 days. The slide cultures were incubated at room temperature (25^{o}C) and the recording of appressoria size and shape took place after 24 hours on a daily basis for up to 8 days.

iii) Conidia characteristics - A mono-conidial culture of each isolate was grown on PDA for 14 days under 12 hours light/dark cycle at 25^{o}C. A conidial suspension was prepared in sterile distilled water, and the shape of conidia of each isolate was determined by examining 100 randomly chosen conidia. The spores were stained with lacto phenol cotton blue before measuring spore size. Conidial size of each isolate was determined by measuring the length and width of 40 randomly chosen conidia from each isolate. The size and shape of the conidia was determined under the compound microscope using 400x magnification. The study was conducted twice.

Molecular examination

i) Genomic DNA isolation - Total genomic DNA of the 14 *Colletotrichum* isolates was extracted from mycelia using the DNeasy Extraction kit (Qiagen Inc., USA). To increase mycelium density, cultures were grown in 50 ml volumes of Czapek-Dox liquid medium

(Oxoid Ltd., England) in 250 ml flasks and incubated at 25^{o}C for 7 days in 12 hour light/dark cycle. Mycelial mats were removed after centrifuging the cultures at 5000 rpm for 5 minutes. DNA was quantified by comparing against 5 µL of *Eco*RI *Hind*III lambda marker (Promega Inc., USA) and the DNA was stored at –20^{o}C.

ii) PCR amplification of DNA - Inter-simple sequence repeat (ISSR) analysis was used to examine DNA polymorphism among the 14 *Colletotrichum* isolates. A single primer was used in each ISSR-polymerase chain reaction (PCR) to amplify the sequence between two simple sequence repeats (SSRs). Seventy four random primers from the University of British Columbia (UBC) primer set #9 were screened on isolates F5-5A and BRIP 26974 to select primers exhibiting maximum polymorphism. The 3'-anchored primers contained a single dinucleotide motif, either GA, AG, AT, AC, CA, TG or GT repeated eight times, with one selective nucleotide (A, C, T or G) following the repeat motif. For anchoring to the 5' border of the simple sequence repeat, a tail consisting of the bases YT, YC, YG, YA, was incorporated into the design of the primers.

PCR amplification was carried out using a 12.5 µl sample which consisted of PCR buffer, 2 mM $MgCl_2$, 0.24 mM each of dATP, dTTP, dGTP & dCTP, 0.6 U *Taq* DNA polymerase (Scientifix, Cheltenham, Victoria, Australia), 0.3 µM of primer (Biotechnology Laboratory of University of British Columbia (UBC), Vancouver, Canada) and 20 ng of genomic DNA. PCR amplifications were performed in a DNA Engine DYAD thermal cycler (MJ Research, Inc, MA, USA) programmed as follows: initial denaturation step at 94^{o}C for 2 minutes, followed by 39 cycles of 94^{o}C for 1 minute, 55.5^{o}C for 1 minute and 72^{o}C for 4 minutes, and a final extension step of 72^{o}C for 7 minutes. The final ISSR amplification products were separated by electrophoresis in 1.4% agarose gels (Scientifix, Cheltenham, Victoria, Australia) in Tris-Acetate-EDTA (TAE) electrophoresis buffer, stained with ethidium bromide and viewed with a UV transilluminator. The molecular weight marker 1KB Plus ladder (Scientifix, Cheltenham, Victoria, Australia) was included in every gel.

iii) Data Analysis - Morphological data was analysed with analysis of variance (ANOVA) using Tukey's pairwise comparison test ($P<0.05$)

(MINITAB release 14, Minitab Inc., Boston, MA, USA). Molecular data was analysed using the NTSYSpc program (Rohlf 2000). Each ISSR band of a particular molecular weight was scored for presence (1) or absence (0) in each isolate. A similarity matrix was generated from the binary data using Jaccard similarities coefficient in SIMQUAL program of NTSYSpc Package. Cluster analysis was done with the unweighted pair group arithmetic mean method (UPGMA) in the SAHN program of NTSYSpc package and the dendrogram with best fit to similarity matrix was generated. Bootstrap analysis of the data was also used to examine phylogenetic relationships (Felsenstein 1985). Bootstrap analysis of the data was performed using 100 resamples of the data using PHYLIP 3.5 (Phylogeny Inference Package, http://evolution.genetics.washington.edu.au/phylip.html).

RESULTS AND DISCUSSION

Results

Colony characteristics

Colony shape, color and growth rate varied considerably among the *C. capsici* isolates (Table 2). Most of the isolates did not resemble each other for every character although some isolates showed similarity in either colony growth or colour or acervuli formation. Based on the similarities among the isolates they could be grouped into six morphological types as follows: Type 1 (F1-2E and F3-1A) had a dark brown flat culture without any aerial mycelium, produced lots of acervuli with setae and also produced lots of salmon-coloured spore pustules (Table 2) which were not present in other isolates. Type 2 (BRIP 4739, BRIP 28371 and BRIP 25478) had a greyish culture with white aerial mycelium. They produced very little acervuli and thus less sporulation compared to other isolates. Type 3 (F5-5A, F6-5A and F8-3B) had a brown culture and produced zig zag colonies with numerous acervuli with setae. Type 4 (BRIP 26974 and the *C. capsici* reference isolate 1511) had a grey culture and possessed circular colonies with distinct zonation and lots of acervuli. Type 5 (BRIP 25584) had a black culture with lots of acervuli and setae. Type 6 (F2-1E and F4-2C) had a brown culture with very few acervuli. The out group *C. gloeosporioides* had dense, cottony, white mycelium with a few orange conidial masses near the inoculum point.

There was a statistically significant difference in colony growth rate among the 14 isolates. The out group *C. gloeosporioides* grew significantly faster than all the *C. capsici* isolates. Among the *C. capsici* isolates, BRIP 28371 grew the fastest followed by the reference isolate 1511. Isolate F5-5A grew the slowest. There was no correspondence between the morphology type and the growth rate of the isolates. The colony diameter of different isolates ranged between 4.12 cm and 6.73 cm after 7 days incubation.

Appressoria characteristics

All the *C. capsici* isolates produced appressoria although the appressoria formation by isolates varied from 24 to 72 hours after inoculation (Table 2). Based on the time taken to appressoria formation, the isolates could be separated into three categories (Category 1 consists of 5 isolates which produce appressoria within 24 hours, Category 2 consists of 5 isolates which produce appressoria within 48 hours and Category 3 which consists of 4 isolates which produce appressoria within 72 hours). Isolates which produced appressoria within 24 hours tended to have shorter germ tubes (visual observations). There was a significant difference in appressoria length and width. The longest appressoria were produced by BRIP 4739 and the broadest appressoria were produced by *C. gloeosporioides* (1508) followed by F4-2C. There was no strong correlation between the length of the appressoria formed and the duration it took to produce appressoria ($r = 0.56$) and between the width of the appressoria and the duration it took to produce appressoria ($r = 0.50$).

Conidia characteristics

Conidia of all the isolates had the characteristic shape of *C. capsici* i.e. falcate with acute apex and truncate base, but the sizes of conidia were significantly different. Average length and width of conidia varied between 12.9 – 23.0 μm and 2.2 – 3.7 μm, respectively. The conidia length of all the *C. capsici* isolates fell within the range of CMI Descriptions i.e. 16 - 30 μm, except for BRIP 25478, which had shorter conidia (12.9 μm). The conidia width of most of the isolates fell within the range of CMI Descriptions i.e. 2.5 - 4.0 μm. The exceptions were 1511, F6-5A, F3-1A which had slightly thinner conidia.

Table 2. Morphological characteristics of 13 isolates of *C. capsici* and 1 *C. gloeosporioides* on Potato Dextrose Agar

Isolate No.	Diameter (cm)	Growth rate (cm/day)	Ventral Color	Reverse Color	Acervuli & Setae	Appressoria length & width (µm), duration*	Conidia length & width (µm)	Conidia
BRIP 25478	6.18 c	0.88 b	Greyish white	Greyish white	Few	7.8 c / 4.3 f (48 hrs)	12.9 h / 2.7 c	Less sporulation
BRIP 26974	6.18 c	0.88 b	Grey	Grey	Lots of acervuli	7.7 c / 5.2 ef (24 hrs)	20.8 def / 2.6 cd	Lots of buff coloured conidia
BRIP 28371	6.73 b	0.96 b	Greyish brown	Greyish brown	Few acervuli	8.8 c / 6.4 bcd (24 hrs)	19.8 f / 3.7 a	Less sporulation
BRIP 25584	4.52 f	0.65 c	Black	Black	Many	7.9 c / 5.7 de (24 hrs)	22.5 ab / 2.6 cd	Lots of buff coloured conidia
BRIP 4739	4.99 e	0.71 c	Greyish yellow	Yellow	Few	14.3 a / 6.9 bcd (72 hrs)	23.0 a / 2.7 c	Less sporulation
Thai F1-2E	5.57 d	0.80 b	Dark brown	Greyish black	Many	8.5 c / 6.8 bcd (24 hrs)	20.1 ef / 2.5 de	Lots of salmon-coloured conidia
Thai F2-1E	5.07 e	0.72 c	Brown	Brown	Some, Few setae	11.2 b / 6.3 bcde (24 hrs)	21.3 cd / 2.5 de	Light pink coloured conidia
Thai F3-1A	4.26 gh	0.61 c	Dark brown	Greyish black	Many	11.3 b / 6.7 bcd (72 hrs)	17.2 g / 2.4 e	Lots of salmon-coloured conidia
Thai F4-2C	4.30 g	0.61 c	Brown	Brown	Some, No setae	12.8 ab / 7.3 b (48 hrs)	21.8 bc/ 2.6 cd	
Thai F5-5A	4.12 h	0.59 d	Brown	Greyish brown	Many	8.9 c / 5.9 cde (72 hrs)	22.1abc / 2.6 cd	Lots of buff-coloured conidia

Thai F6-5A	5.11 e	0.73 c	Brown	Brown	Few	8.2 c / 6.3 bcde (48 hrs)	21.9 bc/ 2.4 e	Lots of buff-coloured conidia
Thai F8-3B	5.49 d	0.78 c	Brown	Brown	Many	8.4 c / 6.4 bcd (48 hrs)	22.5 ab / 2.5de	Lots of buff-coloured conidia
1511	6.23 c	0.89 b	Grey	Grey	Many	11.1 b / 6.6 bcd (48 hrs)	21.9 bc/ 2.2 f	Lots of buff-coloured conidia
1508	8.30 a	1.19 a	Greyish white	Greyish white	No acervuli & setae	11.9 b / 9.4 a (72 hrs)	11.8 i / 3.2 b	
p-value	<0.001	0.001				<0.001	<0.001	
Lsd	0.18	0.18				1.8 µm / 1.2 µm	1.03 µm / 0.18 µm	

Molecular characteristics

Of the seventy four primers initially used for amplification, 40 primers successfully amplified the loci but good amplification and clear banding profiles were obtained from only 16 primers producing 5-19 bands consistently, with an average of 11 bands per primer. Therefore, sixteen primers (Appendix 1), which produced distinct and consistent banding patterns, were used to amplify the DNA of the 13 mono-conidial isolates of *C. capsici* and 1 isolate of *C. gloeosporioides*. Number of bands per primer ranged between 5 (UBC810) and 19 (UBC826) (Figure 3.4-B) with an average of 11 bands per primer. UBC primers 826 and 850 showed the maximum polymorphisms. In total 174 ISSR bands were scored for presence or absence among the isolates. Information on banding pattern for all the primers was used to determine genetic distance between isolates and to construct a dendrogram.

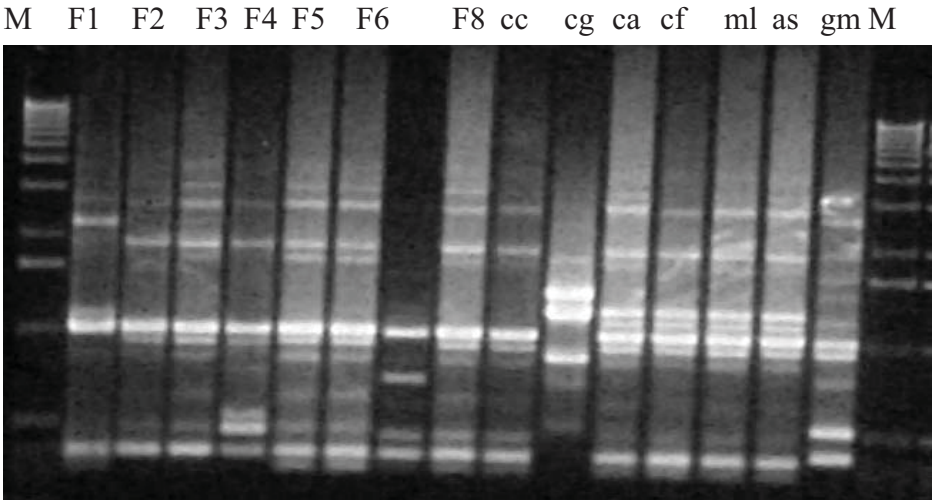

Figure 1 Inter simple sequence repeats polymorphic DNA profiles of 14 *Colletotrichum* isolates generated using PCR with primer UBC 826 (B); the lanes label M on the left and right are the 1KB Plus ladder, lanes F1-F8 (*C. capsici* isolates from Thailand, lane cc (*C. capsici* referral isolate from Thailand), lane cg (outgroup), lane ca (*C. capsici* from *C. annuum,* Queensalnd), lane cf (*C. capsici* from *C. frutescens*, Queensland), lane ml (*C. capsici* from *Manettia luteo-rubra*, Queensland), lane as (*C. capsici* from *Annona squamosa*, Queensland) and lane gm (*C. capsici* from *Glycine max*, Queensland).

UPGMA cluster analysis of the ISSR data using the Jaccard's similarity coefficient resulted in a dendrogram that distinguished 2 clusters that encompassed most of the isolates (Figure 5). Cluster 1 consisted of seven of the eight Thai isolates and cluster 2 consisted of four of the five Queensland isolates. The two isolates that were not associated with either cluster were F4-2C and BRIP 4739. Within cluster 1, F5-5A and F6-5A appeared to be the most similar with genetic similarity of 0.86 and in cluster 2, BRIP 28371 and BRIP 25584 appeared to be most similar with genetic similarity of 0.87 (Table 3).

The dendrogram derived from the bootstrap analysis of 100 replicas of the ISSR data set showed similar groupings of the isolates to the UPGMA dendrogram (Figure 2). The values at the nodes represent the number of times out of 100 that the species beyond that node clustered together. Higher values indicate greater confidence in the clustering of isolates. 1508 (*C. gloeosporioides*) was selected as an out group.

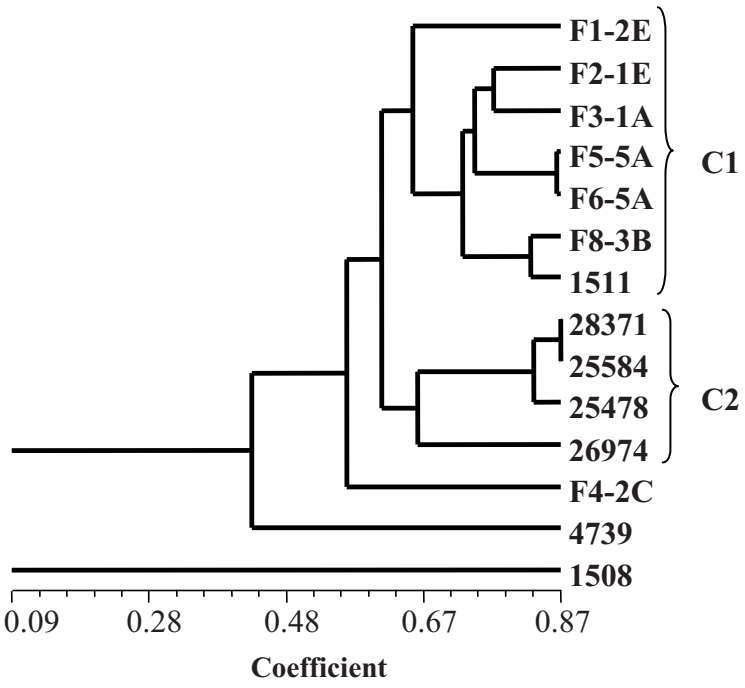

Figure 3.5. Dendrogram derived from a UPGMA cluster analysis using the Jaccard's similarity coefficient based on 174 polymorphic ISSR bands showing the associations among 14 *Colletotrichum* isolates.

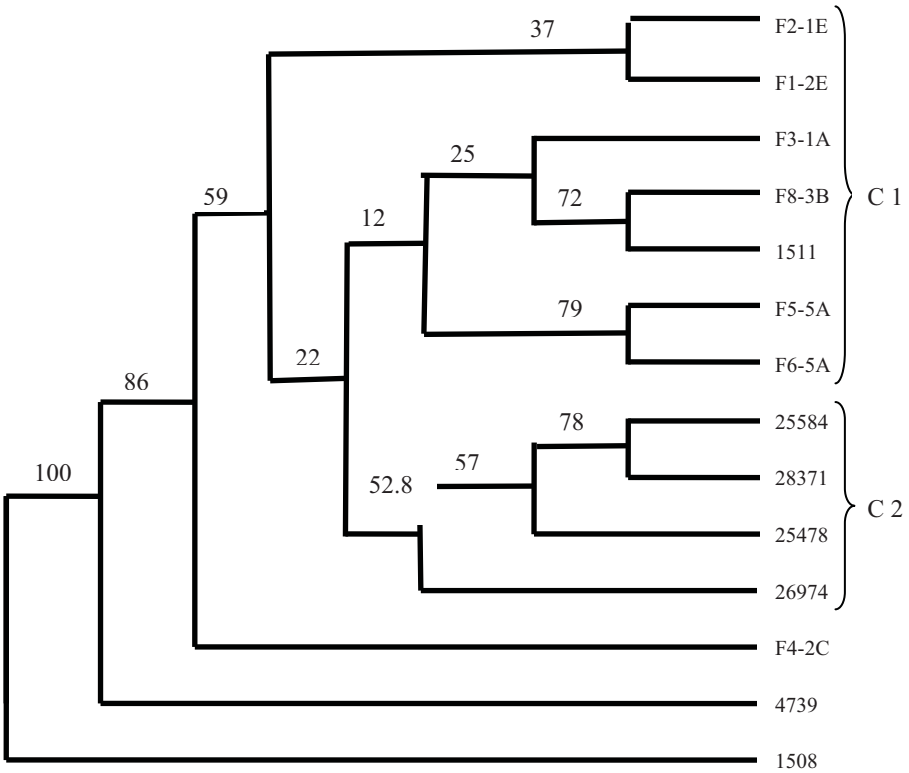

Figure 2 Dendrogram showing the genetic relationships between *Colletotrichum capsici* isolates and *Colletotrichum gloeosporioides*, produced from the bootstrap analysis. Bootstrap confidence levels, based on 100 resamples are given on the appropriate branches.

Table 3 Average genetic similarity of *Colletotrichum capsici* isolates, calculated using Jaccard's coefficient of genetic similarity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. F1-2E	1.00													
2. F2-1E	0.71	1.00												
3. F3-1A	0.68	0.77	1.00											
4. F4-2C	0.54	0.66	0.58	1.00										
5. F5-5A	0.67	0.75	0.75	0.56	1.00									
6. F6-5A	0.66	0.72	0.76	0.55	0.86	1.00								
7. F8-3B	0.63	0.69	0.80	0.57	0.71	0.73	1.00							
8. 1511	0.60	0.75	0.73	0.60	0.71	0.72	0.83	1.00						
9. 1508	0.08	0.10	0.08	0.09	0.11	0.08	0.09	0.08	1.00					
10. 28371	0.56	0.66	0.65	0.56	0.62	0.59	0.64	0.66	0.10	1.00				
11. 26974	0.55	0.61	0.59	0.53	0.59	0.60	0.59	0.61	0.09	0.67	1.00			
12. 25478	0.59	0.65	0.67	0.53	0.62	0.61	0.64	0.65	0.09	0.84	0.68	1.00		
13. 25584	0.54	0.62	0.65	0.52	0.58	0.56	0.62	0.64	0.08	0.87	0.65	0.82	1.00	
14. 4739	0.45	0.43	0.43	0.45	0.42	0.39	0.43	0.42	0.09	0.43	0.44	0.43	0.42	1.00

Table 4 Phylogenic groupings of *Colletotrichum capsici* isolates based on morphology and molecular characteristics

Morphological Types		Colony growth rate groupings		Molecular similarity groupings	
1	F1-2E, F3-1A	1	1511, F1-2E, BRIP 28371, BRIP 26974, BRIP 25478	1	F1-2E, F2-1E, F3-1A, F5-5A, F6-5A, F8-3B & 1511
2	BRIP 4739, BRIP 28371, BRIP 25478	2	F8-3B, F6-5A, F2-1E, F3-1A, F4-2C, BRIP 25584, BRIP 4739	2	BRIP 28371, BRIP 26974, BRIP 25478 & BRIP 25584
3	F5-5A, F6-5A, F8-3B	3	F5-5A	3	F4-2C
4	BRIP 26974, DoAC 1511	4		4	BRIP 4739
5	BRIP 25584				
6	F2-1E, F4-2C				

Discussion

The morphology and molecular analysis showed that the *Colletotrichum capsici* isolates from Thailand and Queensland were quite variable. There was significant variation within the species at both morphological and molecular level. The phylogenetic groupings of *C. capsici* based on ISSR polymorphism did not appear to be congruent with morphological pattern and the rate of growth of isolates in culture. Such morphological and cultural variation in *C. capsici* has also been observed by other researchers including Sharma *et al.* (2004) in chilli-*C. capsici* combination and Pring *et al.* (1995). Sharma *et al.* (2004) mentioned huge morphological, pathogenic and molecular variation without any congruency with each other among *C. capsici* isolates collected from North-western India. Pring *et al.,* (1995) reported that although the conidia of *C. capsici* isolates were of similar shape, being falcate with an acute apex and a truncate base, the sizes of conidia were significantly different from one another.

However, this varies from reports for other *Colletotrichum* species. For example, Photita *et al.* (2005) investigated the diversity of the genus *Colletotrichum* isolated from tropical plants in Thailand and found that there was a good correlation between groupings based on sequence data of rDNA internal transcribed spacer regions (ITS1 and ITS 2) and morphological characters. O'Neill *et al.* (1997) reported that the diversity detected by AFLP among and within *Colletotrichum* species from alfalfa and other crops corroborated their published taxonomy based on morphology, ribosomal DNA sequence, and random amplified polymorphic DNA analysis. Lu *et al.* (2004) reported that the major clusters formed from UPGMA analysis of both ISSR-PCR and RAPDs of *Colletotrichum* species corresponded broadly to the morphological species aggregates. However, none of these studies included *C. capsici* in their studies and the isolates that they have used are *Colletotrichum* with several species.

Molecular analysis of the 13 *C. capsici* isolates resulted in a dendrogram that showed two main clusters based on the geographical origins of the isolates. One cluster consisted of the Thai isolates and the other consisted of isolates from Queensland, which indicated some associations between the genetic diversity of the isolates and their

country of origin. However, two isolates, one from each region did not cluster with any group. The dendrogram also showed that the isolates obtained from *Capsicum* did not cluster separately from *C. capsici* isolates from other host (*Manettia luteo-rubra, Annona squamosa)*. Thus, the results indicated that further analysis with a larger sample of *C. capsici* isolates from a range of countries is required to determine if the clustering based on geographic origin is occurring and to determine if clustering based on host plant occurs.

The large variation between the isolates in terms of morphology as well as ISSR banding pattern may not be due to sexual reproduction as the sexual stage in the life cycle of *Colletotrichum capsici* has not been reported. The possible mechanism by which genetic diversity may be generated is through vegetative compatibility, which is the ability of individual fungal strains to undergo mutual hyphal anastomosis, resulting in viable fused cells containing nuclei of both parental strains in a common cytoplasm (Katan 2000). Since reproduction in many *Colletotrichum* populations is mainly or exclusively vegetative, the only means of exchanging genetic material between two strains would be anastomosis and heterokaryosis (Chacko *et al.* 1994). These processes occur between some *Colletotrichum* isolates and it could also have occurred in *C. capsici* isolates. Isolates that can anastomose with one another and form viable heterokaryons are placed in the same vegetative-compatibility group (VCG) to indicate this fact. They may potentially share a common gene pool, and are isolated from other strains or VCGs within the species by the incompatibility mechanism (Katan 2000). Highly variable populations are more adaptable to changing conditions than are those with little variation and sexual reproduction and recombination is a major factor that contributes to the genetic variability observed in fungal populations (Ciser *et al*. 1994).

DNA profiles based on ISSR markers have revealed potential diagnostic fingerprints for the isolates and these polymorphic bands can be potential candidates as novel markers for use in linkage-map construction in *Colletotrichum*.

CONCLUSIONS

There was significant variation within the *Colletotrichum capsici* species at both morphological and molecular level. There was no congruency between the phylogenetic groupings and the morphological pattern. However, based on molecular analysis, there were two main clusters based on the geographical origins of the isolates, one cluster consisted of the Thai isolates and the other consisted of isolates from Queensland. The isolates obtained from *Capsicum* did not cluster separately from *C. capsici* isolates from other host (*Manettia luteo-rubra, Annona squamosa)*.

A high degree of molecular variability among the *C. capsici* isolates when evaluated by ISSR analysis indicated that ISSR markers can be well utilized for the intraspecific characterization of *C. capsici*. The ISSR technique complemented the morphological analysis and the variance between the results of both techniques indicated that a combined approach should be used for taxonomic identification and determination of genetic diversity of *Colletotrichum capsici* populations. Future research should attempt to include as many isolates as possible. Attempts should be made to collect *C. capsici* from all the host plants that are known to be infected by *C. capsici* and also to collect from different regions in order to determine if the clustering based on geographic origin is occurring and to determine if clustering based on host plant occurs. In addition, pathogenicity testing of the isolates on different host plants is required. Therefore, it may also be concluded that ISSR techniques be employed to look into the similarities and differences between *Colletotrichum* isolates.

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Effect of harvesting time on grain quality parameters of rice

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ABSTRACT

Delayed harvesting of rice crop can result in high shattering losses in the field and the quality of milled rice will be affected with a high percentage of broken grains. Trials conducted to confirm the most appropriate time of harvesting at Wengkhar in 2006 and 2007 season using the mid altitude rice variety Wengkhar Ray Kaap indicated that harvesting 45 days after flowering upto 60 days is most appropriate for mid altitude areas. The percentage of green grains significantly decreased as the harvesting was delayed. The percentage of green grains was more than 10% until 50 days after flowering, but after 55 days to 65 days after flowering it reduced to around 5%, with the lowest of 1.8% recorded at 70 days after the flowering. Different harvesting time also had significant effect on the percentage of cracked or broken grains. The percentage of cracked grains at 40 days after flowering was more than 20%, but from 45 to 55 days after flowering it reduced to about 15%. However, it again started to increase from 60 days after flowering. There was no significant effect of time of harvesting on 1000 grain weight. Taking into consideration all the parameters such as moisture content, percentage of filled grains, green grains, cracked or broken grains and 100 grain weight , harvesting 45 days after flowering upto 60 days after flowering seems appropriate in mid altitude areas. Further investigations on harvesting time for different varieties at different elevations using larger sample sizes and higher capacity milling machines are suggested.

