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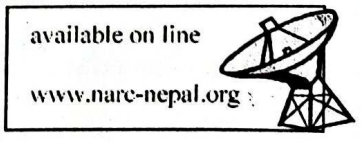

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Contents

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Contribution to Book/Proceedings
Yuan, LP, ZY Yang and JB Yang. 1994. Hybrid rice in China. Pp.143-147. In: Hybrid rice technology: New development and future prospects (SS Virmani, ed.). IRRI, the Philippines.

Annual Report
NWRP. 1980. Rice-wheat system: Opportunities and constraints. Pp.60-65. In: Annual Report-1980, National Wheat Research Program (NWRP), Nepal Agricultural Research Council, Bhairahawa, Rupandehi, Nepal.

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Serials
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Thesis/Dissertation
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Status of Tomato Yellow Leaf Curl Virus in Tomato in the Western Hills of Nepal

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Abstract

Tomato cultivation in rainy season is being endangered and is becoming less profitable because of tomato yellow leaf curl virus (TYLCV) infection. Studies were conducted at Agriculture Research Station, Lumle to assess the incidence of TYLCV and associated yield losses in various commercial tomato growing pockets of the western hills during the period of 1995 and 1997. The studies revealed a high incidence of the disease in most tomato growing pockets and yield losses of 40% or even higher have been reported in some areas like Risingpatan, Tanahun and Kudule, of western hills of Nepal. Laboratory analysis of the diseased samples by Asian Vegetable Research and Development Center revealed the presence of three different strains of TYLCV: Banglore I, Banglore II and Sri Lanka in the western hills. The TYLCV vector, whitefly (Bemisia tabaci Gen.), was found active throughout the crop growing period in some commercial tomato growing pockets. Therefore, research on the development of effective TYLCV management technology is needed to sustain rainy season tomato cultivation in the western hills of Nepal.

Key words: Bemisia tabaci, Lycopersicon esculentum, tomato, yellow leaf curl virus, whitefly

Introduction

Tomato (Lycopersicon esculentum Mill.) is one of the most important vegetable crops grown from subsistence to commercial scale in Nepal. Tomato can be grown in winter, spring and rainy seasons. The crop is grown in winter in the terai and inner-terai and can be grown in two seasons, spring and rainy in the low and middle hills of Nepal. Tomato was used to be grown only in the rainy season in the hills at subsistence level. However, the introduction of improved exotic varieties made it possible to grow the crop in the spring season as well. Both spring and rainy season tomatoes are a major source of income to the vegetable growers of the hills, since they get an off-season market price. Total area and production of this crop in Nepal is 10,530 ha and 72,657 t, respectively with an average productivity of 6.9 t ha^{-1} (Shrestha and Ghimire, 1996), which is very low as compared to the experimental yield of tomato in the country. There are several factors limiting tomato productivity among which tomato yellow leaf curl virus (TYLCV) has been identified as one of the most important biotic constraints for rainy season tomato cultivation in Nepal.

Both spring and rainy season tomatoes are found infected with TYLCV and a high incidence of the disease was observed during the rainy season in the western hills of Nepal. The popular tomato variety, NCL 1 for the rainy season is highly susceptible to TYLCV. Since this variety covers majority of tomato growing areas during rainy season in the western hills, large losses due to the disease have been experienced by the farmers every year. The presence of the disease in the western hills was for the first time, suspected in 1992 in CL 1131 (now NCL 1) a popular rainy season tomato variety at Kudule, Baglung. However, the authentic confirmation of the disease was done only in 1994 (PPD, 1995). The incidence of the disease was just 1 to 2% at the third and the fourth picking in 1992 and hence, it was considered as a disease of very low profile at that time. The subsequent annual monitoring of the disease in commercial tomato growing areas of the western hills revealed an increased incidence and severity associated with significant yield losses up to 95% because of an early

appearance of the disease right from pre-flowering stage. Since then the disease has been considered in high profile at Agriculture Research Station, Lumle. Extensive monitoring of the disease has been carried out to find out the incidence of the disease, associated yield losses and to assess the need for research on TYLCV management. This paper presents the results of the field monitoring and laboratory analysis work on TYLCV in the western hills during the rainy seasons from 1995 to 1997.

Materials and Methods

Field surveys were conducted in different commercial tomato growing areas of the western hills during Sept-Oct 1995 and 1997. Purposive sampling of three commercial rainy season tomato growing areas, Kudule (900 masl), Baglung, Danubase (650 masl), Shyangja and Risingpatan (400 masl), Tanahun of the western hills were done during 1995 survey. Three more sites Yampaphant (475 masl), Tanahun, Baumara (600 masl), Kaski and Phorse (750 masl), Baglung were added in 1996 survey. Two sites from Kaski district, Arghaun (750 masl) and Malepatan (850 masl) were also added in 1997 survey.

At least ten tomato growing farmers, each having minimum of 100 plants were randomly selected from each site in 1995 and 1996 surveys. The number of TYLCV infected plants in each field were counted and average incidence of the disease in the respective sites in the given year was calculated. Only TYLCV infected leaf samples were collected for laboratory analysis in 1997 study. Ten farmers were interviewed for their perception about the disease and associated yield losses in tomatoes in 1995. During the survey, a total of eight infected leaf samples per site were collected in 1996 and 5-11 leaf samples per site in 1997. Samples collected in both years were analyzed at the Asian Vegetable Research and Development Centre, Taiwan. The probe used were Banglore I and Banglore II for 1996 and Sri Lanka and Banglore I for 1997.

The dynamics of TYLCV vector, whitefly (Bemisia tabaci Gen.), was studied from early July 1996 to early Oct 1996 at Yampaphant and Risingpatan using yellow sticky trap. Trapped insects were counted at weekly intervals.

Results and Discussion

Field surveys

There was not a single field free from TYLCV at all three sites visited in 1995 survey. During the visit, the crop was at pre-flowering to late bearing stages and the percentage of TYLCV infection was variable among and within sites. The highest incidence of the disease was recorded at Risingpatan (ranged from 40 to 95%) followed by Kudule (ranged from 40 to 90%) and the least from Dhanubase (ranged from 10 to 30%). Tomato variety, NCL 1 was grown at all sites except at Risingpatan. There was a small plot found with Pusa Ruby, which was at pre-flowering stage and was associated with TYLCV incidence of about 40%. Discussion with farmers about the disease and associated yield loss revealed that such symptoms had started to appear about 4-5 years ago but the incidence than was very low (around 1-2%). It was known that the symptoms appeared only at later stages of the crop and hence there was no damage. However, the incidence of the disease increased over years and the disease started appearing right from pre-