KEYWORDS : harvesting time, flowering days, moisture content, filled grains, green grains, 1000 grain weight

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INTRODUCTION

Rice crop is considered to be physiologically matured when at least 85% of the upper portions of the panicles turn into straw colour. However, Bhutanese farmers normally judge the time of harvest when the colour of the straw turns yellow, drooping of panicles, shattering of grains, crackling sound of grains upon biting and the start of the 9th month of the lunar calendar (Duba *et. al*, 1995). When this is estimated in terms of number of days it comes to about 60 days from the date of flowering. Apparently, farmers seem to harvest their crop quite late after it has attained the physiological maturity resulting in high shattering losses in the field, higher percentage of cracked grains while milling. It is very important to harvest the crop at the right time in order to avoid crop losses mainly from damage by rodents, birds, insects, lodging and shattering (Datta, 1981). So far very little information is available on the time of harvesting and the effect on rice quality. Trials conducted in 2006 season showed that harvesting 40 to 50 days from flowering was suitable. In order to recommend a more precise time, harvesting times were further narrowed down from 40 days to 70 days after flowering at every five days interval.

MATERIALS AND METHODS

The trial was initiated in 2006 at RNRRC Wengkhar using white rice variety Wengkhar Ray Kaap (Khumal 6). Nurseries for the trial in both the years were established using semi-dry bed method in May and transplanting took place in June. The exact dates for different operations are summarized in Table 1. Seedlings in both the years were transplanted in lines drawn with the help of a marker. Marker is a simple device with wooden wheels that can be dragged on puddled field to mark lines on which seedlings can be transplanted. It can make two pairs of lines with a line to line spacing of 21 cm and pair to pair distance of 36 cm. Three seedlings were transplanted in each hill. A basal application of 35:40:20 kg NPK per ha was done at the time of transplanting. This was followed by two top dressings, first with 20 kg N/ha followed by second with 10 kg N/ha. For weed control, Butachlor 5% G was applied four days after transplanting. Two sprays of Chloropyriphos 20% EC were given in July and August to control Leaf roller (*Cnaphalocrosis medinalis*).

When 50% heading was observed in a plot, this was taken as the date of flowering. Although the days to flowering is scored by considering 50% heading from the whole plot, usually there is some variation in heading within the plots. To avoid any bias, we divided the large plots into three sub-plots depending on the heading stage. The three sub-plots included those plants heading early, medium and late. For samples, we harvested one line of 1.5 m length from each sub-plot on the desired dates. In 2007, based on experiences gained from 2006, we divided the large plot into three different blocks depending on uniformity in flowering. The first block comprised of early flowering plants, the second block plants with medium flowering and maturity and the third block consisted of plants which flowered and matured late. From these three blocks, we harvested 10 hills each making a total of 30 hills at designated harvesting dates. The different harvesting treatments for both the years are presented in Table 2. In both the years, sample plots and plants were manually threshed in a plastic sheet and cleaned. Moisture content was measured and standardized at 14%.

Table 1: Dates of seeding, transplanting, topdressing and 50% heading in 2006-07.

No	Categories	2006	2007
1	Nursery date	22nd May	21st May
2	Date of transplanting	29th June	23rd June
3	First top dressing	9th August	9th August
4	Second top dressing	9th September	9th September
5	Days to 50% heading	29th September	19th September

Table 2: Different harvesting treatments in 2006 and 2007

Treatments	Number of days after flowering	
	2006	2007
First harvest	20	45
Second harvest	30	50
Third harvest	40	55
Fourth harvest	50	60
Fifth harvest	60	65
Sixth	-	70

For estimating the percentage of filled and unfilled grains, about 50 gm sample replicated three times was taken from each treatment. These samples were dipped in water. The grains that floated were counted as unfilled grains, while those that sank were counted as filled grains. From the filled grains, 1000 grain weight was estimated by adjusting the moisture content at 14%.

For estimating milling recovery and grain quality, 50 gm sample of filled grains, replicated three times which will have approximately 7000 grains, was taken from each treatment. The samples were dried and their moisture content at the time of husking ranged from 14.2 to 14.5. The samples were husked using a manual husker. The husked samples were cleaned and separated into cracked grain and whole grain. From the whole grain, green grains were separated to estimate the ratio of green grains. The whole grain recovery in percentage was calculated from the weight of whole and cracked grains. The main objective of this trial was to assess the optimum time of harvest and not to compare the different types of milling machines and therefore only data of brown rice was used.

In 2007, we also used an electrically operated husking machine to mill the samples. We then compared the quality of brown rice obtained from manually and electrically operated husking machines.

RESULTS AND DISCUSSION

As anticipated, percent moisture content of the grains decreased as the harvesting was delayed. The percentage of filled grains increased up to 50 days from flowering and it started to decrease at 60 days after flowering.

The percentage of green grains drastically decreased from about 71% to 7% when the crop was harvested 20 days after flowering as compared to harvesting 40 days after flowering. As the harvesting was delayed the percentage of green grain continued to decrease and at 60 days after flowering no green grains were observed (Table 3).

Table 3: Effect of harvesting time of Wengkhar Ray Kaap on moisture content, filling percentage and 1000 grain weight, 2006.

Harvesting Days	Moisture content (%) at Harvest	Filled grain (%)	Green grain (%)	Cracked grain (%)	1000 grain weight (gm)
20 days after Flowering	30.3	36.2	70.7	30.8	22.5
30 days after Flowering	25.4	63.4	30.0	22.0	23.4
40 days after Flowering	26.9	71.1	6.9	11.3	23.7
50 days after Flowering	22.8	72.7	1.5	18.5	23.9
60 days after Flowering	20.9	69.6	0.0	19.3	23.2

Harvesting 50 days after flowering appears to be optimum to avoid high percentage of green grains (Figure 1). The percentage of cracked grain gradually decreased up to 40 days after the flowering, but thereafter it increased again. The increase in the percentage of cracked grains could possibly be attributed to over drying of the grains and a concomitant reduction in moisture content. This indicates that harvesting 40 days after flowering minimizes grain cracking.

When all the parameters are compared together, 40 days from flowering appeared to be the most suitable time to harvest as the percentage of filled grains is high while the percentage of green grain and cracked grains is low as compared to other treatments (Figure 1). The 1000 grain weight increased constantly from 20 days to 50 days but after 50 days it started to decrease (Figure 2).

Figure 1: Effect of time of harvesting on different parameters of rice variety Wengkhar Ray Kaap, 2006.

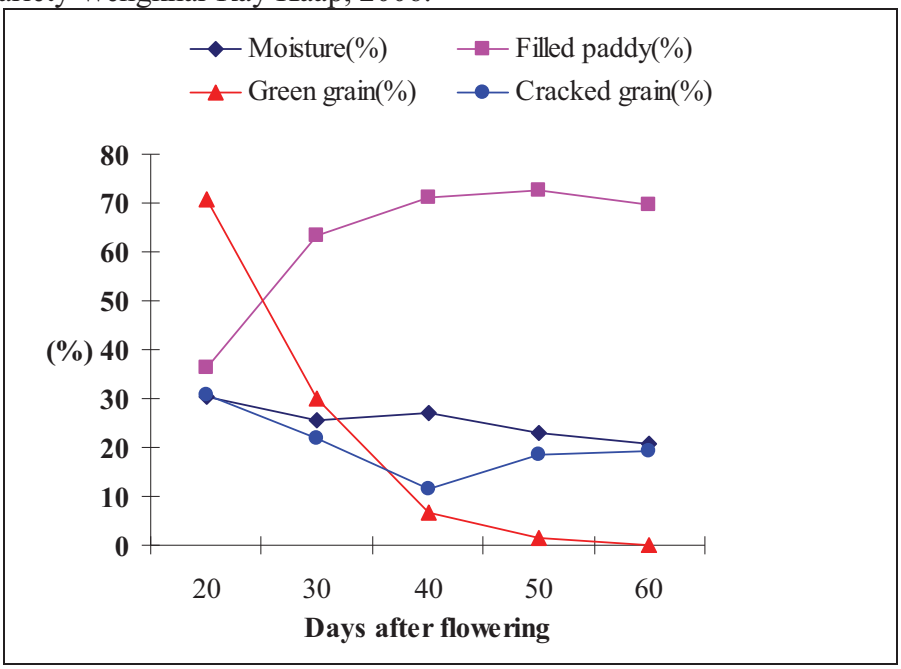
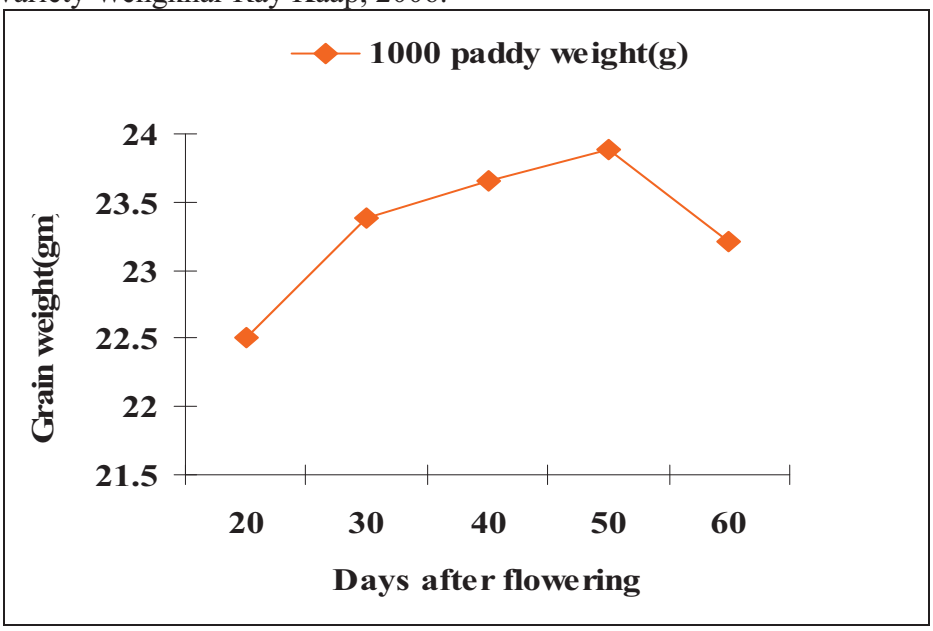


Figure 2: Effect of time of harvesting on 1000 grain weight of rice variety Wengkhar Ray Kaap, 2006.

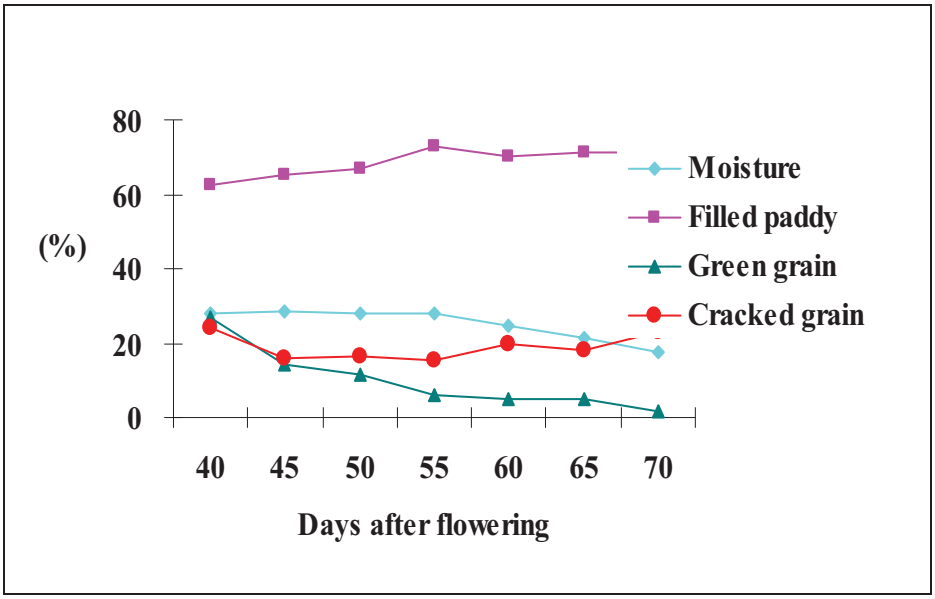


In order to recommend a more precise time for harvesting, harvesting treatments were further narrowed from 40 days to 70 days after flowering at a five-day interval in 2007 season. The changes in the moisture content and the percentage of filled grain as affected by the harvesting time are shown in Figure 3 and Table 4.

Table 4: The effect of harvesting time on different parameters of rice variety Wengkhar Ray Kaap

Harvesting Days	Moisture % at harvest	% Filled paddy	% Green grains	% Cracked grain	1000 grain weight(g)
40	27.8	62.33	20.98	24.24	24.02
45	28.7	65.33	14.22	15.87	24.27
50	28.0	67.00	9.29	16.75	24.19
55	28.2	72.67	4.68	15.53	24.52
60	24.9	70.33	3.96	19.83	24.77
65	21.2	71.33	3.82	17.86	24.04
70	17.4	71.00	1.48	22.83	24.59
F pr	**-**	**0.003**	**<.001**	**0.010**	**0.346**
LSD	**-**	**4.617**	**4.531**	**4.971**	**0.785**
CV (%)	**-**	**4**	**30**	**15**	**-**

Figure 3: The effect of time of harvesting on different parameters of rice variety Wengkhar Ray Kaap (2007)



The results indicated a significant effect of harvesting time on percentage of filled grains and green grains (Table 4) but there was no significant effect of the different harvesting time on moisture content, percentage of cracked grains and 1000 grain weight. The moisture content followed a similar trend as in 2006 but there was no significant difference among the treatments. The decrease in moisture content from 40 days to 55 days after flowering was insignificant, however, we recorded notable decrease in the moisture 60 days after flowering with the lowest moisture content of 17.4% recorded 70 days after flowering (Table 4). It is suitable to harvest rice when the field moisture content is in the range of 22 to 26% (Tanaka, T., personal communication, 2008). Looking at the decrease in the moisture content alone 60 days after flowering appears to be the optimum time for harvesting, but other quality parameters will be adversely affected due to delayed harvesting.

The time of harvesting had significant effect on the percentage of filled grains. Maximum percentage of filled grains was recorded at 55 days after flowering (Table 3). After 55 days from flowering, however, the difference in the percentage of filled grains was small, even upto 70 days after flowering.

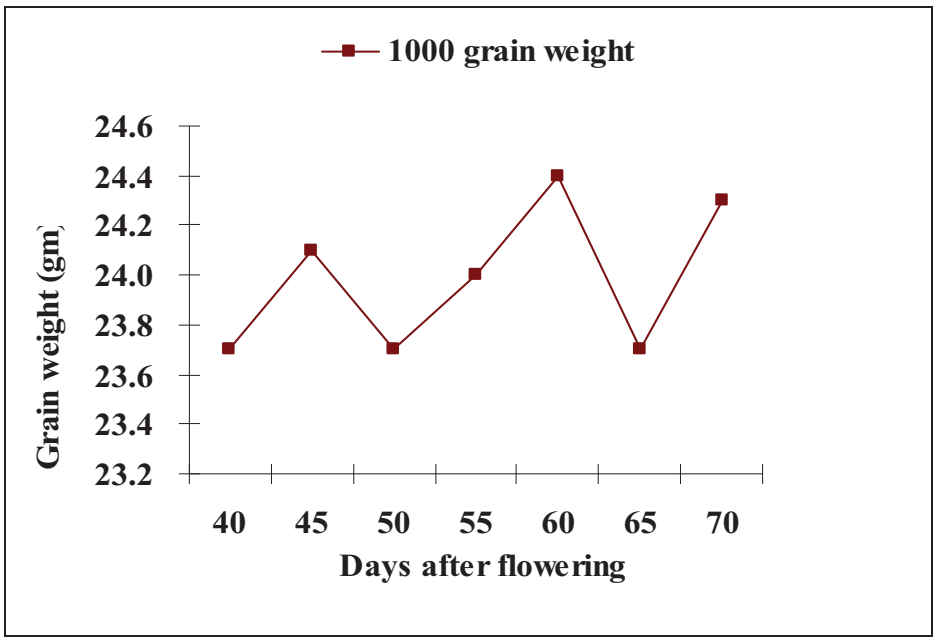
The percentage of green or immature grain which is an important quality parameter was significantly affected by the time of harvesting. The percentage of green grains decreased as the harvesting was delayed (Table 4). The percentage of green grains was more than 10% until 50 days after flowering, but after 55 days to 65 days it reduced to around 5%, with the lowest 1.8 % recorded 70 days after flowering (Table 4). Looking at the percentage of green grains in isolation, 55 days after flowering appears to be the optimum time of harvest.

The different treatments had significant effect on the percentage of cracked or broken grains. The percentage of cracked grains at 40 days after flowering was more than 20%, but from 45 to 55 days after flowering it reduced to about 15%. The percentage of cracked grains, however, again started to increase from 60 days after flowering and it reached about 22 % at 70 days after flowering (Table 4).

No significant treatment effects were observed on the 1000 grain weight. In 2006, we observed a decrease in 1000 grain weight 60 days after

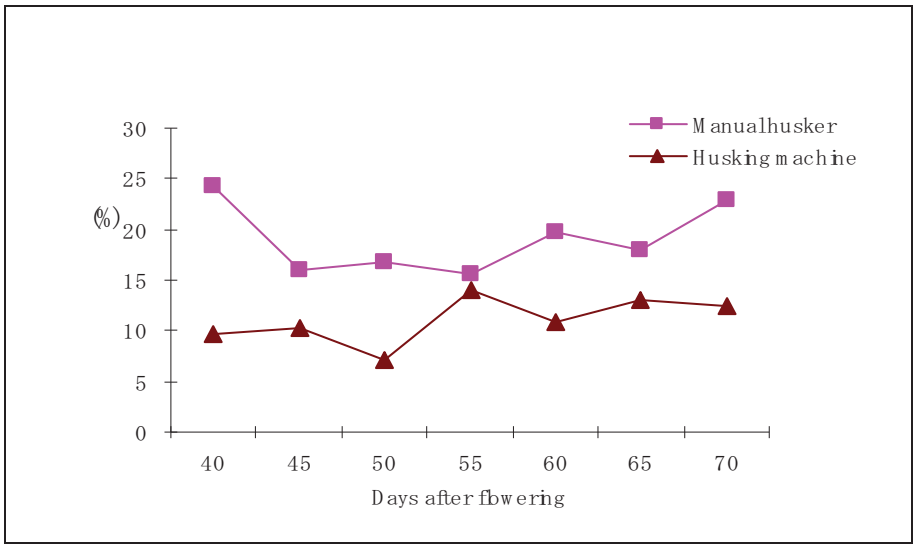
flowering (Figure 2) but this trend was not observed in 2007 (Figure 4). Grains would have attained physiological maturity at 40 days after flowering and changes in the 1000 grain weight would be insignificant.

Figure 4: The effect of time of harvesting on 1000 grain weight of paddy in variety Wengkhar Ray Kaap (2007)



The percentage of cracked brown rice estimated after husking using manually and electrically operated husking machine was compared and the results are presented in Figure 5. The percentage of cracked grains using both the machines showed a similar trend. However, the percentage of cracked grain was 10% less when electrically operated husking machine was used (Figure 5). This indicates that type of milling machine can affect the percentage of cracked grain. Trials on quality assessment of rice were initiated for the first time at RNRRC Wengkhar and comparisons could not be made with the machines used by farmers.

Figure 5: Percentage of cracked grain from manual and electrically operated husking machines for rice variety Wengkhar Ray Kaap (2007)



CONCLUSION

Taking into consideration all the parameters such as moisture content, percentage of filled grains, green grains, cracked or broken grains, harvesting 45 days after flowering seems quite appropriate. Although we can observe an increase in the percentage of filled grains and a decrease in the percentage of green grains at 60 days after flowering, the gains are quite small compared to the loss in quality due to cracked or broken grains. It can therefore be concluded that harvesting 45 days after flowering is appropriate and recommended. It has to be noted that the trials were conducted at mid altitude conditions at RNRRC Wengkhar (approximately 1500 masl) and the results may not be appropriate to be extrapolated to higher and lower elevations where the climatic factors vary significantly. Further, the sample size used to determine the percentage of cracked grains was quite small and data was taken only for brown rice. In order get more reliable information on the quality parameters, further investigation using larger samples and bigger machines is suggested.

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FORESTRY

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Change in forest structure and diversity after the human disturbances in the cool montane evergreen broad-leaved forest

Pema Wangda[1], Lungten Norbu[2], Sonam Tashi[3], Dorji Gyaltshen[4]

ABSTRACT

We studied the changes in forest species diversity and structure of plantation forests in relation to natural forests after human disturbances in the cool, humid evergreen broadleaf forest of Gedu-Darla. The quantitative vegetation and soil survey of the 13 vegetation sampling plots showed that artificial plantation reduces tree species diversity and soil organic matter.

A total of 96 tree species belonging to 44 families were recorded. The entire forest was classified into four different forest habitats/types using cluster analysis; 1. Alnus neplanensis plantation and natural pioneer forest, 2. Natural relict forest, 3. Natural forest and 4. Cryptomeria japonica plantation forest, respectively. Three major life forms of deciduous broad-leaved, coniferous and evergreen broad-leaved type of the natural forests were depicted.

The change from natural to plantation forests as a result of human disturbances caused a huge loss in species diversity, structure and soil properties. The species diversity was reduced to almost 90 % in plantation forests while structurally the forest becomes poorer. Similarly, the soil formation was greatly affected and no definite soil layers were formed in the plantation forest. The plantation forest showed no relict and a few species when compared to the natural forest.

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The study found that if present forest operation is not controlled, intact forest and its resources will be depleted in the near future leading to the loss of rare flora and fauna species.

KEY WORDS: Relict species, Biodiversity, Humid forest, Structural features, Species richness, Forest soil

INTRODUCTION

The forests of Gedu-Darla fall under the subtropical/warm-temperate vegetation zone (Ohsawa, 1987) situated roughly between 1500 and 2300 m a.s.l. just above the southern lowland plains of the Brahmaputra river basin (Van Ijssel, 199l). This forest harbors high diversity of flora and fauna and it is also the last remain of subtropical to warm-temperate evergreen broad-leaved forest of the Himalaya and Bhutan and warrants protection and conservation for future. This complex forest forms one of the reservoirs of natural resources for the people around as they depend on forest directly or indirectly. Accordingly, the abundant palatable grasses, shrubs, and fodder trees in the forest have encouraged farmers to keep large number of cattle.

Climatically, the study area falls under mesic type forest receiving a total mean annual rainfall of 3350 mm (1986-2000). The peak rainfall occurs in June, July and August. Similarly, the mean annual minimum and maximum temperature vary from 2°C in January to 30°C in July, respectively. The mean annual relative humidity changes from 66 % in January to 90 % in July, respectively. Gedu forests are enveloped in fog during most times of a year and they form transition between tropical and temperate zones. The combination of diverse forests and climatic conditions contributes to the formation of complex forest ecosystems. Interestingly, however, very little is known about the ecology of these forests and the impacts of human actvities on the forests.

The evidence of human disturbances in these forests commenced when forest industries were established in Gedu. The industries include wood based saw mill in 1969, a plywood mill in 1982, and a Bhutan Board Particle Limited (BBPL) in 1990. Further, the logged areas were planted (over 100 ha per year) by BBPL with either fast growing species of

Alnus nepalnensis or exotic conifer species of *Cryptomeria japonica*. A total area of 1691.61 ha was planted with either *Cryptomeria* or *Alnus* (BBPL, 2007). Therefore, the present study aims to achieve the following specific objectives:

- to study the forest ecosystem using vegetation survey of the area;
- to investigate the impact of human disturbance on the natural forest and soil properties and,
- to classify vegetation types for different habitats

MATERIALS AND METHODS

Study site

The study area is located in Gedu-Darla (longitude 89°31'44''E, and latitude 26°55'39''N) under Chukha District/Dzongkhag (Fig. 1A,B). The study was carried out in 13 different forest sites (Fig. 1) covering different forest types within an altitudinal range of 900-2540 m a.s.l. including plantation forests and forest habitat for Rufous necked hornbill.

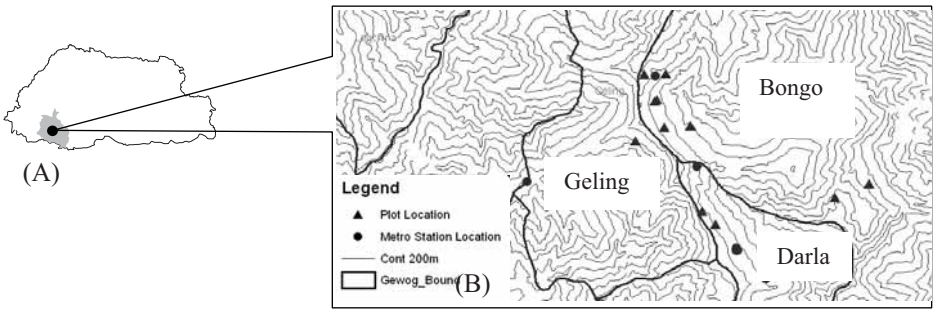

Fig. 1. (A) Map of Bhutan showing the study area (Chukha), and, (B) topography map showing the 13 vegetation plots and HOBO ONSET data loggers.

Temperature and relative air humidity measurement

Air temperature and relative air humidity were recorded using HOBO Onset data logger (Onset Computer Co. MA, USA) mounted on the trees in the forest to avoid human disturbances. In addition, 20 years of meteorological data (1986-2006) at Gedu, 2000 m a.s.l. was collected from Agro-Met division, Council for RNR Research of Bhutan, Ministry of Agriculture to investigate the change in climate conditions over the time span of twenty years.

Litter and soil sampling

Litter and soil samples were collected by setting small quadrats (0.5 m x 0.5 m) in each sampling plot (Fig. 2A, B). The samples were separated into litter, fermentation-humus, and soil surface layer. The samples were measured for the fresh weights (FW) and oven dry weights (ODW) at 45 ^{o}C after 48 hours. Dried samples were then analyzed at the Soils and Plant Analytical Laboratory (SPAL), Semtokha, Bhutan.

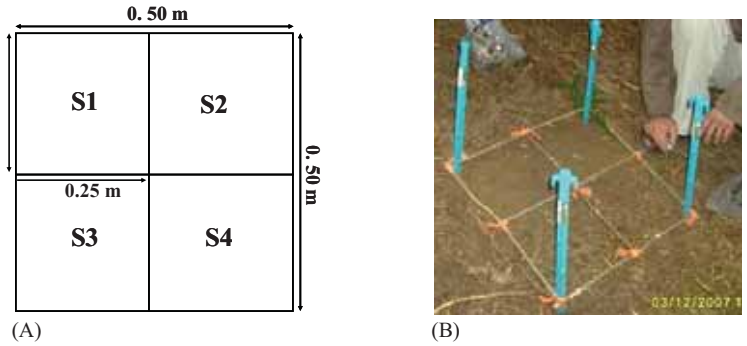

Fig. 2. Litter and soil sampling methods; (A) quadrat sampling with four sub-quadrat (S1-S4) and, (B) taking samples from the plots under *Cryptomeria japonica* forest (P4).

Vegetation survey

Vegetation survey was conducted using quadrat sampling method for tree layers. All the tree individuals occurring within the plot measuring 30 x 40 m attaining a height greater than 1.3 m (H ≥ 1.3 m) were measured, (H, m) and diameter at breast height (DBH, cm at 1.3 m above ground).

Fieldwork was carried out from November 2007-October 2008. Nomenclature of plants followed after Flora of Bhutan (Grierson and Long, 1983, 1984, 1987, 1991, 2001; Noltie, 1994, 1999, 2000). The Orchids of Bhutan (Pearce and Cribb, 2002), Weeds of Bhutan (Parker, 1992), Wild Rhododendrons of Bhutan (Pradhan, 1998), Flowers of the Himalaya: A supplement (Stainton, 1988), Flowers of Bhutan (Nakao and Nishioka, 1984), Flowers of the Himalaya (Polunin and Stainton, 1984), and Photo-album of plants of Eastern Himalaya (Hara, 1968).

Vegetation data analysis

Species basal area (BA, cm^2) was calculated from DBH data of tree individuals and calculated the relative proportion of each species' basal

area in percent (Relative Basal Area, RBA %). The RBA of each species was used as abundance measure of species in a community. The dominant species of the altitudinal plots were determined based on the dominance analysis (Ohsawa, 1984; Kikvidze & Ohsawa, 2002).

The preliminary data was processed using pivotal table of the Microsoft Excel. Once the data was processed, analysis was carried out by using PC-ORD version 4 (McCune and Medford, 1999) and cluster analysis was performed using distance measure of Sorensen (Bray-Curtis method).

RESULTS AND DISCUSSION

Environmental/Climatic conditions

The climate data for the past 20 years (1986-2006) showed slight increase in temperature while the rainfall fluctuates during the recent years (Fig. 3A). Gedu receives fairly high rainfall throughout the year with a total mean annual rainfall of 3504 mm and the peak rainfall occurred during July-August months of the year. This data suggested that Gedu-Darla remains under humid and wet conditions throughout the year round. The average annual temperature of Gedu-Darla was 16.7 ^{o}C and reached a maximum temperature of 28.4 ^{o}C in July with a minimum of 3.4 ^{o}C in December. The combination of high rainfall and relatively high/moderate temperature contribute to the humid high diversity evergreen broad-leaved forest ecosystem of Gedu-Darla region.

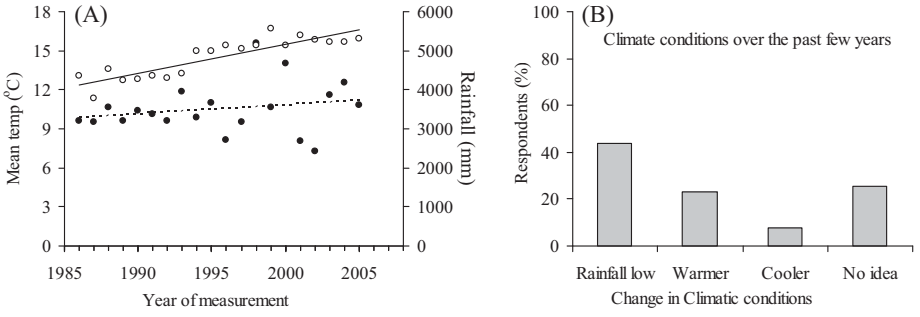

Fig. 3. Climatic conditions of the study area. (A) Measured mean air temperature (open circle) and total rainfall (closed circle) of Gedu (1986-2006 Agromet-CoRRB, MoA, 2008); and (B) Farmer's perception of climate change over the past decades.

The farmers from eight villages were also interviewed to understand their perception of the climate change during the past decades. More than 40 % of the farmers interviewed said that the rainfall has reduced in the area while about 20 % as warmer and about 10 % as cooler (Fig. 3B). There were also some farmers who were ignorant of the climate changes (Fig. 3B). Farmer's perception of climate change correlates with the actual meteorological data of the area (Fig. 3A,B).

Floristic composition and life-form distribution of the sampling plots

A total of 96 tree species comprising of 44 families were recorded in the 13 sampling plots of the study area. On comparison between the natural forest and the plantation forest, plantation forest (P4) has only 9 species while the natural forest (P8) showed 35 species. Similarly, plantation forest has only 1 dominant while natural forest showed 6 dominants. Natural forest was mainly dominated by oak-laurel species while plantation forest composed of either exotic species Cryptomeria or fast growing Alnus. This clearly revealed the low diversity of tree species in plantation

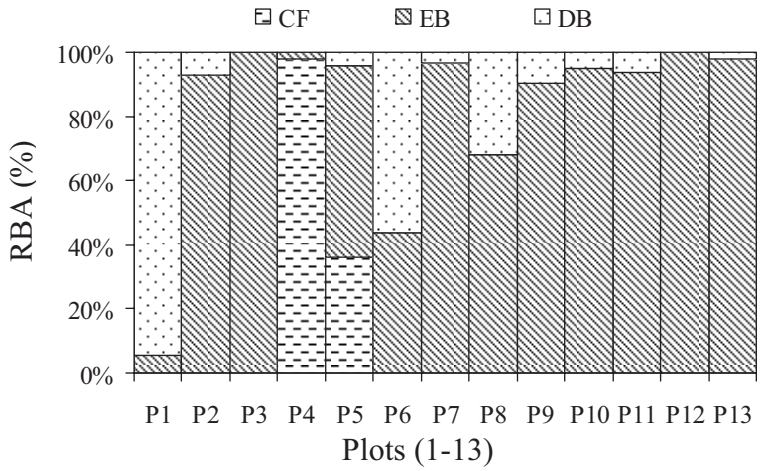

Fig. 4. Life-form distribution of the sampling plots. Five life-forms are compared between the 13 plots.

Three major life-forms of coniferous (CF), deciduous broad-leaved (DB) and evergreen broad-leaved (EB) types were depicted in the study area (Fig. 4). Plots (P1, P6, P8) showed deciduous broad-leaved life-form. These plots were planted with fast growing deciduous broad-leaved

species (*Alnus nepalensis*) while P4 and P5 were planted with exotic conifer species (*Cryptomeria japonica*) and they emerged as conifer life-form in the humid evergreen broad-leaved forest (Fig.4). Except P1, P4, P5, and P6 rest all the other plots were established in the natural forest. Natural forest plots appeared as evergreen broad-leaved life-form including the Rufous necked hornbill habitat forest (P8). The figure clearly revealed the impact of human activities changes the life-form spectrum of the forest (Fig. 4).

Forest types and habitats

The three major life-forms were classified into forest types by cluster dendrogram arbitrarily at 40 % similarity threshold (Fig. 5). Four forest types were defined by their dominants and natural habitats i.e., 1. *Alnus* plantation and young natural pioneer forest (P1, P6, & P7) of *Alnus, Macaranga, Rapanea, Syzygium, Croton,* & *Elaeocarpus* types, 2. Natural forest type (P2, P3, P10, P11, P12, & P13) of mainly oak-laurel types (Fig. 5), 3. Rufous necked hornbill habitat type forest (P8 &P9) of *Lindera, Betula, Schima, Terminalia, Lithocarpus, Castanopsis, Ficus,* and *Cinnamomum,* and 4. *Cryptomeria* plantation forest (P4, &P5). Plot (P4) was established in the old Cryptomeria plantation forest (c. 30 years) while P5 in the young Cryptomeria plantation forest (c. 6 years) (Fig. 5).

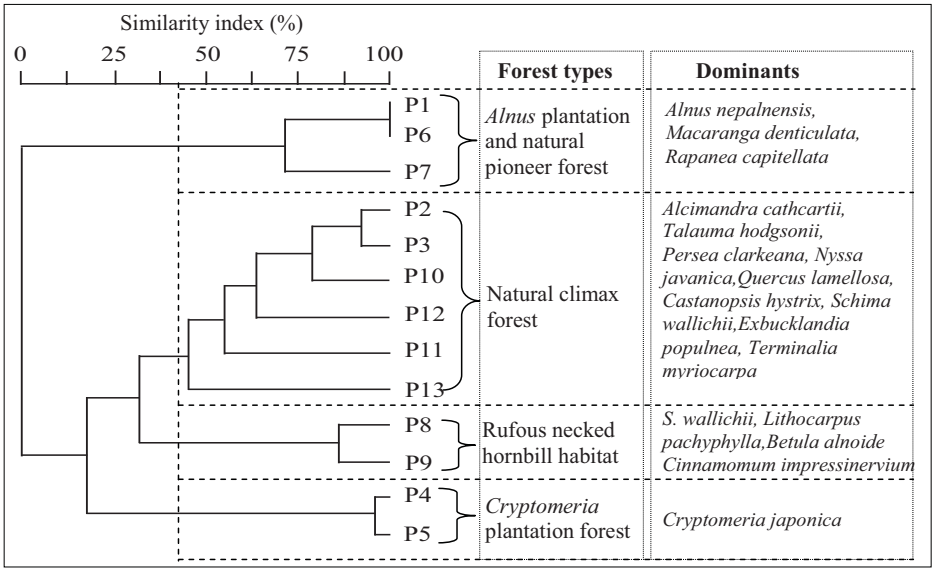

Fig. 5. Cluster dendrogram showing different forest ecosystem in the study area. Four forest ecosystems (habitats) were identified using cluster analysis.

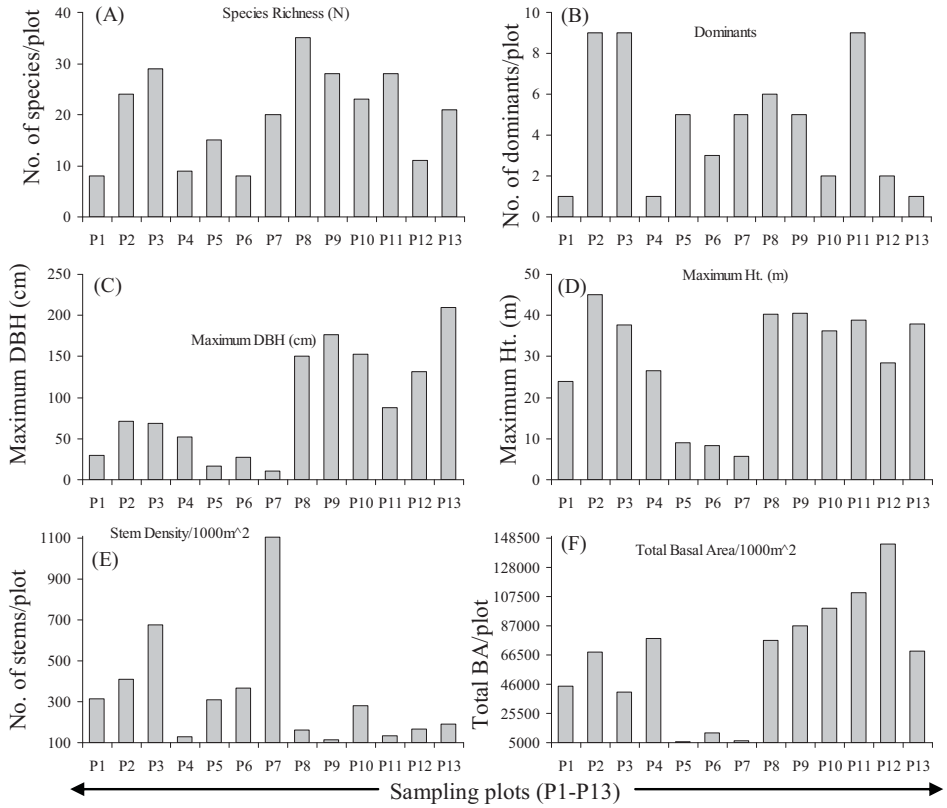

Fig. 6. Forest structural features (A-F) of the sample plots around Gedu-Darla study sites.

The impact of the plantation was also investigated by using the forest structural traits (Fig. 6). Forest structural features represented in figure 6A-F showed the difference in structures of different forest ecosystems including plantation forest and natural forest of the study area. Floristically, the plantation forest showed limited species (P1, P4, P5, P6, P7) compared to natural forest including the Rufous necked hornbill habitat forest with a species number of 35 (P8) (Fig. 6A). Similarly, the dominants were found higher in the natural forest but plots (P10, P12, P13) also showed lower dominants (Fig. 6B). The emergent trees contributed to the low dominants because of higher basal area as can be seen in figures 7C,D & F. The maximum dominants were recorded in P2, P3 & P11 with 9 dominants each followed by other natural forests (Fig. 6B). The maximum DBH of 210 cm was recorded in P13

(*Lithocarpus pachyphylla*) followed by 176 cm of *Terminalia myriocarpa* in plot 9 (Fig. 6C). The maximum height of *Daphnephyllum chartaceum* tree (45 m) was recorded in plot 2 compared to the plantation forest. However, the stem density was found relatively higher in plot 7 (young natural regenerated forest) (Fig. 6E). Accordingly, total basal area was found low in the newly created plantation forest and higher basal area in the natural forest (Fig. 6F).

Litter and soil conditions under different forest types

Litter and soil samples were collected under each forest types (P1-P13) and analyzed for chemical properties. The samples were collected from different layers (Litter, Fermentation-humus and Organic) (Fig. 7A). Old *Cryptomeria* plantation forest (P4) did not show any organic matter and fermentation-humus layer (Fig. 7A). However, when the plantation was young (P5), a well developed layers were observed, due to mainly associate species that helped to accumulate litters and organic matter respectively (Fig. 7A). Whereas, a very few under story species were observed and recorded under the old growth *Cryptomeria* plantation forest (P4).

On the other hand, plots established in the natural forest showed all three layers (litter, Fermentation-Humus & Organic layer) including the *Alnus* plantation forest (Fig. 7A). The intact forest (P11, P12) showed even higher accumulation of organic matter. The result clearly revealed that plantation forest is inferior to natural forests in the development of the soil organic matter.

The soil pH revealed slight acidic in the plantation forest compared to the natural forest (Fig. 7B). Similarly, the available phosphorous was found higher in the natural forest though it fluctuates (Fig. 7C).

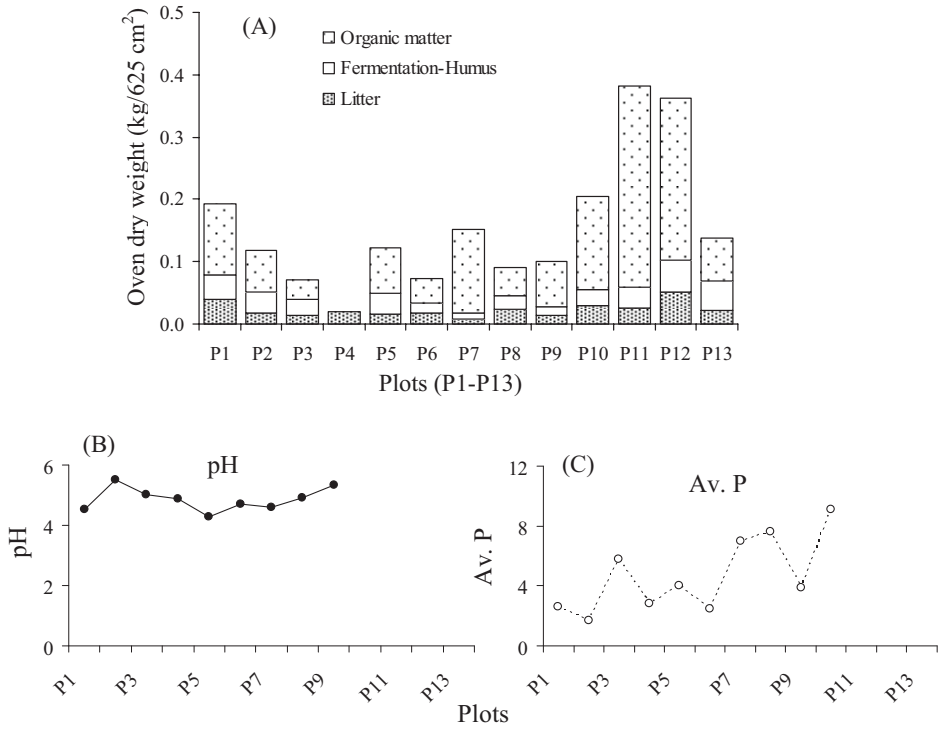

Fig. 7. Litter, organic matter accumulation and soil properties of the studied forest. (A) Accumulation of three layers, (B) Soil pH and (C) soil available phosphorus under each forest types. *(Note; Samples from plots 10-13 are not included.*

CONCLUSION

The study found out that Gedu-Darla forest harbor diverse flora and fauna. Floristically, the forest was found diverse in the natural stands compared to the plantation forest. The similar diverse forest was also found in the inner Bhutan Himalaya (Wangda and Ohsawa, 2006a). The study found that human impact on the natural forest at Gedu-Darla caused loss in species diversity as well as loss of habitats for many fauna including the rare Rufous necked hornbill. On comparing the natural forest (Hornbill habitat) with two types of plantation forest (exotic *Cryptomeria japonica* and *Alnus nepalnensis*) revealed clear difference in diversity and structure (Fig. 8). There were no relict species recorded in the Cryptomeria plantation forest and a few in the Alnus plantation forest. Similarly, the species richness in the natural forest was found four times higher than in the plantation forest (Fig. 8A). The dominant

species were also observed high in the natural forest forming complex canopy structure whereas, only one dominant was observed in the plantation forest. The figure clearly revealed strong human impacts in terms of diversity. Accordingly, Wangda et al (2006b) reported similar human impacts in the evergreen board-leaved forest at Lamperi where the cultivation of Wasabi drastically reduces the forest diversity both at the canopy and ground level.

Structurally, natural forest appeared superior compared to the plantation forest (Fig. 8B). Maximum diameter of the tree in the natural forest was about three times bigger than the diameter of the planted trees. Similarly, the maximum height of the tree was also found taller in the natural forest than the plantation forest. The high complex forest was found suitable habitat for the rare bird (Rufous necked hornbill). Severe human impacts on such natural forest lead to loss of endemic fauna species from the area. Gedu-Darla oak-laurel forest also serves as grazing grounds for the local community's cattle and combined with humid climate favors presence of high species diversity. Such cloud evergreen broad-leaved (relict) forests are the only remaining forest in the Himalayan region and need to accord high priority for conservation.

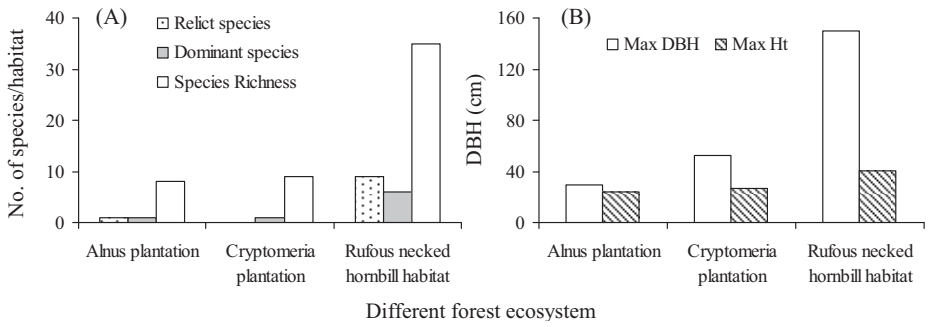

Fig. 8. Comparison of natural forest with that of plantation forest of the study area. (A) Diversity difference and (B) structural difference.

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Pine Die-back in the Plantation forest along the slopes of Pachu- Wangchu Dry Valley

Pema Wangda[1], Lungten Norbu[2], Dorji Gyaltshen[3], Dal Bahadur Chhetri[4]

ABSTRACT

Periodic pine die-backs were observed five times in nearly one and a half decade from 1994 to 2008 along the Pachu-Wangchu dry river valley. Initial investigations were focused on the biotic factors of pine die-back and ruled out any entomological or pathological association with the pine die-back. The later research was focused on the abiotic factors mainly rainfall, temperature, and soil moisture contents.

The adverse environmental conditions of very low soil moisture content (6 %), high evapo-transpiration ratio (1.4) and a low aridity index (24.7) were the possible factors causing pine die-back in the plantation forest.

A strong correlation was observed between tree growth and monthly mean rainfall of the previous autumn. Upon analysis, it was concluded that mean monthly rainfall of the previous autumn plays an important role in the die-back event of the current year. Thus, the pine die-back is predictable based on the findings of the dendrochornological analysis.

KEY WORDS: Barren, Drought, Windy, Plantation, Periodic die-back, Autumn Rainfall

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INTRODUCTION

The dry valleys are prominent in the middle reaches of big rivers extending from south to north dissecting the mid mountains between southern mid Himalaya and northern greater Himalaya (Ohsawa, 1987a; Eguchi,1997; Wangda and Ohsawa, 2006a). Accordingly, the vegetation distribution patterns along these dry valley slopes showed that ridges or mountain tops are wetter than the lower slopes due to subsidence over valleys in the slope wind circulation. One such example of dry valley is the Pachu-Wangchu valley. These dry barren slopes along the Pachu-Wangchu valley covering an area of 497ha were planted in the mid 80s by the Department of the Forest with a total cost of Ngultrum 17.234 million over a period of 10 years (Project Report, 1984-1997). The main objective of the plantation project was to convert the degraded barren land into a productive forest and to reduce the soil erosion. The species planted comprised of mainly three conifer species of *Pinus wallichiana* (bluepine), *Pinus roxburghii* (Chirpine) and *Cupresuss* sp. (Tsenden). Since then, periodic die-backs were observed five times in nearly one and a half decade (1992-2008) in the established plantations particularly during the early spring months of 1994, 1999, 2001, 2003 and 2008. Accordingly, the pine die-back problem was investigated by the visiting scientists from Japan, Europe and South Africa and found out no indication of biotic factors (disease, pest including nematodes or any out breaks). Similarly, the entomological and pathological investigation conducted by Research Centre Yusipang also ruled out the biotic factor for Pachu-Wangchu Plantation pine die-back (Chhetri, 1999; 2001; Chhetri and Gurung, 2002). These results led to the present investigation using dendro-climatological study supported by the past climate data.

The study was conducted with the objective to understand the cause of pine die-back in the plantation along the slopes of Pachu-Wangchu valley. Specifically, the study aims to answer the following objectives:

- to understand the environmental conditions of the study area,
- to study the growth behaviour of different species planted,
- to understand the main cause of pine-die back using tree ring analysis (dendrochronology) technology and finally,
- to analyze and integrate the factors leading to the pine die-back in the plantation forest.

MATERIALS AND METHODS

Study area

The dry valleys of the Bhutan Himalaya stretch from west to east along the mid hills (Wangda and Ohsawa, 2006a) including the present study area (Fig. 1A). Specifically the study was conducted in the pine die-back sites opposite to confluence (Chunzom) and above the Khasadrapchu hydel, respectively (Fig. 1 A, B).

Climatically, the study area falls in one of the typical dry valleys of the western region. Floristically, the study area comprised of xeric species such as *Cotoneaster microphyllus, Leptodermis kumaonensis, Zanthoxylym armatum, Rosa sericea, Berberis aristata, Prinsepia utilis, Colquhounia coccinea, Artemisia* sp., and *Ceratostigma griffithii.*

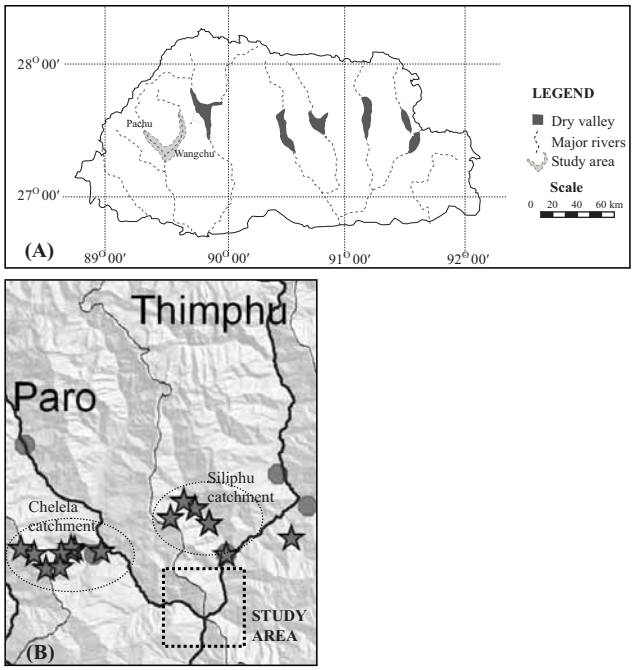

Fig. 1. Map of the study area, (A) Map of Bhutan showing dry river valleys including the study area and, (B) location of the study area along Pachu-Wangchu valley.

Environmental data collection

Meteorological data (temperature and rainfall) was recorded using HOBO Onset data logger enclosed in the solar radiation shield (Onset Computer Co. Ma, USA) and tipping bucket type rain gauge installed since 2002 at 5 locations in the affected sites. The loggers were set at 1-hour interval recording and were downloaded after every six months using BoxCar Pro series for windows, version 4.3.

Climate data recorded were analyzed using thermal and humidity indices:

1. Climate diagram was drawn by using Walter and Lieth's method (Walter et al., 1961-1967; Lieth et al., 1999);
2. Potential Evapo-transpiration Ratio (PER) was calculated by Holdridge's method (1967); PER = (ABT*58.93)/PPT where, ABT = annual biotemperature (^{o}C) and PPT = precipitation (mm);
3. Aridity Index (AI) was calculated using De Mortonne method (1926) where, I = P/(T+10), P = total annual precipitation (mm) and T = annual mean temperature (^{o}C)

Soil moisture content was measured by HydroSense (CD 620+CS 620) (CAMPBELL SCIENTIFIC INC. Logan, Utah) bearing 12 and 20 cm probes during the every field survey. Similarly, soil hardness was measured by push cone called Yamanaka's soil hardness tester (Kiya Seisakusho Ltd. Tokyo).

Tree growth study using tree ring method

The tree core samples were taken from three sites differentiated into upper, middle and lower altitudes. The samples were taken to the Tree-Ring Laboratory at the Lamont-Doherty Earth Observatory, Columbia University, NY for tree ring analysis. The results of the tree growth were also correlated with the climate data to understand the effect of environmental conditions on the tree growth for further investigation.

Vegetation study

Three vegetation sample plots each measuring 40 m by 40 m (1600 m^2) were established in 2000 located at opposite to Chunzom (confluence), Sisina and Khasadrapchu, respectively. In addition, three sample plots

each measuring 10 m x 10 (100 m^2) at Chunzom were established in 2009. Inside the plots, trees were measured for their diameter, height and general assessment. All species within the plots were measured and identified. The vegetation data was analyzed using pivotal table in excel spreadsheet and simple correlations were performed.

RESULTS AND DISCUSSION

Environmental conditions: Climate, soil moisture content and soil hardness

Pachu-Wangchu valley including the present study site falls under the dry temperate type of climate with a total annual rainfall of 590 mm (1986-2006) and the annual mean temperature of 13.6^{o}C (2002-2008). The maximum mean temperature of 19.6^{o}C was recorded in July and the minimum mean temperature of 5.7 ^{o}C was recorded in December. The annual mean temperature slightly increased from 13.5 ^{o}C in 2003 to 13.7 ^{o}C in 2007. Similarly, winter temperature also increased from 4.8 ^{o}C in 2003 to 5.1 ^{o}C in 2007. However, summer temperature remains constant. Interestingly dry months (Jan, Feb) mean temperature increased from 4.8 ^{o}C and 6.7 ^{o}C in 2003 to 6.8 ^{o}C and 8.6 ^{o}C in 2009, respectively. These increasing trends of temperature tend to have severe implication on the growth of the vegetation.

The study sites also experienced a prolonged dry spell over a period of 4 months (Jan-mid Mar) when compared to pine growing sites with slightly less dry months (Fig. 2 A, B). The dry period (water stress) occurred when the temperature curve exceeded the precipitation curve as shown by the climate diagram (Fig. 2). Comparison of the two climate diagram clearly revealed that die-back sites received relatively low precipitation with higher temperature contributing to the prolonged drought period (Fig. 2). Several climate indices revealed that the die-back sites suffer severe water stress. De Mortonne's aridity index was 24.7 corresponding to the wood land type rather than forest. Similarly, the potential evapotranspiration ratio (PER) was 1.4 indicating a very dry forest type.

Accordingly, several scientists and geographers described the area as dry and barren. British botanist Griffith, (1839) described the area as extremely dry and devoid of vegetation. Other scientists from Japan and Germany (Ohsawa & Eguchi 1987, Schweinfurth 1992) also described the valley as extremely dry and windy.

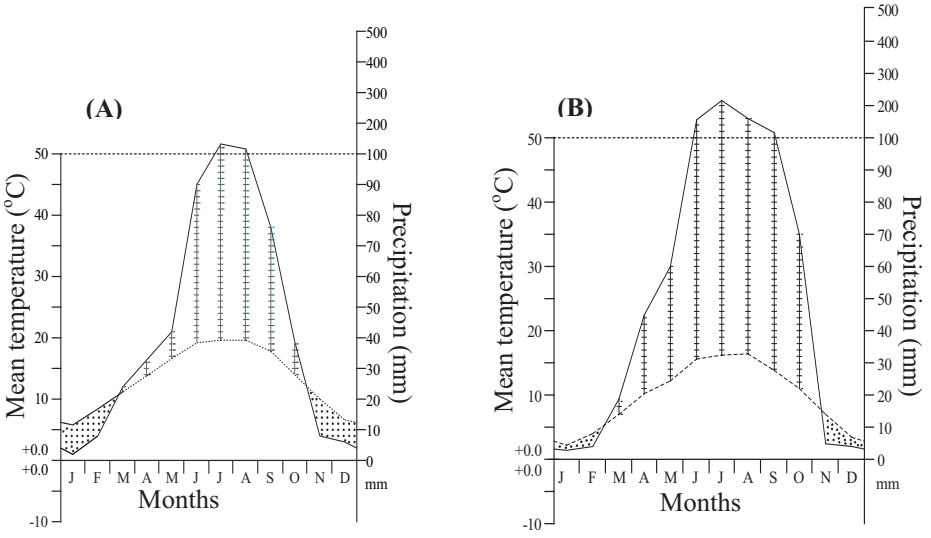

Fig. 2. Walter climate diagrams, (A) Climate diagram of the die-back sites (Khasadrupchu) and, (B) Climate diagram of the natural forest (Yusipang).

Soil moisture contents and soil hardness were measured long the altitudinal gradients of the study site including the die-back area located in the mid-altitudes of the profile (Fig. 3). The data showed low soil moisture content (c. 5 %) and high/compacted soil around the pine die-back sites when compared to river bank and upper natural forest (Fig. 3 A,B,C). The result clarified low soil moisture content and compacted soil contributed to the harsh environmental conditions of the die-back area. In addition, strong wind along the narrow river valley also contributed to the high evapo-transpiration ratio in the area.

In line with the findings of the soil moisture content and the soil hardness, the two photographs in the Pachu-Wangchu die-back plantation (Photo 1A,B) clearly showed the species preference to moisture. In photo 1A, trees were concentrated along the moist slopes while pine die-backs occurred on the drier sites. Similarly, photo 1B revealed well grown blue pine trees near the river bank while upper slopes remained dry and without tree growth.

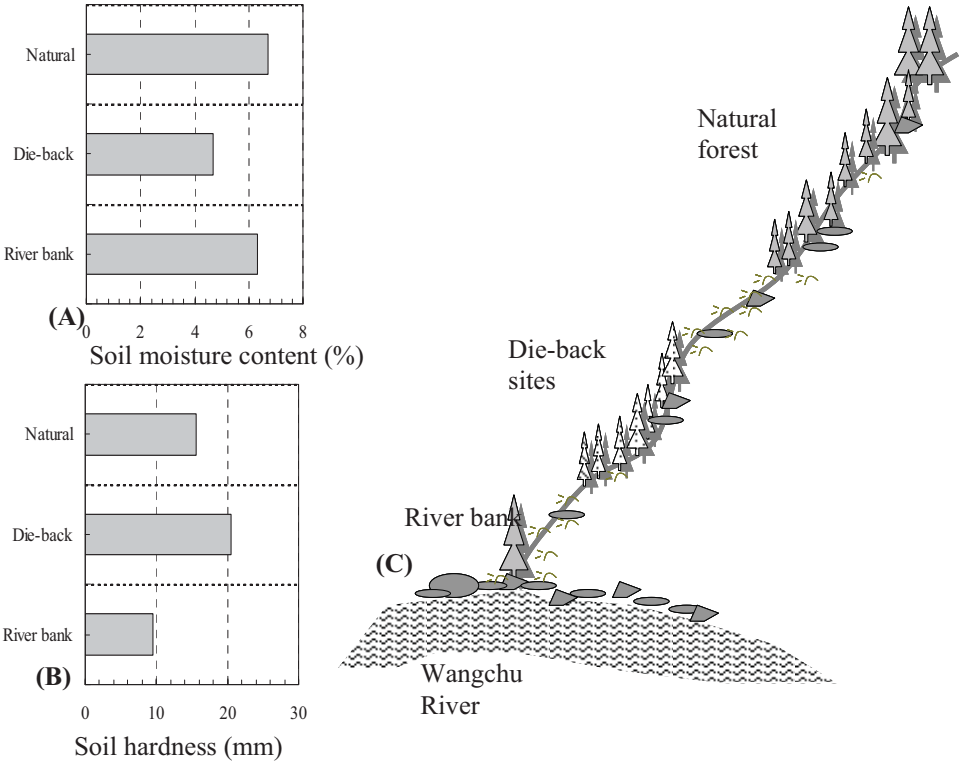

Fig. 3. Environmental conditions of the study area. (A) Soil moisture content (SMC %), (B) soil hardness (kg/cm^2) and (C) schematic profile of study area where the measurements were taken along the altitudes of the die-back affected sites.

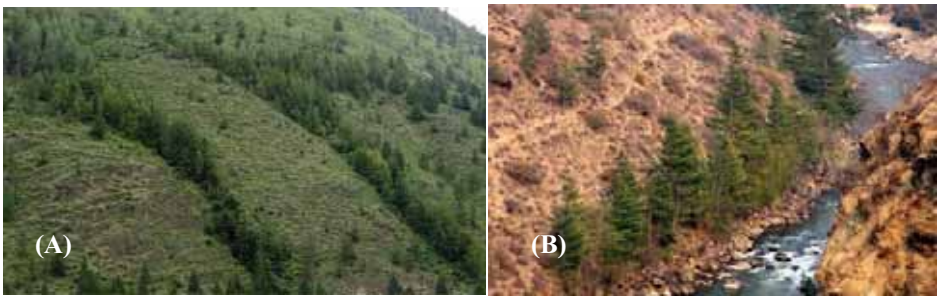

Photo 1. Soil moisture plays an important role during the establishment of the conifer plantation. (A) Living trees concentrated towards the water canal while trees away from the water canal dies up and (B) pine grows well near the river side.

Growth behaviour of *Pinus wallichiana* (Blue pine) and *Pinus roxburghii* (Chir pine)

Tree ring analysis from three different locations (upper, middle and lower altitudes) of the two sites at opposite Chunzom and Khasadrapchu were studied. The result showed an interesting growing pattern of the two pine species (*Pinus wallichiana* & *Pinus roxburghii*). Initially *P. wallichiana* (Blue pine) grows faster than the *P. roxburghii* (Chir pine) until 1999. There was a sharp drop in the growth of *P. wallichiana* after 1999 and on the contrary the growth of *P. roxburghii* was faster than the *P. wallichiana* (Fig. 4) even though *P. roxburghii* was planted outside its ecological range.

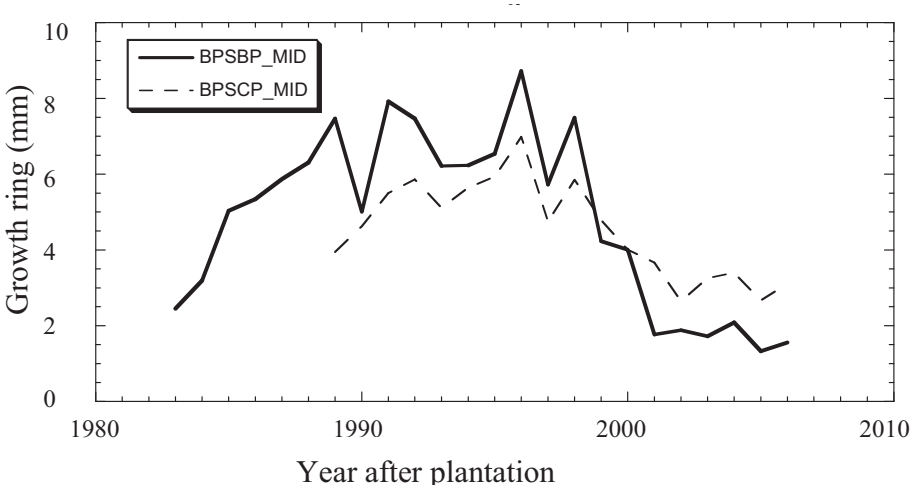

Fig. 4. Comparison of tree growth in the plantation forest along the die-back affected sites (BPSBP_MID with solid line refers to growth of Blue pine in the mid altitude and BPSCP_MID with dash lines refers to growth of Chir pine in the mid altitude of the plantation forest.

Accordingly, the research results from the three vegetation sample plots revealed similar results. *P. wallichiana* was found to be severely affected by the die-back compared to *P. roxburghii* in the Chunzom plantation site (Fig. 5). The plot survey clearly revealed that the survival rate of the *P. wallichiana* was very low. In plot 1, *P. wallichiana* showed only 30 % survival while *P. roxburghii* and *Cuppresus* sp. showed 100 % survival (Fig. 5A). Plot 2 showed 100 % mortality of *P. wallichiana* while other two conifer species (*P. roxburghii* & *Cuppresus* sp.) has shown 100 % survival (Fig. 5). Particularly, *P. wallichiana* showed high

mortality rate when planted in combination with *P. roxburghii* a sub-tropical, dry and fire resistant species.

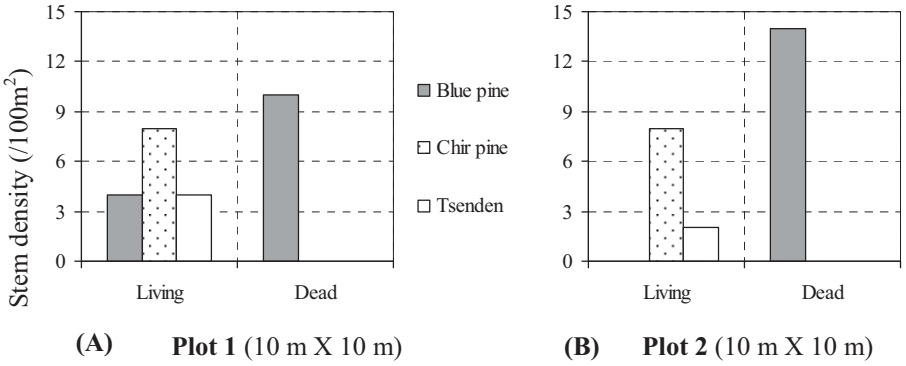

Fig. 5. Species performance after plantation in the two established experimental plots at opposite to Chunzom (2250 & 2280 m).

Structural features of plantation forest such as maximum diameter at breast height and maximum height showed *P. wallichiana* and *P. roxburghii* were of similar age compared to *Cuppresus* sp. (Fig. 6 A, B). However, *P. wallichiana* could not compete with *P. roxburghii* after attending maximum DBH of 12 cm and a maximum height of 8 m respectively. Structural features clarified that growth performance of *P. roxburghii* was better than the other two conifer species.

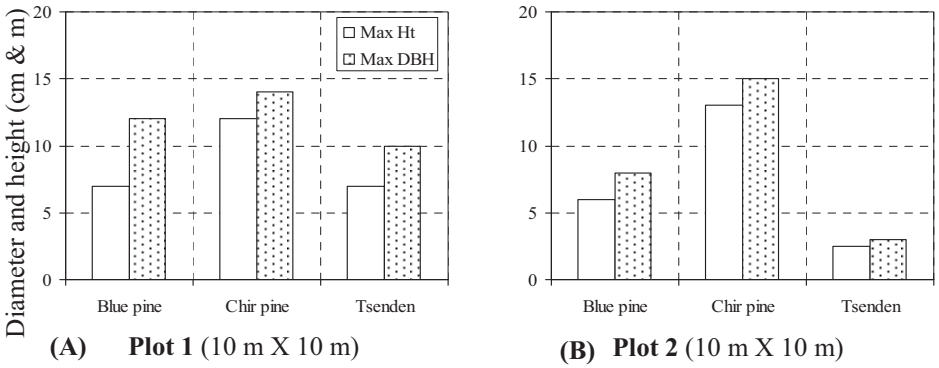

Fig 6. Structural features of the species planted. (A) Plot 1 & (B) Plot 2 showing structural features of maximum diameter at breast height (DBH) and maximum height in the two studied plots.

Relationship between tree growth and mean monthly rainfall

Pine tree growth was analyzed using tree ring (dendrochronological study) and the result was correlated with the monthly mean rainfall in the die-back affected sites along Pachu-Wangchu valley. The analysis showed significant correlation (65 %) between the tree growth and the climate factors particularly the monthly mean rainfall from November to December of the previous year (Fig. 7). This explains that autumn (post monsoon) rainfall is responsible for the growth of the pine trees of the current year in the studied area. Similarly, low rainfall during the spring or growing months (April-May) lead to reduced growth of the pine trees as shown by the significant (negative) correlation between tree growth and April rainfall (Fig. 7).

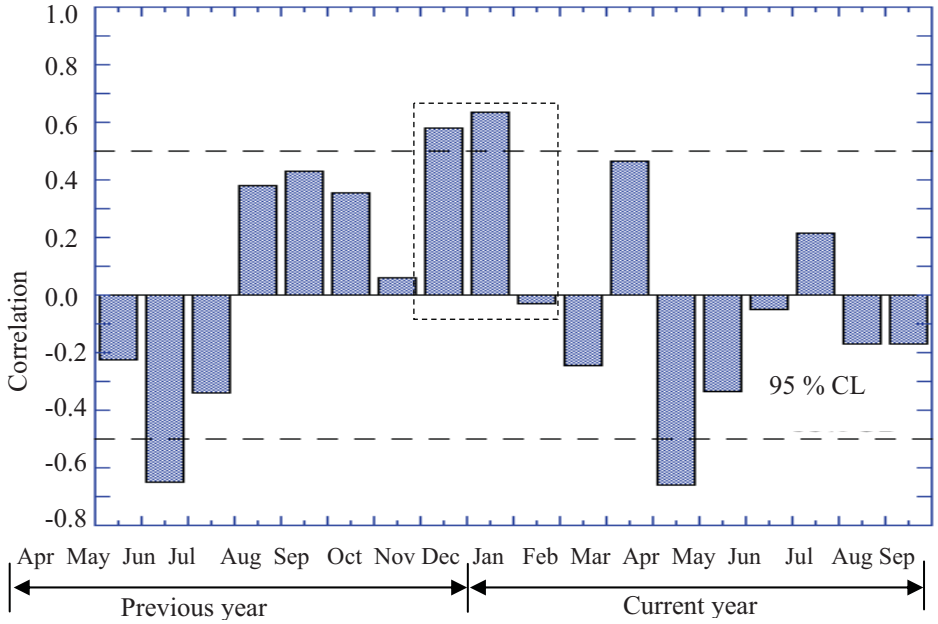

Fig. 7. Correlation between total monthly rainfall and the tree growth (tree ring) of the die-back affected sites. A dotted box shows positive correlation between tree growth and rainfall of the previous year's Nov-Dec.

Correlation between total rainfall (Oct-Dec) and pine die-back events

Based on the significant result of the correlation between tree growth and total rainfall of the previous year (Oct-Dec), further investigation was carried out. On correlating the total rainfall of the previous year's

autumn (Oct-Dec) against the year of rainfall and temperature measurements from 1992-2008, clearly showed that die-back event of the current year always followed the low or no rainfall of the previous year's autumn (Fig. 8). In addition, the low or no rainfall of the previous autumn (Oct-Dec) always coincided with the higher annual mean temperature leading to specific dryness causing prolonged drought in the study area (Fig. 8). Thus this result clarified that the pine die-back is strongly correlated/dependent on the rainfall of the previous autumn.

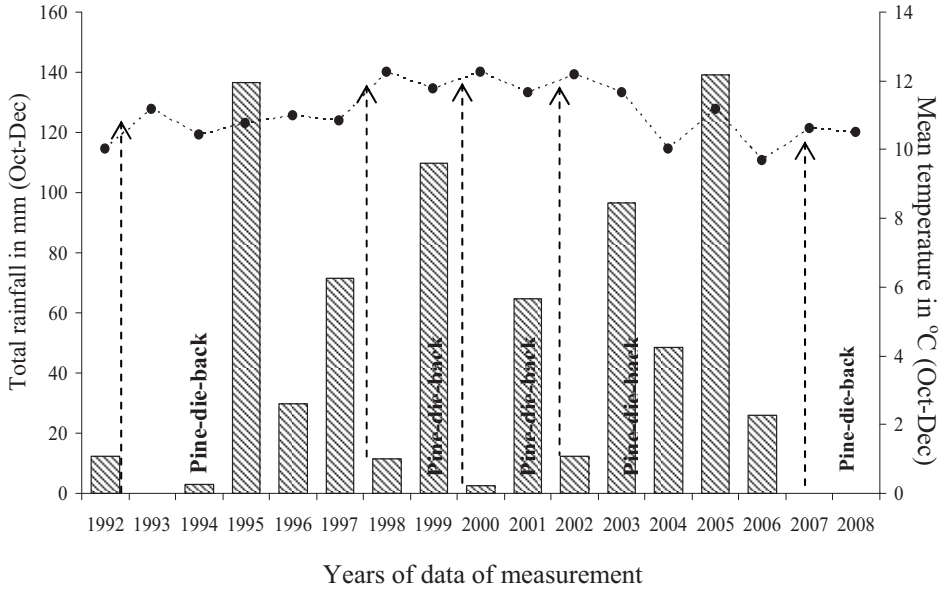

Fig. 8. Effects of previous rainfall (Oct-Dec) on the plantation forest of the current year. Die-back events follow low rainfall and high temperature of the previous year. Dotted arrow line shows high temperature and low precipitation prior to the die-back events.

CONCLUSION

The die-backs are consequences of both abiotic and biotic factors. Along the Pachu-Wangchu valley, abiotic factor plays a vital role such as adverse climatic conditions coupled with poor soil condition. A total of 5 periodic pine die-backs were occurred from 1994 to 2008. However, it is only concentrated in the western region of Bhutan particularly in the plantation area along the Pachu-Wangchu valley.

The adverse climatic factors of very low soil moisture content, a low aridity index and a very high potential evapo-transpiration ratio are some of the factors leading to specific dryness of the study sites causing drying of planted pine trees. The findings of the present study were also supported by the findings of the visiting scientists from Europe, Japan and South Africa (Personnel communication 2003-2008).

The present study showed significant correlation between the tree growth and the climate factors particularly the rainfall of autumn (Oct-Dec) of the previous year. On correlating the rainfall of the previous year's autumn (Oct-Dec) against the year of rainfall measurements of 17 years (1992-2008), clearly showed that die-back of the current year consistently followed either no or very low rainfall of the previous year's autumn. Similar finding was also reported by Chhetri, (1999). In addition the low rainfall was always followed by high temperature leading to the incidence of die-back in the current year. This result clearly showed that the die-back is strongly dependent on rainfall of the previous autumn. Based on the result, the pine die-back along the Pachu-Wangchu valley probably is predictable.

Further, the die-back affected areas were natural barren slopes affected by strong valley wind from the narrow river valleys. Strong upstream river valley wind in combination with strong sun shine during the day increases evapo-transpiration leading to specific local dryness. These complex factors favours only xeric plant species to survive thus plantation in such extreme sites should be carried out with extreme care. The Department of Forests should take in to account species-site matching during re-afforestation planning and implementation.

ACKNOWLEDGEMENT

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Buffalo farming in Bhutan – vanishing before it's full potential is explored

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ABSTRACT

Over the last two decades, there had been a drastic decline in buffalo population in Bhutan. But so far no studies have been conducted to know the reasons for the decline. Further, buffalo farming and it's role to farming communities have not been documented. This study therefore, was undertaken to understand buffalo husbandry practices in Bhutan, it's role to improve rural livelihood and to investigate reasons for fast decline in it's population.

Data was collected from 80 buffalo farming households in four Dzongkhags of Southern Bhutan.

Results indicated that buffaloes are reared mainly for milk, meat, manure and draft power. The other reasons for keeping it were: tastier dairy products due to high fat content, ability to withstand adverse weather conditions and survive on coarse feed.

Buffaloes are found to have better productive and reproductive efficiency than local Siri cattle. But farmers in some areas intentionally reduced reproduction rate through controlled breeding of buffaloes. Though such measures increased lactation yield, it prolonged calving interval, decreased the calf crop and reduced herd size which resulted in population decline. Besides, frequent culling, unavailability of quality breeding bull and inbreeding resulted in the decline.

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Study concluded that though buffalo rearing is more profitable than keeping equal number of local cattle, no attention have been given to improve them. Necessary policy and technical support to revamp buffalo farming could broaden farmer's income-base and save the buffalo population from possible extinction. Support in terms of supply of breeding bulls and introduction of artificial insemination could mitigate inbreeding problem and control buffalo population decline thereby sustaining buffalo farming.

KEYWORDS*:* Buffalo farming, sub-tropical belt, controlled breeding, population decline.

INTRODUCTION

Buffalo (*Bubalus bubalis*) farming is popular in south-east Asia and south Asia. The buffaloes are multipurpose animal and contributes to a large portion (55 percent) of milk for dairy industry in India (Banerjee, 1991). Buffalo meat popularly known as "*buff"* and *"sikuti"* when fresh and processed respectively, is sold in supermarkets in Nepal fetching premium price.

In contrast to cattle, with their ubiquitous distribution all over the globe where there is human settlement, buffaloes are found only in certain regions. The distribution of buffaloes is principally in Asia and some Mediterranean countries and part of Eastern Europe. Similarly, buffaloes in Bhutan are found only in warmer sub-tropical belt *viz.* Chukha, Samtse, Sarpang, Tsirang and Dagana *Dzongkhag* (district).

The buffalo population in Bhutan was 28,000 heads in 1984 (FAO, 1984). But over the last two decades the number has gone down drastically by 26,200 heads. The present population is only about 1,800 heads (MoA, 1999). Joshi (2007) also pointed out that there has been significant decline in buffalo population in Bhutan from 1986 to 1999, which calls for an investigation.

In Bhutan, buffaloes are used for milk, meat, manure and are also used as draft animal. They give higher milk yield and have high fat content in milk as compared to local Siri cattle (*Bos indicus*). Higher fat percent enables farmers to obtain more butter for sale thereby increasing their income.

Despite several benefits farmers derive from buffaloes, in the recent years, farmers are giving up buffalo farming for unknown reasons. Moreover, buffalo husbandry practices and it's importance are not documented. It is therefore, felt that some research based studies on buffalo husbandry practices, it's roles to the farming communities and reason for buffalo population decline needed to be carried out. The objectives of the study were:

- to describe traditional buffalo husbandry practices in selected *Dzongkhags* of Bhutan
- to understand the role of buffalo to farming households/communities
- to understand the reasons for rapid decline in buffalo population.

MATERIALS AND METHODS

Study area

The study was carried out in two *geog* (block) each of Sarpang, Tsirang, Dagana, and Samtse *Dzongkhags*. The *Geogs* and villages were selected based on buffalo population density and farmers involvement in buffalo farming.

Sampling techniques and data collection

Two *Geogs* with high buffalo population from within the buffalo farming *Dzongkhags* of Bhutan were selected. Ten buffalo farming households each from the two selected *Geogs* of Sarpang, Tsirang, Dagana and Samtse were purposively sampled (table 1). Targeted sampling was done by listing farmers who rear buffaloes in that particular areas and only these farmers were interviewed to get authentic information.

Table 1: Sampled *dzongkhags*

Dzongkhag	***Geog selected***	***Households* (*n*)**
Tsirang	Kikhorthang, Cholingkhor	20
Dagana	Goshi, Tshandagang	20
Samtse	Dorokha, Ghumauney	20
Sarpang	Bhur, Umling	20

Semi-structured questionnaires were administered to collect information on buffalo husbandry practices and it's role. Informal discussions were held with some of the resourceful farmers to gather additional information on constraints and reason for decline in buffalo farming tradition in Bhutan.

Data Analysis

- Quantitative variables such as production and reproduction parameters were entered in spread sheet. mean, standard error (SE) was calculated.
- Qualitative data acquired through informal discussion, answers in many cases that fall into patterns with the same answer appearing frequently was coded and entered in spreadsheet. The frequency of each answer was sorted, counted manually and, when appropriate, converted to a percentage.

RESULTS AND DISCUSSION

Buffalo breed and herd dynamics

Breed types

In buffalo rearing *Dzongkhags* mostly local non-descript and their crosses with Indian *Surti* breed, a riverine type of buffalo is reared. Within the available buffalo population, farmers in some areas (*e.g.* Dorokha, Samtse) categorize the buffaloes into three types: *Kagay* with entire black body, *Hyakulae* with white or light grey stripes below the neck region and *Dobla* the cross between the local and *Surti* buffalo breed which has comparatively shorter horns than other types. As per fifth five year plan review document, 17 *Surti* buffalo bulls were supplied to farmers in the southern foothills by the then Department of Animal Husbandry (Planning Commission, 1986). Most of the existing *Dobla* type buffalo is believed to be the off-springs of those breeding bulls once supplied.

Herd dynamics

The average herd size in sampled households is around two buffaloes (2.46, n =45). In some lower altitude area (*e.g.* Ghumauney, Samtse) majority of buffaloes (90 percent) reared are males, bought from Sibsoo

and neighbouring India for draft purpose. But in mid-altitude areas such as Tsirang, Dagana and Dorokha there is no tradition of using buffaloes as draft animal. Farmers suggested that buffalo bulls are not suitable for ploughing in slopes and small terraces prevalent in mid-altitude. Thus, only females are retained in the herd while the males that do not have much utility are culled and sold. Only few household keeping large herds also keep good male(s) as breeding bulls.

Reasons for rearing buffaloes

Farmers stated various reasons for rearing buffaloes. Some of the reasons are tastier dairy products: milk, cheese, curd and whey due to higher fat content in milk; more manure production.

At the same time buffalo bulls are reported to have greater strength and are easier to train for draft purpose than cattle. In the plain areas bordering to the Indian towns, endurance of buffalo bull is being capitalized for ploughing fields. Besides, bullocks are also used for pulling carts to transport boulders and timber for construction and to take farm produce to the local markets. However, use of males for draft purpose is uncommon in mid-altitude areas.

Nevertheless, buffaloes have higher market value when sold. They are also used for appeasing deities in a form of sacrificial offering during the local festive occasions and religious ceremonies.

Reasons for population decline

Most farmers interviewed reported decline in buffalo population. The reasons for decreasing trend in buffalo farming practices and decline in population are:

- Unavailability of quality breeding bull
- Inbreeding that resulted from use of scrub buffalo bulls that are never castrated
- Controlled breeding, a common practice that prolongs calving interval, reduced calf crops. This coupled with frequent culling of males and infertile/sterile females for sale, did not allow the herd size to grow.
- Drying-up of wallowing ponds (water pools) due to settlements and other encroachments

- Fodder scarcity as buffalos consume more forage than cattle
- Labour shortage in the farm households

Reproduction and production parameters

Reproduction parameters

Buffaloes are seasonal breeders. The breeding takes place mostly from July to October and calving starts from June till September. But breeding can also take place during warmer months from March to May. However, Payne (1990) reported that autumn and winter season are found to be conducive for manifestation of oestrous in buffaloes in the tropics of India. This conveys that climatic variation in different places could affect the photo-period required for expression of heat in buffaloes.

Some farmers bred their buffaloes only in alternate year and prolonged the calving intervals intentionally (late calvers) while others followed the annual cycle (annual calvers). Through intentional delayed calving interval farmer harvest more milk per lactation. Nevertheless, it was at the cost of a calf crop.

Average age at first calving is 42 months with a range of 36 to 48 months for annual and late calvers respectively. The average gestation period is 311 days and lactation length 315 days (10.5 months). The inter-calving period range from one to two years for both pattern of calving with an average of 540 days (table 2).

Table 2. Reproductive parameters of buffaloes

Reproductive parameters	***Households interviewed (n)***	***Mean ± SE****
Age at first calving (months)	18	42±5.20
Gestation period (days)	10	311±4.70
Calving interval (days)	9	540±19.70
Lactation length (days)	9	315±69.20
Productive life (years)	12	14.3±3.60
Life span (years)	14	21.2± 2.30
No of calving in lifetime (no)	9	10.4± 1.30

* The figures are farmers' best estimates

The above table indicates that reproductive efficiency of buffaloes is better than local Siri cattle. According to Tamang and Perkins (2005) Siri cows bear their first calves at about five years and have short lactation length of about eight months. Payne and Hodges (1997) supported the view that the average age of first calving for *Bos indicus* cattle was about 60-63 months. Thus, parameters *e.g.* shorter age at first calving, longer lactation length makes buffalo a better dairy animal than local Siri cattle.

Production parameters

Milk production

Milk is the primary produce from buffalo. Joshi (2007) also had the opinion that buffaloes in Bhutan are primarily kept for milk production as about two third of total buffalo population consisted of females.

Milk production ranges from 2-5kg per cow per day depending on the level of management. This yield is higher than local Siri cattle which gives an average yield of about 1.5 litres per day (Arbenz and Tshering, 2000).

The estimated average lactation yield is 1260 litres for annual calvers and 2970 litres for delayed (controlled breeding) calvers. However, these yields, if computed to standard lactation period of 305 days, are lower than the *Surti* breed of buffaloes which is reported to yield 1772±10 litres per lactation (Banerjee, 1991). Thus, buffaloes in Bhutan if improved further by crossbreeding with good breeds such as Murrah and Surti, it is likely to produce more milk and compete with exotic-cross cattle in milk production.

Marketing of buffalo and it's products

Dairy and other products

Milk produced is processed locally and converted into curd, cheese, butter and whey. Milk is not reported to be sold as fresh. Buffalo milk and products are also not fit for offerings to deities.

Butter and cheese if produced in surplus is sold in the local market to generate cash income. Depending on accessibility and closeness to market, a kilogram of butter is sold for Ngultrum (Nu) 120-180 per kg

and a cheese ball of approximately 60gms for Nu.7-10. The curd and whey is used mostly for domestic consumption. Although buffalo butter is not different from that of cattle nutritionally, farmers reported that consumers prefer yellow butter and are unable to sell buffalo butter at par with that of cattle. Yellowish butter from cattle is due to traces of β carotene, the precursor of vitamin A while in buffalo milk, β carotene is fully converted to vitamin A which is why it is colourless.

Meat is another product from buffalo. However, many higher caste Hindus who rear buffalo are forbidden to eat buffalo meat (*carabeef)* so they cull and sell males and infertile buffaloes to other lower caste people for slaughter and consumption. Fresh buffalo meat fetches Nu.70-80 per kg.

Hides are very thick and are used in making sole of boots, covers for knives, or make ropes for traction. Hide is used to make ancient warrior's shield (Hasrat, 1980).

Market values of live buffaloes

The farmers reported that buffaloes are sold and bought from among, families, friends and neighbours. An adult buffalo cow fetches as high as Nu.16000 (1 US$= 40 Nu.). The value of heifers and young bulls ranges between Nu.8000-12000. A breeding bull also fetches about Nu.12000. A pair of bullocks could cost as high as Nu. 23,000 (table 3). According to farmers, high price for buffalo bullock is due to it's multiple uses: ploughing, carting and for meat purpose.

Table 3. The market value of different categories of live buffalo

Category	***Price (Nu.)***	***Criteria for pricing***
Lactating buffalo	12000-16000	Milk yield, no of lactation
Pregnant buffalo	12000-14000	Milk yield, stage of pregnancy
Heifers (2-3 years)	8000-12000	Health and dam's milk yield
Young bulls (2-3 yrs)	8000-10,000	Meatiness (live weight)
Dry/infertile	8000-10000	Meatiness (live weight)
Breeding bull	10000-12000	Health and libido
Castrated bullock (pair)	18000-23000	Health
Calves	4000-6000	Health and body weight

The market value of an adult buffalo cow is comparable to *Jatsham*-Mithun (*Bos frontalis* ***x*** Siri, 50:50), which is reported to fetch about Nu.15, 000-18,000, and is almost double to that of local Siri cattle (Tamang and Perkins, 2005).

Buffalo husbandry and health

Feeding

The suckling calves are retained in the shed and stall-fed with tender green fodder. Rice bran, maize grit and hulls are cooked and fed in a form of a porridge/gruel locally called *kholay.* When the calves are about three weeks old, they are provided with some green fodder and this practice is continued till they are weaned.

The adults are let loose to graze with other livestock. Lactating buffaloes are provided with little amount of *kholay* and some green grass/fodder as supplementary feed to the day's grazing. Working bullocks are also provided local grasses/straw, salt and gruel occasionally during working season. Dry cows, heifers and bulls are not eligible for supplementary allowance except some salt once or twice in a month.

Husbandry practices

Many farmers are of the opinion that buffalo need no shelter. But it is found that those farmers in mid-altitude who rear buffalo in the homestead provide shelter especially to milking animals. Moreover, buffalo calves are always kept inside the shed all the time till they are weaned (12- 15 months of age). After weaning they are tethered along with some other buffaloes either inside or outside depending on weather condition and availability of shed. Buffalo bulls, dry cows and heifers are often found tethered in the open farmland without any shelter through-out the year, exposed to harsh weather condition. As revealed by buffalo farmers, such practice cuts down the rearing costs. Farmers can tie the animals in the cultivable land. The dung being directly deposited in the field, the farmers benefit by saving time and labour costs if they have to carry the manure to the field from the shed.

Heat stress management

In the plain areas bordering to the Indian towns, buffalo bulls are used for ploughing and for pulling carts. Farmers reported that buffalo bulls

have greater strength and are easier to train for draft purpose than cattle. But it is also reported that buffalo bullock should be put to work only during mornings and evenings. During the days when the sun is bright, their dark skin and sparse coat of hair absorb great deal of solar radiation and get stressed within few hours. Poor heat tolerance due to inadequate development of body cooling mechanism is attributed to it (Payne, 1990). Access to water and shed is essential to overcome the heat stress. Most farmers thus provide water ponds to let animals wallow during the hot weather.

Buffalo health

Farmers generally believe that buffaloes are more resistant to disease than cattle. However, buffaloes are found to susceptible Foot and Mouth Disease, Haemorrhagic Septicaemia, bloat and parasitic infestation. Mortality of calves due to worm infestation is also is reported. Payne (1990) supported the view that round worm (*Neoascaries vitulorum)* transferable from dam to foetus during pregnancy or through milk to the young buffalo calf causes considerable mortality in buffalo rearing countries.

Management constraints

Genetic improvement

Most farmers believe that availability of good buffalo bull is pre-requisite for buffalo herd improvement and have put up request to the government agencies for the same. But in the absence of policy support to supply buffalo breeding bulls the farmers donot have a good opportunity to rear better buffaloes. Although Artificial Insemination (AI) is possible, buffalo semen was also not supplied to the AI centres and such a facility is not available either.

Feed scarcity

Fodder scarcity is reported to be a problem in the vicinity of the villages. Over the years there had been an increase in the time required for fodder collection.

Shrinking of grazing area and drying up of wallowing pond (water pools) due to human settlements and other encroachments has created inconvenience for rearing buffaloes.

Labour shortage

Many farmers said that labour shortage is an emerging issue. Changing expectation and lifestyles of young people often means they are not keen to remain farmers. They prefer to live in towns and look for easier jobs. Most children who help the family in herding must also attend school. Thus, the limited labourer available in small isolated communities strongly affects management options.

CONCLUSIONS

Buffalo in Bhutan are primarily used for milk production though it also has other utilities. Higher milk yield than local Siri cattle, easy management and market value suggest that rearing buffalo could compliment and supplement dairy development efforts of Royal Government of Bhutan.

However, for the last two decades, little or no attention is given to encourage buffalo farming in Bhutan. This resulted in decrease in quality and number of buffaloes to a significant proportion. Thus there is a danger that rapid decline may result in extinction of buffalo population and loss of domestic animal bio-diversity in Bhutan.

In order to boost farmer's income and at the same time conserve the scarce buffalo population it may be worthwhile to revive buffalo farming in potential areas. This could be done by broadening the scope and prospect of buffalo rearing through policy and technical support.

The study recommends the following:

- Support the policy of supplying quality breeding bulls or progeny tested semen to improve buffalo production.
- Diversify utility of buffaloes to bring greater benefits to the rural communities such as draft, dairy products and dried *carabeef.*
- Provide training on fodder development and buffalo husbandry practices periodically

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Factors Influencing Species Composition Of Improved Temperate Pastures Grazed By Dairy Cattle

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ABSTRACT

A study was carried out in Bumthang to investigate factors influencing species composition of temperate pasture under grazing management.

Four geogs under Bumthang dzongkhag were selected for the study. The principle of plug sampling technique was employed for estimating plant densities of sown species. A total of 1365 turf cores were collected from the sampling sites. Soil samples from 5-15cm depth were collected from the same point where turf cores were collected. The dried samples were analysed for available P (Phosphorus), K (Potassium), pH, moisture, and texture in the Soil and Plant Analytical Laboratory at Semtokha. Slope and elevation of individual fields were recorded.

Soil moisture, available P and soil pH influenced the tiller density of Italian ryegrass in temperate pasture. Available P also influenced the growing point density of white clover. Cocksfoot was not affected by any of the soil and topographic factors; however, the tiller density of cocksfoot was higher on sloping pastures. This indicated the comparative advantage of cocksfoot over other pasture species to thrive under droughty conditions. Old paddocks displayed acidic pH and lower levels of available K, which can be attributed to greater removal of K from old paddocks.

KEY WORDS: White clover, cocksfoot, tall fescue, Italian ryegrass, tiller density, growing point, pasture.

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INTRODUCTION

A desired species composition in forage mixture is vital to achieving better productivity and sustainability of established dairy pastures. Since the initiation of germplasm evaluation in the early 1970s (Roder *et al.* 2001), introduced species were tested for yield and persistence in forage mixtures across a range of conditions in temperate environments of Bhutan. Roder *et al.* (2001) identified and recommended white clover (*Trifolium repens*) cv. Ladino, cocksfoot (*Dactylis glomerata*) cv. Amba, tall fescue (*Festuca arundinacea*) cv. Barcel, and Italian ryegrass (*Lolium multiflorum*) cv. Lipo for inclusion in the standard forage mixture for temperate environments. However, the composition of these species in a mixed stand has been variable across dairy farms. Especially, Italian ryegrass persisted only under specific conditions (Roder *et al.* 2001).

Studies on temperate forage mixture by Roder *et al.* (2001) suggest defoliation management as one of the causes of variation in species composition. Management influences pasture composition via impacts on the competitive interactions between species (Kemp and King 2001). Soil fertility level is also a major determinant of species distribution (Andrew and Johanson 1978). However, the management and edaphic factors associated with variation in species composition have not been adequately explored under the prevailing conditions. Due to lack of scientific evidence, the causalities of inconsistent species composition in temperate pastures in Bhutan remain unclear. Improved understanding is therefore needed as to which factors influence species composition particularly under farmer managed conditions.

This study was carried out to investigate species dynamics at particular point in time in pasture mixture in relation to soil and topographic factors in Bumthang. The objective is to investigate factors associated with the variation in composition of recommended species in grazed dairy pastures under farmer managed condition.

MATERIALS AND METHODS

Sites and pasture establishment

Four *geogs* (administrative blocks) under Bumthang *dzongkhag* (administrative *district*) were selected for the study in October 2004. The field sampling included only those pastures sown with white clover, cocksfoot, tall fescue and Italian ryegrass. Due to rugged terrain, most paddocks were spread across slopes with gradient ranging from 0-35^{o}. Mean monthly temperature and precipitation of Bumthang over the last eighteen years is presented in Table 1.

Table 1. Mean monthly temperature and precipitation of Bumthang (1985-2003)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Tempera -ture (^{o}C)	4.5	6	8.5	11.5	14.5	17.5	18	18	16.5	12.5	8.5	5.5
Rainfall (mm)	7	10	29	50	83	126	141	137	94	53	8	6

Source: RNR Statistics 2003

The recommended quantities of seed of individual species in a seed mixture per acre of land were 4kg white clover, 4kg cocksfoot, 4kg tall fescue and 8kg Italian ryegrass (Roder *et al.* 2001). Species were broadcast sown and the seeding rate varied from 10-30kg per acre. Irrespective of paddock size, 41 pasture fields were selected for field sampling. Botanical assessment and soil sampling were carried out in late summer.

Botanical assessment

Species composition was measured in terms of plant density since measurements of pant density are suited to determine species composition of sown pastures (Grant 1981). The principle of plug sampling technique (Mitchell and Glenday 1958) was employed for estimating plant densities of sown species. The metal sampler was modified to suit the plant densities of sown species (Mitchell and Glenday 1958; Grant 1981). The modified sampler of 2mm thickness, 15cm long and 10cm diameter was used for extracting turf cores. Depending upon the size of pasture field, the sampler was placed randomly 10 to 25 times per field. To avoid bias and to ensure uniform coverage of bigger paddocks, the technique of stratified random

sampling was applied. Approximately, 25 turf cores were collected from a field size of 4000m^2. A total of 1365 turf cores were collected from the sampling sites (Table 2).

Species on the turf cores were counted. An average species count was estimated for each paddock. Growing points m^{-2} is used for reporting plant densities of white clover and tillers m^{-2} is used for reporting plant densities of grass species.

Table 2: Number of turf cores collected from pasture fields in four *geogs* in Bumthang.

Geog	Total pasture fields	Turf cores
Chokhor	51	765
Tang	14	210
Chumey	15	225
Ura	11	165
Total	91	1365

Soil sampling

Soil auger was used for collecting soil samples. Soil samples were collected from a depth of 5 to 15cm as high percentage of root distribution is in 15cm depth of top soil under frequent defoliation (Macklon 1994). Soil samples were collected from the same point where turf cores were collected. Following extraction of turf cores, soil auger was driven into the soil up to the depth of 15cm and auger pulled out. Soil samples from 5-15cm depth were collected. Soil samples (10-25 samples per field) were bulked and thoroughly mixed and a final representative sample weighing 300g was collected. The samples were air dried at ambient temperature. The dried samples were analysed for available P, K, pH, moisture, and texture at the Soil and Plant Analytical Laboratory, Semtokha, Thimphu.

Slope and elevation of individual fields were recorded with clinometer and altimeter.

Interviewing dairy farmers

Dairy farmers were interviewed using semi structured questionnaires. Information on pasture age and management practices were collected. The field interview also gathered information on pasture establishment, fertilizer usage and pasture utilization.

RESULTS AND DISCUSSION

Data were analysed using the Minitab version 14. Field data were tested for equal variance and the normality of a dataset. The data of field variables were also plotted against one another to check whether some variables required transformation. The data for age, P, and K displayed skewed distribution and they were log transformed to meet the statistical requirement.

Regression analysis at 5% significance level was performed to investigate correlation between field variables and botanical components.

Results

Association between field variables

Soil moisture was not significantly correlated with any of the field variables but there was a significant (P<0.05) negative correlation between soil pH and pasture age (Table 3). Available P had a significant (P<0.05) negative relationship with elevation and slope. Elevation and slope together explained about a quarter of the variation in available P, and the interaction of elevation and slope was not significant. A significant negative relationship was also found between available K and pasture age. Pasture age explained over 16 per cent of the variation in available K.

Table 3: Relationship between field variables under grazing management. P and K are measured in mg/kg and moisture in %. Values in each cell represent the slope of a straight line equation.

	pH	Moisture (%)	Log P (mg/kg)	Log K(mg/kg)
Elevation (m)	ns	ns	-0.004*	ns
Slope (degree)	ns	ns	-0.04*	ns
Log Age (years)	-0.13*	ns	ns	-0.29***
pH	-	ns	ns	ns
Moisture (%)	ns	ns	ns	ns
Elevation x Slope	-	-	ns	-
R^2	10.8	0	23.9	16.4

ns, $P > 0.05$ *$P < 0.05$ ***$P < 0.001$

Association between pasture species and field variables

There was a significant (P<0.05) positive correlation between white clover growing point density and available P (Table 4). Available P explained 15 per cent of the variation in growing point density of white clover. There was a significant (P<0.01) positive correlation between cocksfoot tiller density and land slope. Paddock slope explained about a quarter of the variation in tiller density of cocksfoot.

Tall fescue showed a significant positive correlation with elevation (P<0.01) and available K (P<0.05) and a significant negative relationship with land slope (P<0.01) and pH (P<0.05). Overall, elevation, available K, land slope and soil pH explained over 35 per cent of the variation in tiller density of tall fescue.

A significant (P<0.01) positive correlation was observed between Italian ryegrass and soil pH, soil moisture (P<0.001) and available P (P<0.05). However, a significant (P<0.01) negative relationship existed between Italian ryegrass and elevation. Elevation, soil pH, soil moisture, and available P explained over half of the variation in tiller density of Italian ryegrass.

Table 4: Relationship between field variables and botanical components under grazing management. Values in each cell represent the slope of a straight line equation.

	White clover	Cocksfoot	Tall fescue	Italian ryegrass
Elevation	ns	ns	0.89**	-1.66**
Slope	ns	28**	-10**	ns
Log Age	ns	ns	ns	ns
pH	ns	ns	-222*	620**
Moisture	ns	ns	ns	252***
Log P	266*	ns	ns	120*
Log K	ns	ns	119*	ns
R^2	15.0	23.6	35.5	53.7

ns, $P > 0.05$ *$P < 0.05$ **$P < 0.01$ ***$P < 0.001$

Relationships between botanical components

Tall fescue was not significantly correlated with any of the botanical components (Table 5). There was a significant (P<0.05) negative relationship between white clover and cocksfoot. Cocksfoot explained over 11 per cent of the variation in growing point density of white clover.

Cocksfoot had a significant negative relationship with Italian ryegrass (P<0.01) and sedge (P<0.05). Altogether, Italian ryegrass and sedge explained a quarter of the variation in tiller density of cocksfoot.

Italian ryegrass was significantly and negatively correlated with cocksfoot (P<0.05) and local grass species (P<0.01). Cocksfoot and local grass species together explained over a quarter of the variation in tiller density of Italian ryegrass. The interaction effects were not statistically significant for the sown species and therefore they are not included in Table 5.

Table 5: Relationships between botanical components in grazed pastures. Values in each cell represent the slope of a straight line equation.

Species	White clover	Cocksfoot	Tall fescue	Italian ryegrass
White clover	-	ns	ns	ns
Cocksfoot	-0.59*	-	ns	-0.41*
Tall fescue	ns	ns	-	ns
Italian ryegrass	ns	-0.37**	ns	-
Sedge	ns	-0.28*	ns	ns
Broadleaf	ns	ns	ns	ns
Local grass	ns	ns	ns	-0.58**
R^2	11.1	25.2	0	28.8

ns, $P > 0.05$ *$P < 0.05$ **$P < 0.01$ ***$P < 0.001$

DISCUSSION

Among temperate grass species, Italian ryegrass displayed a strong response to soil moisture as indicated by significant increase in tiller density with increasing level of soil moisture. Italian ryegrass is

generally intolerant of drought (Singh 1986) and the study result shows the importance of moisture for persistence of Italian ryegrass in temperate pastures of Bhutan.

Italian ryegrass also showed positive relationship with available P. The response of Italian ryegrass to pH could be attributed to the increasing availability of P along a gradient of increasing pH from acidic to near neutral. Although this study did not show any significant association between pH and available P, Busman *et al.* (1998) mentioned that there is linear increase in available P with soil pH. Hopkins and Ellsworth (2005) mentioned that the P availability increases with pH from acidic to neutral. The positive association between the available P and the tiller density of Italian ryegrass would mean that Italian ryegrass is positively responding to the increasing level of available P along a gradient of increasing pH. A significant positive response to available P of Italian ryegrass could also be attributed to its efficient P utilization. Kemp and Blair (1991) reported Italian ryegrass as the most efficient species in terms of P uptake, which was associated with the high relative growth rate.

The finding of this study that the growing point density of white clover is positively associated with increasing P level supports the review findings of Roder *et al.* (2001) that adequate P level is necessary for successful white clover based pastures in Bhutan. White clover is well known as a plant that requires adequate levels of plant available P (Jackman and Mouat 1972). P is an important element in white clover nutrition and the amounts of P required varies among white clover lines (Caradus *et al.* 1980). Generally, in mixed swards, white clover is a poor competitor for soil nutrients owing to its shallow root system but the surface rooting habit of white clover results in higher P uptakes on P deficient soils (Caradus 1990).

Cocksfoot density increased while the density of tall fescue declined with increase in steepness of the land slope. Our findings show that the available P declined with increasing steepness of the slope indicating that the sloping pastures are of low fertility. Thus, the persistence of cocksfoot on sloping pastures can be attributed to its ability to tolerate soils of poor to medium fertility. The low fertility on slope could also be a factor for decline in density of tall fescue on sloping pastures, since tall fescue based pastures require high fertility for better yield and are

usually invaded by other grasses if soil fertility is medium to low (Kemp *et al*. 1999).

The amount of available P decreased with increasing elevation. This is because farmers at higher elevations practiced inadequate SSP application due to inaccessibility to commercial fertilizers, lack of motor able road and financial constraints.

Old paddocks had lower levels of available K and acidic pH. Single superphosphate (SSP) is the most popular fertilizer applied by the Bhutanese dairy farmers. Due to sufficient reserves of K in most areas in Bhutan's Himalaya zone, and based on the poor results obtained earlier with K in the temperate regions (Roder *et al.* 2001), K fertilization in pasture has not been recommended. Consequently, all households interviewed under cutting and grazing management systems did not apply K fertilizers following establishment. As a consequence, without external nutrient inputs, the pasture fields may have suffered from K decline over the years. However, K is an element that is taken up in excessive (luxury) amounts by plants under favourable conditions (Brady 1995). Continued defoliation over the years without K inputs to replace losses may explain the negative association between available K and pasture age in grazed pastures in Bumthang. In an experiment conducted by Moloney and Conway (1963), the amount of K extracted by the older pasture was greater than that of the new pasture especially when the sward height was 8-12 inches. Thus, removal of herbage from older pasture would remove more K from the pasture field and reduce the level of K in the soil.

CONCLUSION

Nutrient status influences the species composition of dairy pastures and nutrient level of pastures is influenced by management. In most fields, the species composition is rather unbalanced and calls for better management in terms of soil nutrient and grazing management. Emphasize is required in the future research to quantify the effect of potassium level on species composition as the present study revealed significant decline in potassium level in older pastures. As emphasized, research in the future should continue to focus on phosphorus with

refined strategies to embark on organic sources of phosphorus. Above all, the changing aptitude of farmers and economic opportunities needs equal consideration since the associated social and cultural factors play important role in the overall pasture production and management.

The study is conducted in Bumtahang dzongkhag and findings should not be considered applicable in the other dairying regions. However, it indicates the need to conduct similar studies in the major dairying regions where improved pasture is a major source of fodder.

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Epidemiological investigation of the 2005-2006 Rabies outbreaks in animals in Eastern Bhutan

Tenzin[1], Jambay Dorjee[2] and Karma Rinzin[3]

ABSTRACT

The objective of the study was to describe the epidemiology of rabies outbreak that occurred in eastern Bhutan between May 2005 and November 2006. Using data collected during the entire outbreak period, the epidemic curve and choropleth maps were used to describe the temporal and spatial patterns of outbreaks. Incidence risk of animal rabies, expressed as the number of cases of rabies per 10,000 animal populations at-risk with 95% confidence interval was used to describe the animal pattern of rabies. The florescent antibody test was used to diagnose cases.

The outbreak affected 16 geogs within Tashiyangtse, Trashigang and Mongar districts. There were a total of 136 cattle, six horse and about 110 dog deaths. Of these three districts, Trashigang experienced more cases, followed by Mongar and Tashiyangtse. It was observed that the first case of rabies was reported in two geogs (Khamdang & Toetsho) within Tashiyangtse district that share a common border with Tawang town (Arunachal Pradesh, India). Apparently, the disease, then spread to other nearby geogs within Trashigang and Monger districts located close to the infected areas.

The epidemic (monthly number of cases reported) for the entire eastern region peaked during February 2006 and subsequently subsided by November 2006. The epidemic for Trashigang and Mongar peaked in February 2006, while it peaked in April 2006 in Tashiyangtse.

The incidence risk (IR) in canine was 249 (95% CI: 189-328), 114 (95% CI: 78-166) & 104 (95% CI: 75-144) in Mongar, Tashiyangtse and Trashigang respectively. The incidence risk in bovine was 13.4 (95% CI:

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9.9-18.3), 20.0 (95%CI: 13.8-28.8) and 22.0 (95%CI: 17.4-27.9) and the IR in equine was 11.3 (95%CI: 3.8-33.2), 10.1 (95%CI: 2.8-36.6) and 2.0 (95%CI: 0.3-11.2) in Mongar, Tashiyangtse and Trashigang districts respectively indicating higher high risk of infection in dogs.

KEYWORD: Epidemiology, Rabies, outbreak, eastern Bhutan

INTRODUCTION

Rabies is an invariably fatal zoonotic disease caused by rabies virus. It is transmitted mainly by the bite of an infected host (Kaplan et al., 1986). The disease is widespread throughout the world. Only few countries (mostly island nations) have eradicated it or are historically free from disease. Domestic dogs act as the main reservoir and vector of infection in Asia, Africa and Latin America while sylvatic (wildlife) rabies is common in Europe, North America and parts of Central America (WHO., 2004). In Bhutan, rabies is a notifiable disease and it is mandatory to report the disease even if suspected.

Rabies is still a public health problem. Human death from dog rabies was estimated to be 55,000 deaths per year with 56% of the deaths estimated to occur in Asia and 44% in Africa (WHO, 2004). The annual cost for the post exposure treatment in human was estimated at US$ 583.5 million (Asia: US$ 563 million, Africa: US$ 20.5 million) while the annual cost of livestock losses due to rabies is estimated at US$ 12.3 million in Africa and Asia. The total global expenditure for rabies prevention is over US$ 1 billion annually (WHO, 2004).

In Bhutan, rabies outbreaks are reported annually, particularly in the border towns and villages of Samdrup Jongkhar, Sarpang, Chhukha and Samtse. Rinzin et al. (2006) reported a total of 477 rabies cases in Bhutan (241 cattle, 188 dogs, 25 pigs, 11 horses and 12 sheep and goats) between January 1998 to March 2006. However, three Dzongkhags in the east, Tashiyangtse, Tashigang and Mongar, for the first time experienced a major outbreak of rabies in animals between May 2005 and November 2006 after decades of freedom from the disease. During this outbreak, a total of 252 animals were infected and over 900 humans directly or indirectly exposed to the virus. One boy student in Kanglung

also died of rabies after being bitten by rabid dog. The cost of post exposure treatment in human in eastern Bhutan during 2005-2006 outbreak was estimated to be over Nu. 1.782million and the direct cost for the loss of livestock were estimated to be over Nu. 1.450million. The estimates were based on the number of people exposed to rabies, livestock death and its related costs (cost of vaccine). The Ministry of Health had spent more than Nu. 5.878million in the past five years (Kuensel, February 23, 2007) while the Department of Livestock spent over Nu. 1.800 million for the cost of anti-rabies vaccine for animal prophylaxis alone (Vaccine Production Unit, NCAH records., 2008). Therefore, rabies represents a significant expenditure to the Royal Government of Bhutan due to the cost associated with control measures, loss of livestock, human illness and post exposure treatment.

The objective of this study was to describe the spatio-temporal epidemiological features of the incidence of rabies cases and generate hypothesis for the spread of the disease between May 2005 and December 2007 in eastern Bhutan. The finding of this study is expected to help the policy makers in designing future response plan for rabies.

MATERIALS AND METHODS

Study area

The study area included Tashiyangtse, Trashigang and Mongar districts in eastern part of the country which experienced a major rabies outbreak in 2005-06 (Figure 1). There are a total of 40 geogs with 8, 15 and 17 in Tashiyangtse, Trashigang and Mongar districts respectively. Fourteen of the sixteen affected geogs have major road connections (including motorable roads) and they share common border with each other. Two geogs, Khamdang and Toetsho, which reported the first cases, are located close to the town of Tawang, Arunachal Pradesh, India.

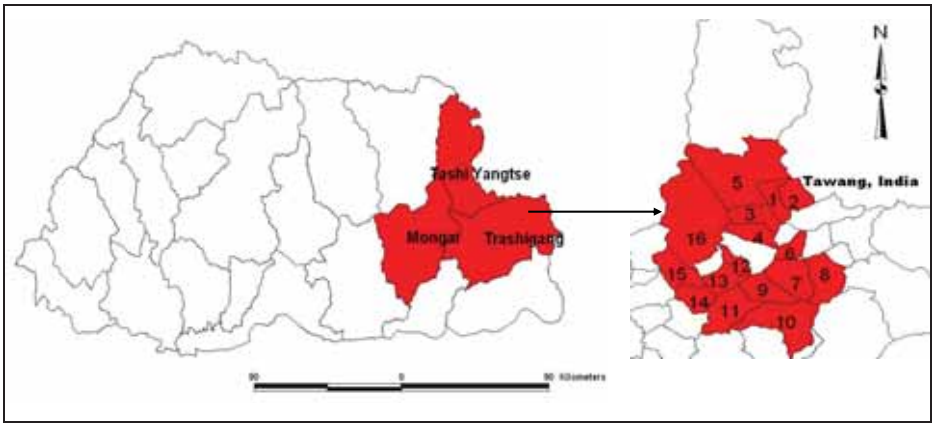


Figure 1: Rabies epidemic study region and 16 affected geogs in eastern *(1) Khamdang, (2) Toetsho, (3)Tongzhang, (4) Jamkhar, (5) Yangtse, (6) Bartsham, (7) Samkhar, (8) Shongphu, (9) Kanglung, (10) Khaling, (11) Uzorong, (12) Narang, (13) Dremetse Tshogom, (14) Chaskar, (15) Ngatshang, and (16) Sherimuhung.*

Case definition

A rabies outbreak was defined as a village/geog from which one or more clinical cases of rabies were reported. An outbreak was classified as confirmed when rabies virus was identified from brain tissue sample by fluorescent antibody test from one or more clinical cases. Only those animals that showed the symptoms of rabies and later died or got eliminated were included as cases in this study. Some dogs in the locality that showed aggressive behaviour and later eliminated before the progression of the disease were also included as the cases.

Data collection

Data related to the number of cases, number at risk, species affected, date and place of outbreak and probable source of infection were collected at the time of disease investigation and routine follow up visits in the affected areas for the period between May 2005 and December 2006. Reports received from the field units were also incorporated for the analysis.

Data analysis

The data were managed using Microsoft Excel for Windows Version 2003 (Microsft Inc, USA). The incidence (risk rate) of rabies in bovine, equine and canine species within the 3 study districts was calculated as (total number of cases) divided (÷) by the (total number of animals at the beginning of the outbreak)(Dohoo et al., 2003). Temporal epidemic

patterns were investigated by constructing an epidemic curve: number of reported cases per month, from May 2005 to November 2006. The spatial distribution was described by mapping the number of cases in each geog on a quarterly basis ((a) May-August 2005, (b) September-December 2005, (c) January-April 2006, (d) May-August 2006, and (e) September-November 2006), using Geographic Information System ArcView for Windows Version 3.2 a; (Environment Environmental Systems Research Institute, Redlands, California).

RESULTS AND DISCUSSION

Results

Animal pattern

Between May 2005 and November 2006, 252 cases of rabies cases were reported: 136 in cattle, 6 in horses and 110 in dogs giving different species specific risk (Table 1). Confirmed rabies cases (bovine and canine) were observed in all of the affected geogs. The incidence risk in dogs was higher than for the other species.

Table 1: Cases of animal rabies and incidence risk (expressed as the number of cases of rabies per 10,000 animal populations) in eastern Bhutan between May 2005 and November 2006.

Dzongkhag/Species	Cases	Population at risk	Incidence risk[a](95% CI)
Mongar			
Bovine	40	29793	13.42 (9.86-18.27)
Canine	49	1968	248.98 (188.84-327.63)[a]
Equine	3	2654	11.30 (3.84-33.18)
Tashi Yangtse			
Bovine	28	14022	19.96 (13.81-28.84)
Canine	26	2290	113.53 (77.59-165.84)
Equine	2	1986	10.07 (2.76-36.64)
Trashigang			
Bovine	68	30894	22.01 (17.36-27.89)
Canine	35	3371	103.82 (74.74-144.05)
Equine	1	5045	1.98 (0.34-11.22)

[a] Interpretation: A total of 249 (188.84-327.63) cases of rabies per 10,000 dogs were reported during the 19 months period (May 2005 to November 2006) in Mongar Dzongkhag.
CI = Confidence interval.

Temporal pattern

Figure 2 (a) shows the overall epidemic curve plotted from the beginning to the end of the rabies epidemic in three eastern districts between May 2005 and November 2006. It also shows the temporal patterns of rabies cases by months in Tashiyangtse, Trashigang and Mongar districts during the period (figure 2 (b,c,d).

The epidemic peaked during the month of February 2006 in Mongar and Trashigang while it peaked during April 2006 in Tashiyangtse. Overall, most cases were reported in February 2006.

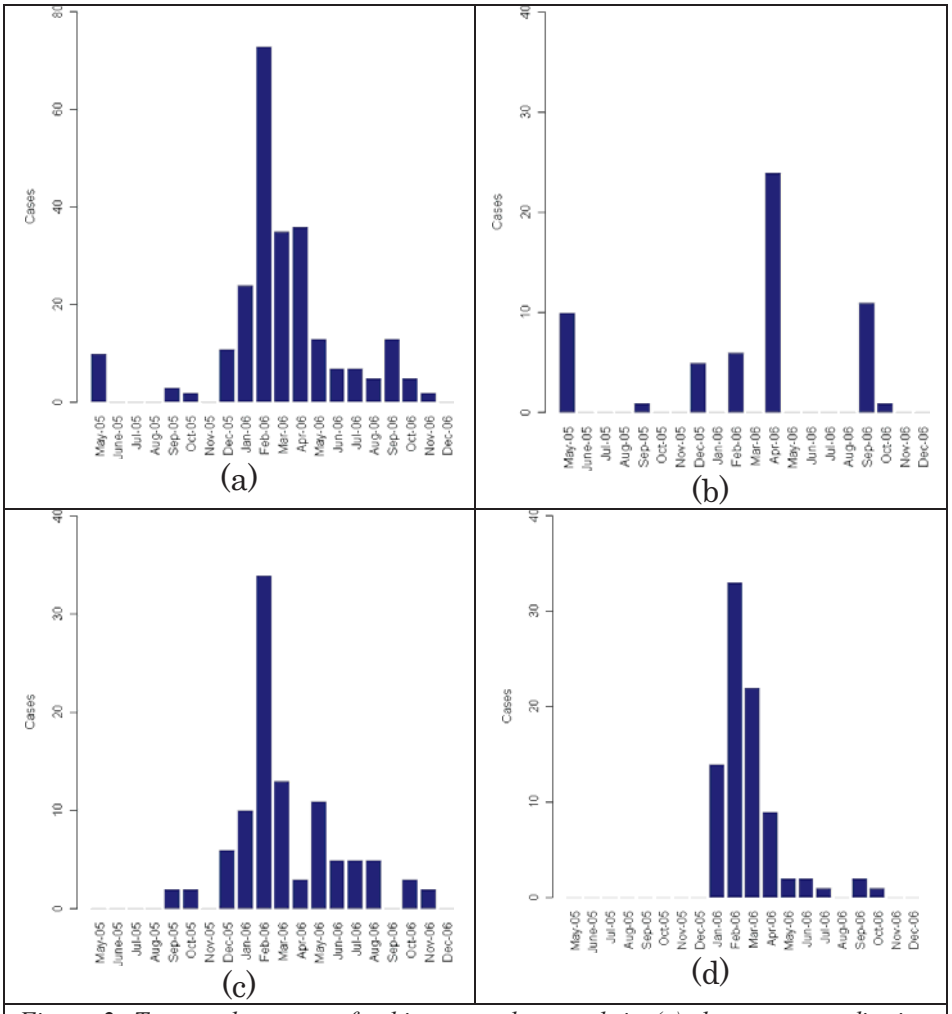
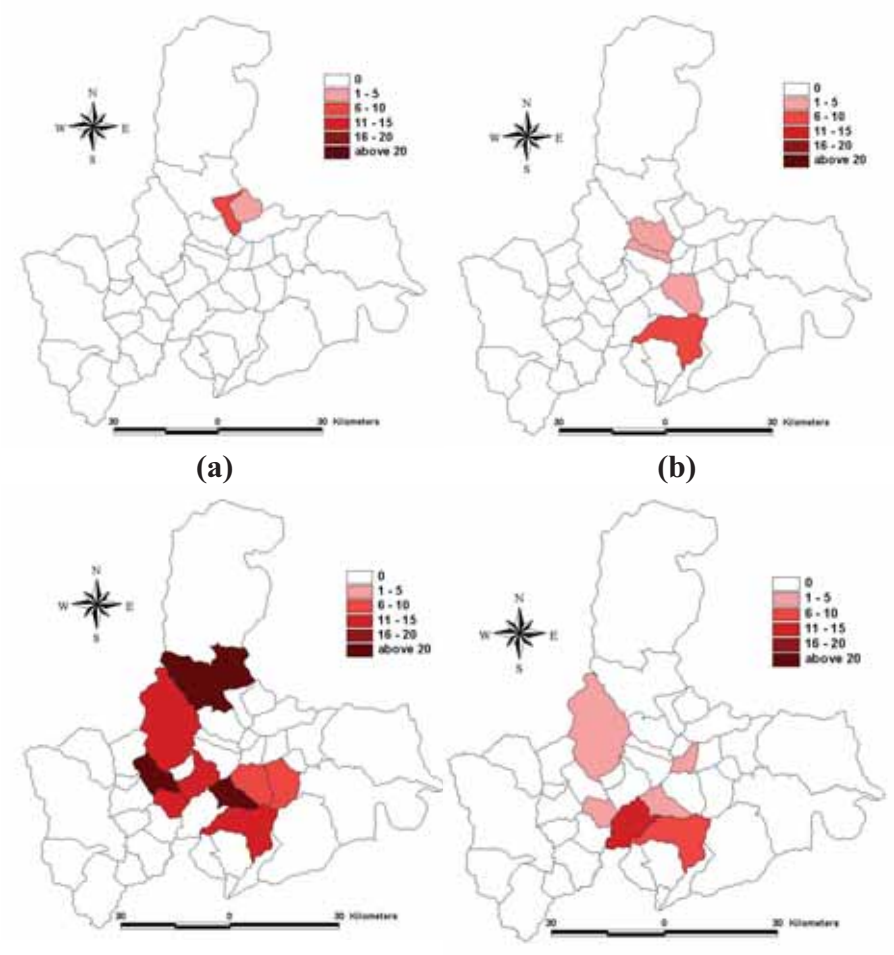


Figure 2: Temporal pattern of rabies cases by month in (a) three eastern districts (overall); (b) Tashiyangtse; (c) Trashigang & (d) Mongar.

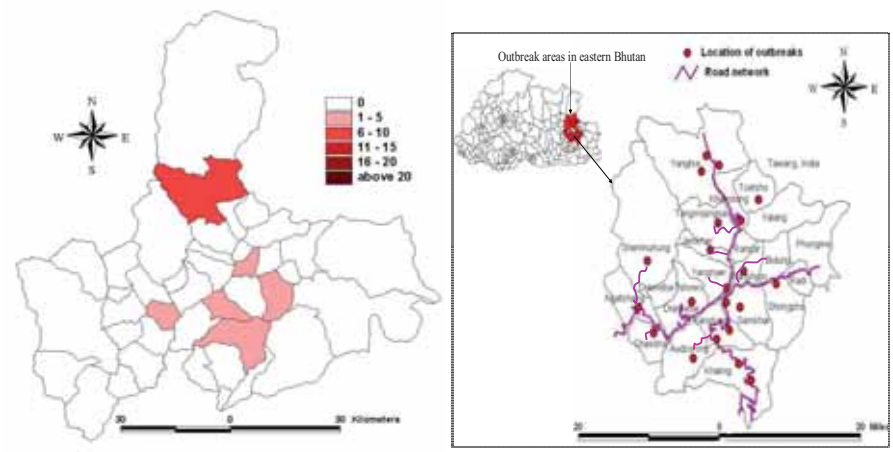
Spatial patterns

Figure 3 shows the number of cases per geog on a four monthly basis to identify the spatial patterns of occurrence of rabies in affected districts.



(a) **(b)**

(c) **(d)**



(e) **(f)**

Figure 3: Spatio-temporal pattern of rabies in animals in eastern Bhutan(May 2005 to November 2006). Choropleth maps showing the number of reported cases in geogs: (a) May-August 2005, (b) September-December 2005, (c) January-April 2006, (d) May-August 2006, and (e) September-November 2006, (f) Location of outbreak in each affected geogs.

The major spread of rabies occurred from December 2005 to April 2006 (figure 3 c). Kanglung, Khaling, Yangtse and Ngatshang geogs reported higher number of cases compared to others geogs (figures 3; c, d). Some geogs reported cases for more than eight months (figure 3; b, c, d & e). Overall, more cases was reported between January and April 2006 (Figures 1 & 2 c. [Please refer figure 1 for identification of geog].

DISCUSSION

Our findings showed that the first case of rabies was reported on 2 May 2005 at Khamdang geog in Tashiyangtse district. Following this, 15 of the 40 geogs in the three eastern districts (Figure 1 & 3) reported cases of the disease until the outbreak subsided in November 2006. Although the exact source of the disease is unknown, anecdotal evidence suggest the likelihood of spread from across the international border during the Gom Kora Tsechu in Tashiyangtse, which is an important religious festival and many visitors come from Tawang, Arunachal Pradesh, India (personnel communication of Trashiyangtse livestock staff). It has also been observed that the nearby geogs or places located close to the outbreak area experienced the additional cases, as is common with infectious disease that spreads spatially (figure 3). There are also

indication of space and time clustering of cases, but the significance of it was not tested in this study.

The incidence risk of rabies in dogs was high compared to other species of animals (Table 1), indicating high risk of infection. However, some measurement bias (possibility of including some non cases) might have resulted in an over estimation of this risk. Another reason for high incidence could be underestimation of the size of the (stray) dog population. For this estimation, we used the approximate population as collected from extension centres which may be not very accurate (stray dog census is not usually done). For accurate estimates of the risk, a reliable estimate of the size of the dog population (denominator) is necessary. This is also essential for instituting proper disease prevention and control program.

More cases of rabies occurred between January and April 2006 and might be attributable to: (i) translocation of stray dogs by people to places where rabies outbreaks were ongoing (often done to avoid the dog problem in their locality), (ii) availability of a large number of susceptible stray dogs in the towns, and (iii) increased bite incidences of domestic cattle by rabid dogs (domestic livestock are let -out to open fields in winter, since the cultivable land is fallow during the winter season). The presence of a major road network (accessibility), close proximity of villages or towns having large number of stray dogs resulted in a fast spreading epidemic. Non enforcement of elimination strategy of infected and exposed dogs from the locality due to aversion of the people against mass elimination was also one important factor for the difficulty in containing the spread of the disease. As an alternative control measure to the elimination program, a number of awareness programs were conducted in the Dzongkhag DYT meetings involving the *Meday Gothrip* (village leaders) and the farmers in each outbreak village. In addition, about 883 stray dogs were impounded in 13 enclosures (pounds) in the different affected geogs during April 2006. This action seems to have resulted in a decline in reported rabies cases in the region (figure 2 and 3). However, many impounded dogs escaped while some died. Impounding is not recommended for future control plans because of logistical problems, and most importantly the risk of introducing the disease into the pound (by dogs that are in incubation period of the disease or already infected dogs) and subsequent disease

spread within the impounded group. No cases were, however, reported within the enclosures during the particular episode.

Conclusion

Although the major outbreak has subsided, few sporadic cases are expected to occur in future as the virus could be maintained in the dog cycle. Sustained vaccination campaign covering more than 75% of the dog population could eliminate the virus from the three districts and regain the free status.

The experiences and the review of this epidemic suggest that there was a lack of clear rabies control policy and strategy in Bhutan or the understanding of the policy by the implementers as no awareness and education on the subjects were carried out.

Effective control of the disease in the future depends on clear policy guidelines, legal support and their implementation. Further, detailed study on rabies virus characterization is needed to identify the sources of the epidemic; risk factors related to it occurrence and the economic impact of the disease. The analyses presented in this paper are descriptive and provide a basis for further analytical investigations into the spatial and spatio-temporal aspects of rabies in Bhutan.

A method used for wildlife population estimatimation (captures, mark and recapture methods) and the recommendation of WHO would be useful to get the estimates of the size of stray dog population in Bhutan (WHO, 1996).

ACKNOWLEDGEMENT

The authors would like to thank the District Livestock Officers & the field staff of Tashiyangtse, Trashigang & Mongar for their support during the time of investigation. We would also like to thank the farmers and the Meday Gothrip for their cooperation and active participation in the control program.

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Design, Fabrication and Performance Evaluation of Wild Pig Repellent Device

Pema Dakpa[1], Ugyen Penjore[2] & Thinley Dorji[3]

ABSTRACT

The indigenous wild boar repellent was developed based on intermittent production of simultaneous shrill sound and bright light. The shrill sound is produced by the electric horn with a frequency of 480 Hertz, sound pressure of 100 decibels and acoustic range of 300m. The bright light is produced by 500-watt inflorescence bulb. After a series of modification by the National Post Harvest Centre, the final prototype developed has overcome the low amperage problem of rural electricity and humid conditions in the farms. The energy consumption of one set of repellent was found be only 0.25 kilo watt per hour, which amounts to 3 kilo watt per 12 hour night. The repellent has proven effective in keeping the wild animals away from the farmers' field since there was no crop damage reported when the repellent operated smoothly. In addition, 95 percent of the respondents (collaborating farmer) rated the performance of the machine as excellent and only 5 percent rated the frequency of breakdown of repellent as high. Crop damage did occur when the repellent malfunctioned, that is, when there was no sound or light. The effect of repellent on domestic animals was reported to be negligible. Thus, this indigenous repellent is suitable as a short-term crop protection measure against wild animals.

KEY WORDS: Wild animal, Crop damage, Repellent, Prototype, Performance, Energy

INTRODUCTION

Bhutanese farmers grow a wide variety of crops including maize, wheat, paddy, barley, potatoes, peas, vegetables, apples, citrus, etc. for their own consumption and for sale in the market. However, wild animals (example: wild boar, deer, sambar, monkey, bear, elephant) depredation

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of crops is one of the main constraints faced by farmers. Farmers spend considerable time guarding crops against wild animals by using devices such as beating empty tins and drums, blowing indigenous horns, etc. during day and night.

The most common and dominant wild animal pests are wild boars. Aaken (1997) found that due to wild boar problem 23% of farmers of Zhemgang stop growing paddy and 39% of farmers there stopped cultivation in dryland.

Wild animals including sambar, bear, elephants, and porcupine normally damage the crops during late night especially when the farmers are asleep. Other pests like monkey damage the crops during the day. The wildlife damage of crops contributes from 31% to 35% of food shortage during the year (Planning Commission Report, 2007). The maximum time spent guarding the crops was 59 days for maize followed by 54 days for paddy (Choden et al., 1996; NRTI, 1996).

Further, many control methods like poisoning, shooting, trapping, fencing, supplementary feeding were tried in areas where wild boar and feral pigs, pigs living wild with domestic ancestry, are considered an agricultural menace but those controls have not always been successfully implemented because of some disadvantages (Izac and O'Brien, 1991).

The target of reducing rural poverty by half within 10^{th} FYP may be difficult to realize if chronic wild animals issue is not properly addressed by looking at both long and short-term control measures. Therefore, the National Post Harvest Centre initiated a study to design, fabricate and evaluate the performance of the repellent against wild animals with main focus on wild boar to prevent crop damage and lessen the burden of night guarding of crops.

The specific objectives of the study were;

- to develop low cost wild boar repellent using electricity
- to assess effectiveness of the repellent against other wild and domestic animals
- to determine the energy cost of the repellent

MATERIALS AND METHODS

Design, description and operation of the repellent

The design principle of wild boar repellent is based on the intermittent production of simultaneous shrill sound with a frequency of 480Hz, sound pressure of 100 decibels and acoustic range of 300m and bright light. The main parts of the repellent were electricity supply source, control box, inflorescence bulb and electric horn (Fig 1). The control box draws electricity power from the main supply source and provides required alternating current voltage to the inflorescence bulb and direct current voltage for electric horn through switch module power supply (SMPS).

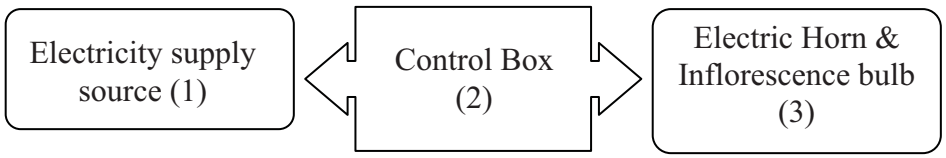

Fig 1. Schematic diagram of repellent

Fabrication of the repellent

The repellent was fabricated using the locally available raw materials. The control box fabrication consists of wooden box with plastic cover, miniature circuit breaker, auxiliary contactor, electric blower, timer and thermostat. The sound and light production unit consists of two 500 watt inflorescence bulbs, SMPS, electric horn, tin and wooden box with plastic cover. The electricity supply was provided from the farm house using weather-proof electricity cables. The 500-Watt inflorescence bulb produces bright light and vehicle horn produces shrill sound with a frequency of 480 Hertz, sound pressure of 100 decibels and acoustic range of 300m intermittently.

Installation of the repellent

Ten sets of repellent were set up at seven different locations in collaboration with interested farmers and concerned Dzongkhag and Geog extension officers (Table1). Four sets were used in Berti Village to encircle the whole village.

Table 1. Location of repellent installation

Sl. No.	Location	No of sets	Installed date	Crops cultivated
1	Pangbisa, Lukni Geog, Paro Dzongkhag	1	May 29, 2008	Peas, Wheat, Potatoes, Apples
2	Berti Village, Trong Geog, Zhemgang Dzongkhag	4	July 27 ,2008	Maize, Paddy
3	Zampaling, Balam Geog, Mogar Dzongkhag	1	August 4, 2008	Maize
4	Bakpa village,Tomajang Geog, Trashiyangtse Dzongkhag	1	August 6, 2008	Maize, Paddy
5	Pam, Kanglung Geog, Trashigang Dzongkhag	1	August 7,2008	Maize
6	Hangay, Sibsu Geog, Samtse Dzongkhag	1	Sept 22, 2008	Paddy
7	Jogimara, Sibsu Geog, Samtse Dzongkhag	1	Sept 23, 2008	Paddy

The performance of the repellent was evaluated by interviewing collaborating farmers using structured questionnaires.

RESULTS AND DISCUSSIONS

Crops damaged prior to the installation of repellent

There were frequent damage of crops by wild boars and other animals such as monkeys, sambar, porcupine, bear and elephants in the pilot sites. Table 2 shows the damage of the crops by the wild boars and other animals prior to installation of repellent at trial locations.

Table 2. Crop damage observed prior to repellent installation

Location	Crops damaged
Pangbisa, Lukni Geog, Paro Dzongkhag	Peas and potatoes were damaged by wild boars
Berti Village, Trong Geog, Zhemgang Dzongkhag	- Mature maize and paddy field were damaged by wild boars - Transplanted paddy was damaged by sambar
Zampaling, Balam Geog, Mongar Dzongkhag	Mature maize was damaged by wild boars and monkeys
Bakpa village, Tomajang Geog, Trashiyangtse Dzongkhag	Matured maize and paddy field were damaged by wild boars
Pam, Kanglung Geog, Trashigang Dzongkhag	Mature maize was damaged by wild boars and porcupine
Hangay, Sibsu Geog, Samtse Dzongkhag	Cassava and transplanted paddy was damaged by wild elephants
Jogimara, Sibsu Geog, Samtse Dzongkhag	Transplanted paddy was damaged by wild elephants

Improvement of repellent

The initial prototype required modification to maximize the light coverage and to make it adaptable to low amperage which is typical in rural electricity supply. Three stages of modification were done. The bulb of the first prototype was stationary and light coverage was only 50% of the field area. There were evidences that wild boars sneaked through the darker area that was not lighted. Thus, a rotating device from an electric fan was incorporated in second prototype to ensure that light covered the entire area. However, after installation of second prototype in different parts of the country, high power fluctuation, insufficient amperage and extremely high relative humidity that is prevalent during the maize maturity and paddy transplantation stage, caused the repellent to malfunction occasionally. To overcome this hurdle, the third prototype used only one inflorescent bulb and an electric fan; while other parts like timer, auxiliary contactor and electric blower were removed.

Support of collaborative farmers

The collaborating farmers provided very good cooperation despite being skeptical about the outcome. They shared the cost by providing free labor to install the pre-fabricated repellent which involved collecting and erecting the necessary wooden poles for electric cables and the repellent. They shared stories of their past failed experiences such as trying to catch the wild boars, setting traps and using solar wires for wild elephants.

Performance of the repellent

Fig 2 indicates that 67% of the twenty one respondents reported no crop damage and 33% reported crop damage as low. There was no damage of crops when the machine was working properly but the damage occurred mostly when the repellent malfunctioned (Fig 3). This clearly indicated that the repellent agitated those wild animals that trespassed farmers' fields.

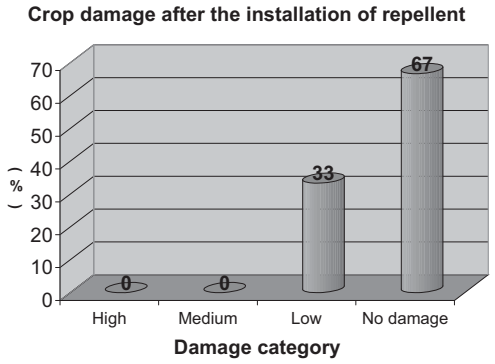

Fig 2. Crop damage condition

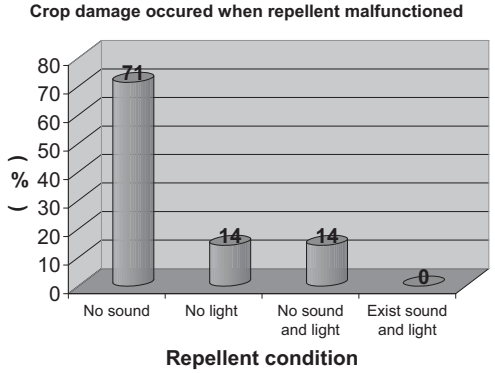

Fig 3. Repellent condition

90% of the respondents rated the performance of the repellent as excellent. No farmers rated the performance as poor (Fig 4). Moreover, only 5 % of the respondents rated break down of repellent as high and according to farmers the effect of the repellent on domestic animals is negligible (Fig 5).

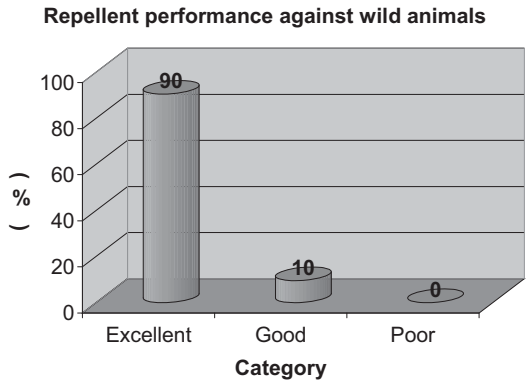

Fig 4. Repellent performance

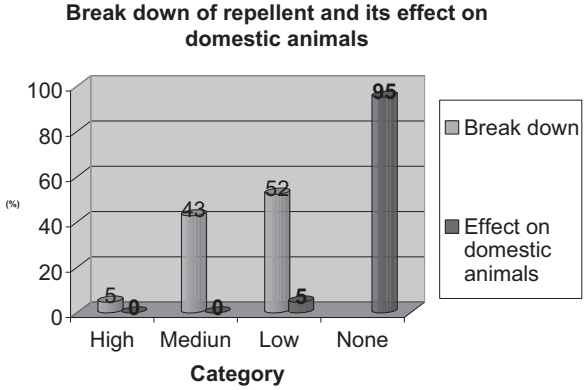

Fig 5. Repellent breakdown

The efficacy of the repellent was successfully tested for many kinds of wild animals including wild boars as shown in Fig 6. The most abundant wild animals in trial sites were wild boar followed by sambar.

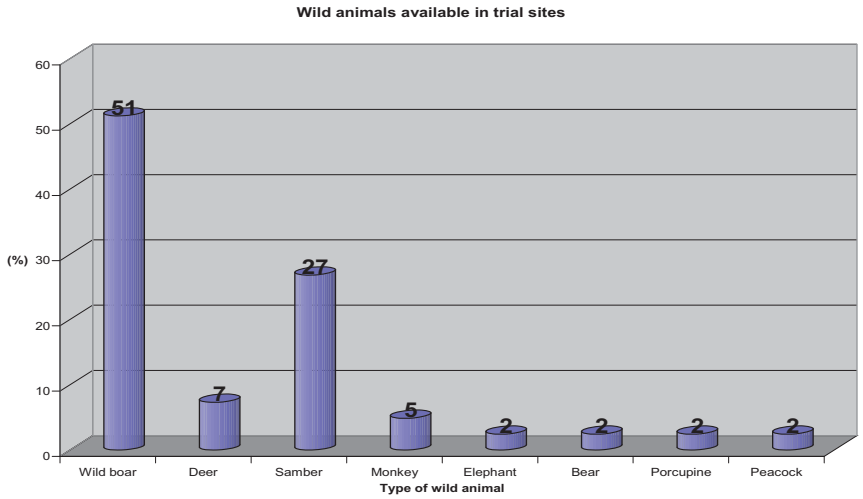

Fig. 6. Availability of wild animals

Nine varieties of crops were cultivated and protected successfully by the repellent in trial sites (Fig 7). The two dominant crops were paddy and maize.

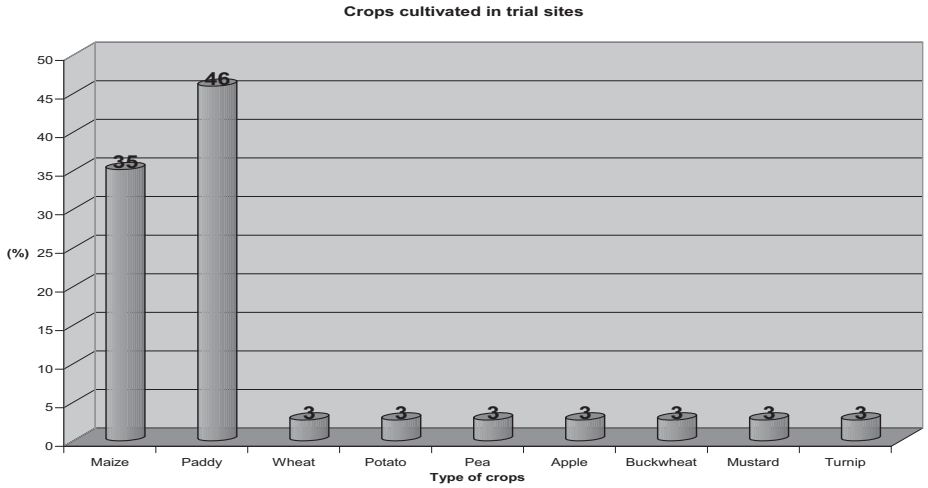

Fig 7. Type of crops cultivated

The repellent had been successfully tested for the last 9 months at Pangbisa, 6 months at Berti Village and 3 to 4 months at other sites. Hence, it can be concluded that wild animals do not get accustomed to the repellent.

In short, the indigenous repellent has overcome the disadvantages experienced in other control methods such as the effect of repeated vomiting prior to death on non-targeted species using poisoning method (O'Brien and Lukins, 1990), disappearance of animals at the sound of the shot and the difficulty of shooting in wooded, wet or marshy terrain (Brown,1985), trap shyness where the animals did not enter the traps but ate the food outside the baited traps in Trapping methods (Saunders *et al.* (1993), high initial cost and heavy maintenance program to prevent the grass and undergrowth shorting out the current in electric fencing (Hone and Atkinson, 1983) and requirement of fencing and facilitating the increase of wild boar population numbers because of availability of additional food conditions in supplementary feeding methods (Mackin,1970).

Installation and energy cost of the repellent

After a series of modification, total cost of materials for one unit was reduced to Nu. 10,000.00; materials (Nu. 6600) and 200 m electricity cable (Nu. 3400.00). The costs of wooden materials and labor were excluded for they were provided free by farmers themselves. If required, two additional units can be installed to the same controller and power supply. The cost of material for one additional unit is Nu.7, 000.00.

The electricity energy consumption of the repellent with single unit was found to be only 0.25 Kilo Watt per hour, which amounts to 3 Kilo Watt per 12 hours night. The total energy consumption for 12 hours of operation (every night) amounted to 90 Kilo Watt Hour in a month. The total energy consumption, after adding this energy consumption to normal household energy consumption falls under the current power tariff range, 80-300 Kilo Watt Hour (Kwh). Thus, the energy cost of running one unit repellent for one month was Nu.122.00 only (Nu. 1.35 per Kwh x 90 Kwh), which is reasonable to most farmers of Bhutan.

CONCLUSION

The indigenous wild boar repellent designed and evaluated has proven effective for keeping the wild animals at bay since there was no crop damage reported when the repellent was operating smoothly. In addition, majority of the respondents rates the performance of the machine as excellent. The damage of crops by wild boars and other animals when

the repellent malfunctioned clearly proved that the repellent distracted those wild pests that have trespassed into the farmers' fields. Furthermore, the effect of repellent on domestic animals was reported negligible.

Through series of modification during the trial, the final prototype suitable to rural farmers of Bhutan was developed. The operation was simplified by removing the timer so that the farmers can manually switch on the repellent in the evening and put off the repellent in the morning. This prototype has overcome the low amperage problem and high relative humidity environment of the farms. Further, the energy consumption of one set of repellent was found to be very reasonable.

Farmers in all trial sites have harvested the crops with very minimal wild animal damage and also enjoyed proper sleep after hard days work. Based on the success of the trial, the following recommendations are made:

- Rubber strip used as controlling the speed of fan need to be improved,
- There is a need to find an alternative for SMPS, since SMPS is very sensitive to humidity,
- There is need to find an alternative to electric horn since electric horn last for about 3 to 4 months only,
- This indigenous repellent is a suitable device to keep wild animals from entering farms and should be adopted as a short-term control measure.

ACKNOWLEDGEMENT

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Pharmacognostic Study of *Vangueria spinosa* (Roxb.) Hook.: An Important Medicinal Drug

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ABSTRACT

Vangueria spinosa (Roxb.) Hook. syn. Meyna laxiflora Robyns. (Rubiaceae) is a medicinally important plant being used in the different indigenous systems of medicine such as Ayurveda, Siddha, Unani, Tibbi and Amchi. Since pharmacognostic study of the plant is not reported so far, the present investigation involves its macroscopic and microscopic studies such as quantitative microscopy, percentage extractives, ash values, fluorescence analysis, histochemistry and phytochemistry of the leaf and stem of the plant. Findings will be useful in correct botanical identification, detection of adulteration and screening of active phytochemicals to increase the efficacy of the drug. Crude extracts of the leaf and stem drugs exhibited the presence of both primary and secondary metabolites. Quantitative estimations prove the plant to be a rich source of the active phytochemicals such as tannins, saponins and alkaloids for pharmaceuticals and for indigenous medicine.

KEY WORDS: *Vangueria spinosa*, Pharmacognosy, Phytochemicals, Indigenous medicine.

INTRODUCTION

Vangueria spinosa Roxb. (Sanskrit: Pindu/ Pinditaka) is a deciduous shrub or small tree of the tropical and sub-tropical regions of the world. It is found to be growing in almost all parts of India. Leaves are used for treatment of diphtheria, dysentery, indigestion and for removal of worms, the root paste is used for treatment of painful urination, the stem bark paste is used as a cure for boils and the seed powder is used as a narcotic (Pal & Jain, 1998). Its use as a strengthener, coolant, an expellant of phlegm and bile, and as anti-venom to snake-bite and scorpion-sting has been mentioned (Chopra *et al*., 1999). Though *V.*

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spinosa has importance in indigenous medicine, pharmacognostic study on it is limited. Hence, a detailed pharmacognostic study of the plant was carried out. The present investigation will help in correct botanical identification, detection of adulteration and screening of the active phytochemicals and hence increase the efficacy of the drug in the indigenous systems of medicine.

MATERIALS AND METHODS

Plant materials were collected from various parts of Maharashtra such as, Mulshi, Bhimashankar and Lonavala areas. Efforts were made to collect the plant materials were collected in flowering and fruiting conditions for the correct botanical identification. The plants were identified with the help of Botanical Survey of India, Western Circle, Pune (B.S.I.) and Flora of Presidency of Bombay (Cooke, 1967). Voucher specimens have been deposited in the Botanical Survey of India, Western Circle, Pune, and the Department of Botany, University of Pune.

Macroscopic study (organoleptic evaluation) was carried out involving the evaluation of drug by means of sense organs. The process included i the observation of the colour, odour, taste and texture of the leaf and stem drugs (Khandelwal, 2005). Microscopic evaluation, hand and microtome sections of the fresh leaf and stem were taken, by dehydrating in different grades of alcohol, stained with safranin and light green and mounted in Canada balsam (Johansen, 1940; Trease & Evans, 1972). Microphotographs of the slide preparations were taken with phase contrast microscope. In order to study the vessels, tracheids and fibres small portions of the leaf and the stem were macerated by using Jeffery's maceration fluid (10% aqueous HNO_3: 10% aqueous chromic acid. Diagrams were drawn using mirror Leitz's Camera Lucida.

Quantitative microscopy of the leaf involved determination of stomatal number and index; palisade ratio, vein-islets and veinlet termination number. Percentage extractives and ash analysis were carried out (Anonymous, 1955). Histochemical tests were conducted to detect the localization of the phytochemicals (Khandelwal, 2005). Phytochemical and fluorescence analysis were carried out using standard methods (Harborne, 1973; Sadasivam & Manickam, 2006; Obadoni & Ochuko, 2001).

RESULTS AND DISCUSSION

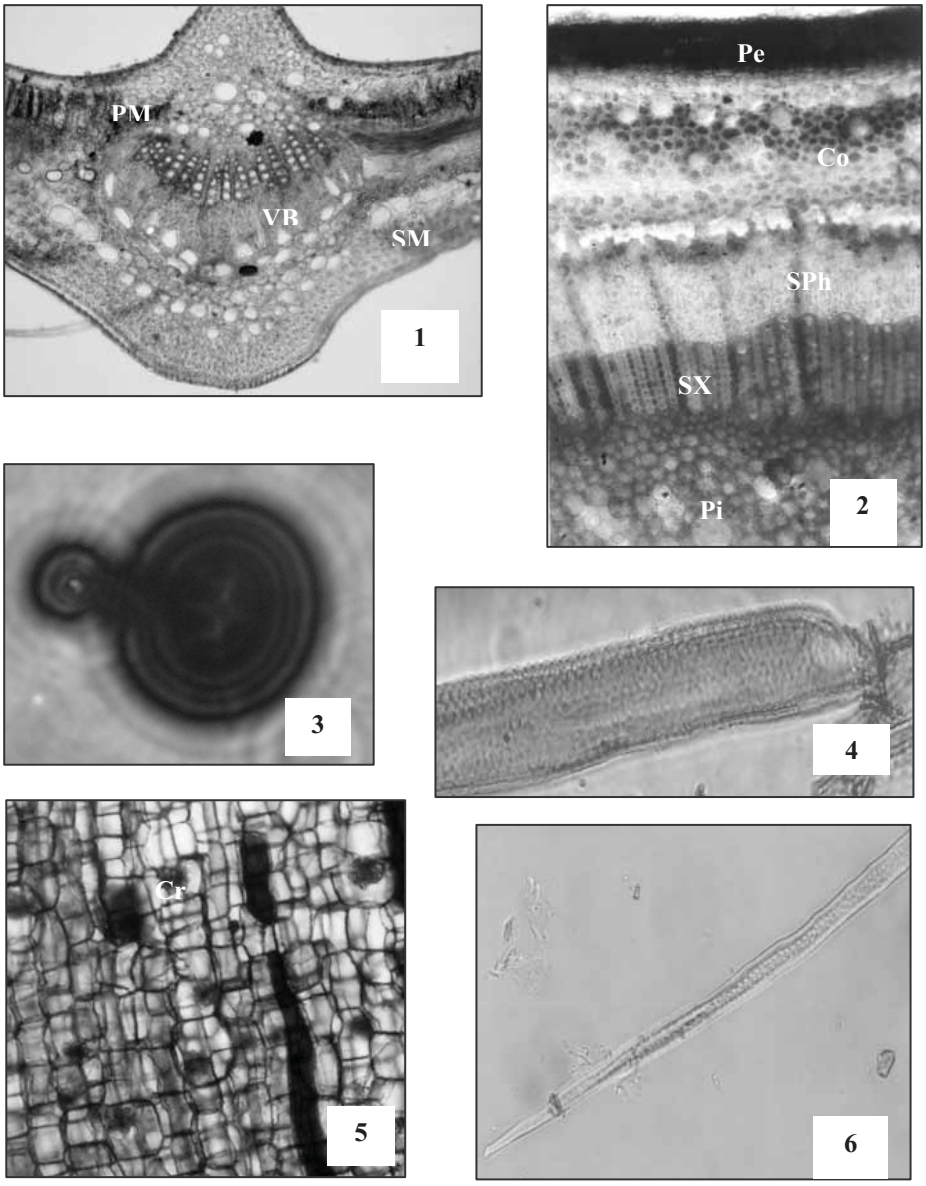
Macroscopic Characters

Leaf: Leaves are light green in colour, simple, opposite, petiolate, ovate or ovate-lanceolate, glabrous and acute. The length of the leaf is between 5.0 to 9.6 cm and the breadth is between 3.0 to 6.8 cm. Petioles are very short and measures 0.6 to 2.0 cm. The leaf exhibits dorsiventral characteristics; stomata are confined to the lower surface only. Lamina is traversed with unicostate reticulate venation. The odour and taste of the leaf are characteristic, while the texture is smooth.

Stem: Stem is dark gray in colour when matured but green when young and armed with spines having lengths ranging from 2cm to 2.5cm. The stem is quadrangular when young. Odour and taste are characteristic while the fracture is rough.

Microscopic evaluation

T. S. of Leaf: The leaf shows the characteristic features of a mesophyte. It has a dorsiventral structure since the mesophyll tissue consists of two layers of upper palisade cells and 3-4 layers of lower spongy cells. Starch grains are either found singly or in groups and have a diameter of (3.388-15.40) μ in this region. The upper and lower epidermis consists of compact, barrel-shaped cells with cuticle. Stomata are confined only to the lower surface of the leaf and are paracytic (Rubiaceous). The venation is unicostate reticulate. Midrib shows collenchyma below upper and lower epidermis, 4-6 layers of cells and a crescent -shaped vascular bundle with a single-layer of barrel-shaped endodermal cells. The xylem is surrounded with phloem. A few cluster calcium oxalate crystals, secretory cells and starch grains are found in the parenchyma cells of this region. The xylem is lignified and, consists of scalariform vessels and tracheids, aseptate lignified fibres and xylem parenchyma. Tracheids are (86.24-110.8) μ in length and (3.08-2.772) μ in breadth. The length and breadth of the fibre is 150.92 μ and 3.08 μ, respectively. Lengths of vessels range from 20.02 μ-135.52 μ and their breadths from 3.08 μ - 4.5 μ. Phloem is formed of sieve tubes, companion cells and phloem parenchyma cells (Figs 1, 3 & 6).



Figures. 1-6: Vangueria spinosa: 1.T.S. of leaf (x 10); 2.T.S. of stem(x 50); 3. Starch of leaf (x 66); 4.Vessel of stem (x 66); 5. L.S. of stem (x 66); 6. Tracheid of leaf (x 66).

(PM- Palisade mesophyll, SM- Spongy mesophyll, VB- Vascular bundle, Pe- Periderm, Co- Cortex, SPh- Secondary phloem, SX- Secondary xylem, Pi- Pith, Cr- Crystals)

T.S. of Stem: Transverse section of the stem is tretragonal to ovoid in outline for young stems and is circular in older stems. The older stem has a periderm made up of cork or phellem, phellogen or cork-cambium and phelloderm. The cork is 2-3 celled thick, compact and the cells are rectangular in shape. Lenticels and tannins are also seen in this region. Inner to the cork is the phellogen consisting of 2-3 rows of narrow, thin-walled, rectangular or flattened and compactly arranged cells. Next to the latter is the phelloderm which consists of 3-4 rows of parenchymatous, isodiametric cells. The younger stem has a single-layered epidermis consisting of columnar cells. Epidermal trichomes are absent. Just below the phelloderm or the epidermis is the cortex which is composed of two types of cells namely, 3-4 layers of collenchymatous cells with chloroplasts on the outer side and 5-6 rows of parenchymatous cells towards the inner side. Numerous secretory cells, tannins, starch and cluster calcium oxalate crystals are present in the cortex. A single layer of endodermis is present which is followed by a distinct layer of pericycle which consists of sclerenchyma cells (Fig.2).

The vascular bundle is tetragonal in young stem and is conjoint, collateral and open. The phloem consists of many layers of compact parenchyma cells, sieve tube cells and companion cells. These cells are rich in both primary and secondary metabolites. Cambium is composed of 1-2 layers of thin-walled rectangular cells. Radially elongated, uni-seriate medullary rays traverse through xylem till the cortical cells. Xylem is endarch and is made up of tracheids, fibres and xylem parenchyma. The xylem elements are lignified and thick-walled. The xylem vessels are arranged radially in 3-6 rows and exhibit unequal growth and size. Vessel lengths and breadths range from (93.94-123.2) µ and (3.08-3.696) µ, respectively. Lengths of tracheids range from (106.26-197.12) µ and their breadth from (3.08-3.696) µ and have pits. The fibres are lignified and are (135.52-163.24) µ in length and (3.08) µ in breadth. In the centre of the stele is pith which consists of isodiametric parenchyma cells. These cells are rich in tannins, glycosides, alkaloids, proteins, calcium oxalate crystals, starch and other secretory cells (Figs. 3 & 5).

Quantitative microscopy

Stomata were shown to be present only on the lower surface of the leaf. The results of stomatal number and index, palisade ratio, vein-islet number and veinlet termination number are given in Table 1.

Table 1. Quantitative microscopy of the leaf of *V. spinosa*

Parameters	**Values**
Palisade Ratio	13.00 per cell
Stomatal Index	19.17 per mm^2
Stomatal Number	13.00 per mm^2
Vein-islet Number	23.20 per mm^2
Veinlet Termination Number	69.2 0 per mm^2

Values are the mean of three different observations.

Histochemistry

Very thin hand cut sections of fresh leaf and stem were taken and treated with chemical reagents and observed under a microscope for detection and localization of chemical constituents in their tissues. Histochemical tests for starch, proteins, fats, tannins, sugars, glycosides, alkaloids and saponins were carried out. The tests were shown to be positive for all the chemicals tested (Table 2).

Table 2. Histochemical tests of the leaf and stem of *V. spinosa*

Tests	**Reagents**	**Localization of phytochemicals**	
		Leaf	**Stem**
Starch	I_2KI solution	PH, PR.CY, CO.CL, PL.CL, SP.CL, Ep. CL	PH,CT,CO.CL, PT, PD.
Protein	i) Eosin ii) KFeCN + H_2O+ glacial $CH_3COOH+C_2H_5OH+FeCl_3$	XY, PR.CY, PH, PL.CL, SP.CL, Ep. CL	XY, PH, MR, PR.CY, CT, PD
Tannin	i) Acidic $FeCl_3$ + Clove oil ii) Aq.$FeCl_3$ +	CO.CL, PL.CL, SP.CL	XY, PH, CT, CO.CL, PD.

	Na_2CO_3		
Saponin	i) Conc.H_2SO_4 ii) $Ba(OH)_2$+ $CaCl_2$+ $K_2Cr_2O_7$	PR.CY, PL.CL, SP.CL, Ep. CL	PT, XY, PH, CT, CO.CL.
Fat	i) Sudan III ii) Sudan IV	Ep.CL, CO.CL, PR.CY, PH, CT, SP.CL, PL.CL.	PT, XY, PH, CT, CO.CL.
Sugar	Aq.NaOH+ Copper tartarate	XY, PH, PL.CL, SP.CL, Ep.CL	PT, MR, PH, XY, CO.CL, CT, PD.
Glycosides	Guignard's Test	XY, PH, PL.CL, SP.CL, PR.CL, CT, Ep.CL.	PT, XY, PH, C, PR.CY, CT, CO.CL, PD.
Alkaloids	i) Mayer's reagent ii) Wagner's reagent iii) Dragendorff's reagent	Ep.CL, PH., XY, PR.CY, PL.CL, SP.CL	PH, XY, CT, PD.

PH - phloem, XY – xylem, PR.CY – pericycle, CO.CL –collenchyma cell, PL.CL –palisade cell, SP.CL – spongy cell, Ep.CL - epidermal cell, CT – cortex, PD – periderm, MR – medullary ray, PT – pith

Phytochemistry

The percentage of total ash was 6.7 % and 5.7 % while, that of acid insoluble ash was 2.5 % and 1.6 % for the leaf and stem, respectively. The results of percentage extractives are given in Table 3. The results of fluorescence analysis are shown in Table 4.

Table 3. Percentage extractives of the stem and leaf of *V. spinosa*

Solvent (100 ml)	**Stem**	**Leaf**
Distilled water	4.8 %	9.6 %
Absolute alcohol	5.0 %	8.0 %
Solvent ether	3.8 %	1.8 %
Petroleum ether	0.4 %	0.8 %

Table 4. Fluorescence analysis of the leaf and stem of *V. spinosa*		
Treatment	**Leaf**	**Stem**
Natural powder as such	Greenish gray	Gray
Powder + UV light	Grayish green	Grayish
Powder + nitrocellulose	Dark green	Greenish
Powder + 1N NaOH in	Greenish yellow	Dark green
Powder + 1N NaOH in methanol, dried ½ hour + nitrocellulose	Light green	Light green

Phytochemical tests were performed using water extracts for starch, proteins, tannins, saponins, sugar, fats, flavonoids and anthraquinones, while alcohol extracts were used for alkaloids and glycosides. Qualitative tests showed the presence of all the phytochemicals analysed except for anthraquinones and flavonoids (Table 5).

Quantitative tests showed comparatively, the concentration of the non-reducing sugar, carbohydrate, protein, saponin and alkaloid was more in the leaf than in the stem while, it was the reverse for reducing sugar and tannins. Among the phytochemicals, the amount of carbohydrates was highest (16.746 and 13.588) mg/g, while that of non-reducing sugars was (13.668 and 8.675) mg/g, proteins (6.413 and 4.038) mg/g and tannins (7.118 and 12.412) mg/g which were relatively more than the others in the leaf and stem, respectively. The concentration of saponins was 2.01 mg/g in the leaf and 0.89 mg/g in the stem which was more than the alkaloids (Table 6).

Table 5. Qualitative phytochemical tests of the leaf and stem of *V. spinosa*				
Extract	**Test**	**Reagents**	**Leaf**	**Stem**
Water extracts	Starch	I_2KI solution	+ve	+ve
	Protein	Millon's reagent	+ve	+ve
	Tannin	Acidic $FeCl_3$	+ve	+ve
	Saponin	Water + $CHCl_3$ + Conc.H_2SO_4 + acetic anhydride	+ve	+ve

	Fat	Sudan III	+ve	+ve
	Sugar	Benedicts reagent	+ve	+ve
	Anthraquinones	C_6H_6+ NH_4OH	-ve	-ve
	Flavonoids	Conc.HCl + Mg turnings	-ve	-ve
Alcoholic extracts	Alkaloids	Mayer's Reagent	+ve	+ve
		Dragendorff's Reagent	+ve	+ve
		Wagner's Reagent	+ve	+ve
		Tannic acid	+ve	+ve
		Folin-Ciocalteau	+ve	+ve
	Glycosides	C_6H_6	+ve	+ve

+ve means positive result. , -ve means negative result.

Table 6. Quantitative estimation of phytochemicals from the leaf and stem of *V. spinosa*

Phytochemicals	**Leaf Dry weight (mg/g)**	**Stem Dry weight (mg/g)**
Reducing sugar	0.425 ± 0.10	1.425 ± 0.24
Non-reducing sugar	13.668 ± 0.33	8.675 ± 0.01
Total carbohydrate	16.746 ± 0.31	13.588 ±1.41
Proteins	6.413 ± 0.28	4.038 ± 0.81
Alkaloids	0.570 ± 0.11	0.468 ± 0.39
Tannins	7.118 ±1.07	12.412 ± 2.05
Saponins	2.010 ± 0.20	0.890 ± 1.12

Results are mean of 3 determinants in mg/g of dry weight tissue

Secondary metabolites such as tannins, saponins and alkaloids have been reported to exhibit various medicinal properties. The high content of tannins and saponins in *V. spinosa* justifies its use as a styptic, antidote for snake-bite and scorpion-sting; anti-tumour and anti-HIV agents mainly due to their property of precipitating proteins (Sodipo *et al*., 2000; Okwu, 2004). The use of the plant in the treatment of fever, diphtheria, dysentery, indigestion, dermatitis, and also for expulsion of phlegm and bile can be associated to the antibacterial, antifungal, anti-inflammatory and cooling properties of tannins (Ansari, 2006; Okuda, 2005 and Kojima *et al*., 2000). Medicinal properties of *V. spinosa* can also be due to the presence of alkaloids which has been reported to

exhibit analgesic, antibacterial and antifungal and antispasmodic actions (Okwu & Okwu, 2004).

CONCLUSION

The pharmacognostic study of the various features such as macroscopy, microscopy, quantitative microscopy, percentage values and fluorescence analysis of *V. spinosa* can be used for correct botanical identification and detection of adulteration in the drug. The qualitative and quantitative analysis of the drug showed it to be rich in phytochemicals, like carbohydrates, starch, proteins, tannins, saponins and alkaloids. Thus, indicating the potential of the plant in pharmaceuticals and in indigenous systems of medicine.

Analysis of essential elements is done on the plant parts, not on the product of the plant (drug). Thus, conclusion made is invalid and is contrary to what is stated.

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BRIEF COMMUNICATION

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Yield Performance of IR 64 variety Using System of Rice Intensification (SRI) Methods at Three Locations in Wandgue and Punakha

Karma Lhendup[1], Mahesh Ghimiray[2], Sangay Tshewang[3]

INTRODUCTION

There is a growing recognition and popularity of the System of Rice Intensification (SRI) methods of rice cultivation around the world since its first trials outside of Madagascar in 1999-2000. As of 4th March 2009, 36 countries have documented the beneficial effects of SRI method in terms of saving seed, water, cost, increased soil health, and grain yields compared with traditional methods of rice cultivation [http://ciifad.cornell.edu/sri/countries/costarica/index.html]. Other benefits of SRI experienced by farmers include earlier crop maturity, higher straw yields and higher rate of milling outturn (Uphoff, 2005). It is now considered as an innovative and cost-effective method of rice cultivation particularly in terms of seed and irrigation water saving, and chemical input reduction.

SRI method of rice cultivation does not involve an entire change in the existing cultivation practices of rice. It involves modification of some agronomic practices on existing methods in rice cultivation providing better growing conditions for plants, particularly in their root zones. Six principles and practices of SRI include: transplanting of young seedlings (at 2-3 leaf stage), transplanting of single seedling per hill, wider spacing, moist but unflooded soil conditions during the vegetative growth phase, early and timely weeding, and organic manuring. Researchers claim that these practices achieve synergistic effects, resulting in higher yield than the conventional rice production methods (Uphoff, 2001).

Success of SRI in many countries has stimulated researchers in Bhutan to conduct trials on SRI methods (initial trials were in Khangma,

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Kanglung and Lobesa - Lhendup, et al. 2008, http://ciifad.cornell.edu/sri/countries/bhutan/bhLobesaRpt07. pdf) to observe their performance under Bhutanese field conditions and examine their associated benefits.

Past Research on SRI methods in Bhutan

Lhendup et al. (2008) found that initial trials at RC Khangma and Kanglung using some of the SRI method such as young seedlings (3-leaf stage), single seedlings, wide spacing (30x30 cm gave better performance than 20x20 cm), and reduced water application (non-flooding) showed better crop performance in terms of yield and various yield parameters than did comparable plots grown with standard methods. For this study, there was no introduction and evaluation of organic soil fertilization (standard chemical fertilizer applications were made), and there was no active soil aeration (only hand weeding, not using a rotary hoe). The average yield increase comparing replicated plot results was 0.65 t/ha, significant at the .05 level of confidence. Profuse tillering was observed, and farmers were impressed with both the plant growth and the cost-saving opportunities. In addition, the first trial using SRI methods at Khasadrapchu by researchers of Yusipang also showed positive results as compared to those with conventional methods (http:// ciifad.cornell.edu/sri/countries/bhutan/bhKhachadrapchuRpt07.pdf).

Further, the results of the follow-up study at the College of Natural Resources (CNR) farm, Lobesa in 2007 showed a positive effect from the SRI method evaluated (http://ciifad.cornell.edu/sri/countries/bhutan/ bhLobesaRpt07.pdf).

The results of the first and second years' trial conducted in 2006 and 2007 by RNR RC Bajo at its station were not very convincing. Thus, a study was undertaken jointly in 2008 by RC Bajo and CNR researchers at three locations: RC, Bajo (1200 masl); CNR, Lobesa (1440 masl); and Sopsokha, Punakha (farmer's field, 1480 masl) to verify the performance of SRI methods. The variety tested was an introduced rice variety (IR64), which is quite popular among the farmers in Wangdue-Punakha valley.

At RC Bajo, the trial was laid out in a large observation plot of 645 squared meters. The trial was not randomized given that our previous

experience showed that it was difficult to follow SRI principles in small contiguous plots especially water management because of lateral spread. Prof. Norman Uphoff, a SRI specialist agreed on single-plot observation rather than randomization during his visit to the station (Personal communication, 2007). A large adjacent plot subjected to normal conventional practices was used as a control.

At CNR farm, a modified simple randomized design was used with three replications. The replicated plots were separated by 50 cm wide line spacing. At Sopsokha, a portion of the farmer's field was cultivated with SRI methods while traditional methods were employed on the rest. A bund was constructed within the field to separate the two areas to avoid spill-over of fertilizers and weedicides.

At Bajo, the SRI plots were raised organically without any inorganic fertilization. Farm yard manure of 2 tonnes per 645 squared meters was applied and incorporated during the last puddling. However, there could have been some residual effects from the previous season's fertilization. At Sopsokha, a total of 7 bags of farmyard manure (FYM), each weighing 30-35 kilograms, were applied on each of the half terraces, 18 m long and 4 m wide, while the FYM application at the CNR farm was slightly more. This is because the animal waste upstream was let into the field until the vegetative phase.

In all the sites, the nursery was established using pre-soaked incubated seeds. At CNR farm and Sopsokha, transplanting of 3-leaf seedlings was done in the first to second week of June at 25 cm spacing. At Bajo, the spacing used was 30 cm. Three weedings were carried out at all the sites.

RESULTS

Table 1 presents the results for a main yield-contributing parameter (number of fertile tillers) and for yield at the three locations: RC Bajo, CNR farm, and Sopsokha. In all three sites, the average number of fertile (effective) tillers per hill was higher in SRI plots than in conventional/control plots. Among the three sites, the highest number of productive tillers was found at RC Bajo (47) followed by Sopsokha (32) and CNR farm (30 tillers). This was an increase of 64%, 66% and 33% compared to conventional methods, respectively.

Similarly, average yield performance was better on the SRI plots compared with conventional plots in all three sites (Table 1). Among the three sites, the 10.1 t/ha yield performance at CNR farm was the highest, followed closely by Sopsokha and RC Bajo, with 9.3 and 8.6 t/ha. The increase in yield compared to conventional methods in all three locations was 14%, 29% and 19%, respectively. Further, the yield obtained for IR 64 variety with SRI methods was more than that obtained from the normal practice in RC station at Bajo, which is usually averaging about 6-8 t/ha. The increased in yield with SRI methods is mainly contributed from the increased number of productive tillers.

Apart from the increase in yield, it was observed that there was a reduction in the prevalence of *shochum* (*Potamogeton distinctus* A. Bennett) at RC Bajo and Sopsokha. *Shochum* is a dominant and perennial fresh-water weed found abundantly in almost all the rice-growing districts at mid-altitude. It is reported to reduce paddy yields by about 40% in the country. Among the many districts, Wangdue and Punakha are the most severely affected by the prevalence of this weed. Thus, the possibility that SRI methods will enable farmers to reduce the prevalence of *shochum* simply through changes in crop management is an area where further investigation of the effects of SRI methods is warranted.

Table 1. Yield and yield-contributing parameter of trials at RC Bajo, CNR farm and Sopsokha, farmer's field using IR64 variety.

Sl. No.	Parameters	Study Site		
		RC Bajo	CNR farm	Sopsokha
1	**Fertile tillers/hill**			
	SRI method	47	30	32
	Conventional method	17	20	11
2	**Plant height (cm)**			
	SRI method	88	91	92
	Conventional method	90	90	89

3	**Number of hills/6m^2** SRI method Conventional method	 66 NA	 96 110	 96 211
4	**Yield (kg/6m^2) & t/ha** SRI method Conventional method	 (5.4) 8.6 (4.3) 7.0	 (6.1) 10.1 (5.2) 8.7	 (5.6) 9.3 (4.0) 6.6
	Yield calculated at 14% moisture content			

CONCLUSION

The results of evaluations in the 2008 season at three sites showed a positive effect from SRI methods, greater than observed in previous SRI trials. The researchers at RC Bajo as well as farmers were convinced about the potentiality of SRI techniques of rice production in enhancing the yield. Thus, the adoption of these techniques by farmers will not only enhance food security, but will also ensure a sustainable environment and improve the livelihoods of farmers.

Further trials and demonstrations involving farmers are planned for the coming season to verify and demonstrate the benefits of SRI under more varied circumstances, comparing SRI results to conventional practices, thereby helping to build the trust and confidence among farmers to adopt these techniques**.**

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Rice-rice doubles cropping in Changmari

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INTRODUCTION

Rice is the staple food and is the way of livelihood for Bhutanese farmers. Rice is grown from lowlands (200m) in the south to elevation as high as 2600m in the north. More than 50% of the regional paddy area lies in subtropical rice environment. Samtse and part of Chukha Dzongkhag fall under this environment. Ranges of food grain self-sufficiency level in Bhutan is 39 to 56%, therefore domestic production of rice has not been able to meet the demand (Shrestha, 2004).

Rice production in Samtse Dzongkhag is 9,105 MT from an area of 11,858 acre with an average yield of .76t/ac (DoA 2005). About 85% of households in Samtse Dzongkhag face some degree of seasonal food shortages for an average of 2.9 months. The shortages are usually met through purchasing or borrowing from neighbors, bartering with livestock or livestock products, or labour exchange for food (Bhutan national food security strategy paper, 2004). To attain the Millennium Development Goal (MDG) of poverty alleviation, food self sufficiency is through land intensification (rice–rice double cropping), as further expansion of cultivated area is limited.

A survey was conducted in 2001 to study the feasibility of rice-rice double cropping in Samtse Dzongkhag. Looking at the winter temperature ranges from 19.5 to 22.5 ^{0}C, it was found to be optimumal for germination, seedling emergence and rooting of rice crop (Yoshida, 1981) therefore Samtse Dzongkhag was considered suitable for rice-rice double cropping. Through the survey, irrigation facilities were virtually absent with disrupted channels, therefore promotion of wide scale double cropping in whole of Samtse Dzongkhag was not possible.

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Hence, the survey recommended identifying farmers who have provision of winter irrigation facilities to practice rice-rice double cropping.

Rice-Rice double cropping in Changmari

The land area under rice cultivation in Changmari is 1391 acres. Traditionally farmers of Changmari followed rice-fallow, and the cattle were let free after the harvest of main rice crop in which hindered cultivation of winter crop. Farmers grow their traditional varieties of rice like Phakha Dhan, Bali Musli, Chota Dhan, Champasari, Jadu Dhan which is very low in yield with a an average yield of .85t/ac. House hold in Changmari geog experience food shortage for about seven months (February-September) and they met this by borrowing, supplementing with maize/other crops and through labour exchange (Farmers Changmari)

In 2005 cropping season potential area for rice double cropping, with winter irrigation facilities was identified in Changmari geog. An observation trial in 716 m^2 plot area was conducted by RNRRC Yusipang in collaboration with Samtse Dzongkhag Agriculture Extension. The objective of rice double cropping activity was to bridge the gap of food shortage period and to increase the average yield of the region. With adoption of the rice-rice double cropping by the farmers with winter irrigation facilities, perceived benefits was to increase the food self sufficiency level of the household in Changmari. The variety used for first rice crop was IR20913 which is an advance selection from cross between Bhutanese white, its average height is 100cm, matures in 130-140 days and yield potential is 2.8t/ac. At the initial start up of the trial extension staff of Changmari took extra initiative to fence the trial site from stray cattle. During main rice cropping season, after the harvest of first rice crop, farmers' own variety was grown and managed as per the farmers' management practices.

Benefits of Rice- Rice double cropping

Further promotion of rice double cropping was done through field day and training by Dzongkhag extension of Changmari. The farmers practicing rice double cropping obtained an average additional yield of 1.3t/ac from the first crop (IR20913) and .76t/ac the main crop (local varieties). As there was additional yield benefit from the first rice crop, 13 farmers in Changmari geog joined hands to cultivate rice double crop

and area increased from 716 m^2 plot area (initial demonstration) to 5 acres in 2008 cropping season.

Through informal discussion with the farmers of Changmari, it was noted that food from production (rice, maize and other crops) for household consumption only last for five months (October - February) prior to rice – rice double cropping. Farmers expressed, after cultivating rice double crop there is food shortage only for five months. The farmers cultivating rice double crop also mentioned that the problem with stray cattle still prevails and other constraints are damage due to bird attack.

Extension agent of Changmari stated, more, and more farmers are now becoming aware of the benefit of rice-rice double cropping system. Due to the interest shown by the farmers, Dzongkhag Extension of Samtse Dzongkhags in collaboration with RC Yusipang will expand area (30 acres) under rice-rice double cropping to other geog of Samtse Dzongkhag with winter irrigation facilities in 2009 cropping season.

Rice double cropping outside

Although rice-rice double cropping is expanding in Samtse Dzongkhag and food security gap is also partially fulfilled, there is need to study the soil status of farmers cultivating rice-rice double crop. As nutrient uptake of rice crop for one ton of grain yield is 15 kg Nitrogen, 2.6 kg Phosphorus and/or 15 kg Potassium (Yoshida, 1981). Hence intensive cropping of rice (two crops of rice per year) will deplete twice the amount of nutrient uptake required for rice. Consequently soil capacity to provide nutrient will decline leading to severe nutrient deficiencies for growing rice, if nutrients are not replenished properly. Research at IRRI indicated decline in partial factor productivity of nitrogen fertilizer, increased deficiency incidence of phosphorus, potassium and micro-nutrient due to intensive cultivation of rice (De Datta et al, 1988).

Decline in yield trend around 30% in long term experiment (over 20yrs) was observed and documented in areas like Phillipines, Thailand, India and Bangladesh, due to intensive rice cultivation at all nitrogen level, with the use of best available cultivars and scientific management. Countries practicing rice double or triple cropping cautioned, stating that increased amount of input is required to maintain yield level, which indicated declining partial factor productivities (Pingali P.L, et al, 1997).

Double cropping of rice in Bhutan (Wangdue, Punakha area) declined considerably since 1990s as there was inverse correlation between increased rice production (through adoption of modern varieties which sufficed the food need of the family) and double cropping. However, severe rice deficit areas still continue rice double cropping in Rinchengang (Shrestha, 2004).

In china a cropping system consisting of two crops of rice followed by crop of barley or soya bean, practiced over 18 years, and has maintained a high and stable crop yield (Li, 1993, as cited in Khundu and Ladha, 1995).

WAY FORWARD

Looking at the rice-fallow cropping system in Samtse Dzongkha, firstly there is a need to encourage farmers to cultivate winter crop. As winter rice cultivation is gaining popularity among the farmers of Samtse, RNR sector should support farmers to take up this activity, which will in turn reduce damage by stray cattle. Large area under rice double cropping will reduce damage by birds and rat also. After few years of research at RSC Bhur high yielding variety for subtropical environment will be identified, this will fully meet the household food shortage. After that, there is need to diversify high value crops during winter season (horticultural vegetable crops) as the farmers will be familiar with winter cropping practices.

At present, looking at the growing interest of farmers to grow rice double crop there is need to do strategic research, through integration of organic and inorganic nutrient management to replenish and improve soil nutrient and to come up with nutrient management recommendation. Green manuring (*Sesbania aculeate*), cropping of leguminous crop (vegetables or other crops) and bio-fertilizer integration might be the immediate solution to prevent declining of soil capacity to provide nutrient. Therefore, a collaborative research with relevant stakeholders are required to come up with best management practices for rice-rice double cropping which will at least maintained stable crop yield to meet household food security of Samtse Dzongkhag.

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Efficacy of Biological Formulations for the Control of Apple Scab: Towards Providing Alternative Options

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INTRODUCTION

Apple is grown on a semi-commercial scale in Bumthang. Production is mostly low input traditional with little use of chemicals and pesticides. The delicious group of apples is the most commonly grown. A range of activities have been carried out by the Renewable Natural Resources Research Centre in Bumthang with the objectives to identify major constraints in apple production and develop technologies to optimize benefits from apple production in the valley. Current production constraints include limited varieties, fruit borer and scab disease. Surveys have indicated that more than 34% of the fruits are affected by scab disease and is one of the major factors affecting the fruit quality in this area (RNRRC Jakar,1998; Chophyll,K, 2005). With traditional methods adopted by many, fruit quality is poor and therefore fit mostly for local consumption and processing. Several ways of managing apple scab *(V. inaequalis)* include fungicide application, planting resistant varieties, pruning and orchard management. The current scab management is through conventional methods using fungicides sprayed at different stages of the tree. Varieties imported are being tested at research centres for their tolerance to scab disease and their marketability.

The type of farming systems that we adopt could play a role in defining the status of the surroundings we live in. Bhutan presently has a clean image with its pristine and clean environment relatively unpolluted by the ravages of modernization. Because of minimal usage of chemical fertilizers and pesticides in our largely traditional farming system which is true particularly in the far-flung areas, most Bhutanese farms could qualify as natural or organic farming. Crop yields in Bhutan including that of apple are therefore moderate to low. Through slight modifications and improvements upon the traditional system using modern natural and organic farming principles and techniques, there is

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good potential to increase productivity and quality of many crops. With the set up of an organic farming unit within the MoA, the Government is placing high priority to promote organic farming.

Due to geographical limitations, Bhutan has limited competitiveness in the International market for agricultural commodities produced through conventional methods alone. Alternative options such as through the adoption of organic production methods may be able to boost our farmers' income as well as safeguard and protect our environment and our health (DoA, 2005). Due to limited efforts on organic research and information generation in the past, awareness on the concept of organic farming methods has remained minimal. Through verification/adaptation of existing knowledge on natural and organic methods elsewhere, such information need to be disseminated to the farmers to provide them with choice. Studies abroad have revealed water and soil pollution due to increasing use of artificial products could not only lead to negative changes in the environment but also pose dangers to our health. Controlling apple scab requires the application of 10–22 fungicide applications per season depending on weather conditions. Such use of synthetic fungicides can cause environmental problems (Kadir Ilhan et al, 2006).

Our goal therefore of scab management in organic farming is to eliminate or reduce the amount of synthetic fungicides used. One of the ways to achieve this goal may be the use of natural substances that have no known adverse affects on the environment and human health (Kadir Ilhan et al, 2006). Selected organic and inorganic salts are active antimicrobial agents and are widely used as food additives. Among these, sodium bicarbonate and vinegar are believed to have broad-spectrum antifungal activity. A trial was conducted to determine the efficacy of these two formulations and two other organic fungicides viz Mycosan - a sulphur based product (sulfur is still allowed in organics), and an Indian organic formulation called Sambhavi, which were compared against the conventional fungicide Carbendazim with the objective to provide alternatives to conventional spraying options to apple growers for the control of scab disease.

Results

Spraying these on a nine year old orchard of eighteen varieties in Bumthang in 2006 showed that complete control of scab was not possible with all the treatments including the conventional fungicide (table 1). These results tally with those of similar trials conducted earlier in 2004 and 2005 (tables 2 & 3). However, positive inference could be made on the capacity of the four organic formulations for scab control though they showed lower efficacy than carbendazim. Trees sprayed with organic formulations all showed a significantly higher incidence of scab on fruits than those of the conventional chemical spray. Vinegar and Sambhavi sprays resulted in the least number of lesions followed by Mycosan while trees sprayed with Baking soda produced the highest number of lesions. In terms of cost, Mycosan formulation was the most expensive with poor efficacy comparable only to the much cheaper soda.

Table 1. Mean scab lesions per fruit, 2006

Treatment	**Number of lesions/fruit**
Carbendazim	2.15
Vinegar	3.82
Sambhavi	4.09
Mycosan	4.88
Baking soda	5.61
F probability	0.001
LSD	1.02
sed	0.52

Table 2. Mean scab lesions per fruit, 2005

Treatment	**Number of lesions/fruit**
Myco-san	7.45
Vinegar	8.11
Carbendazim	2.12
F probability	0.001
LSD	2.28
s.e.d	1.10
CV%	51.4%

Table 3. Mean scab lesions per fruit, 2004

Treatment	**Scab lesions (Mean)**
Control	6.19
Myco-san spray 5 times	3.92
2 chemical sprays	1.43
5 chemical sprays	1.29
F probability	<0.001
s.e.d	0.291

VARIETAL DIFFERENCES

Scab lesions were found on all the eighteen varieties ranging from 9 to 0 .9 lesions per fruit. Variability of the extent of scab lesion occurrence among varieties was high depending upon the variety and its resistance capacity. Inference therefore could also be made on the scab tolerance nature of each variety. Susceptible varieties had a higher number of scab lesions while tolerant varieties such as Florina, Well spur, Ariwa, Red chief, Kogetsu and Red free had very few lesions (fig 1). The supposedly immune variety Red free also exhibited lesions indicating that under high disease pressure and congenial climatic conditions such varieties can also get infected.

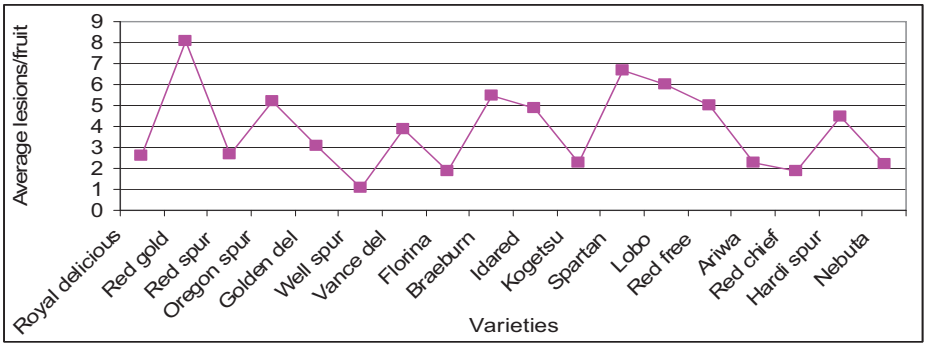

Figure 1: Scab lesions on different varieties, 2006

CONCLUSION

Given the satisfactory results obtained with the Vinegar and Sambhavi sprays and their lower costing, locally available Vinegar followed by Sambhavi may be alternative options for use in organic apple production at least in the short-term range before new information and new products are made available.

However, apple scab disease cannot be addressed through the application of plant protection sprays alone and this holds particularly true for organic apple production. Selection of suitable tolerant varieties combined with proper management such as field sanitation, pruning, use of FYM and intercropping will be the main factors that will have to be adopted by organic enthusiasts.

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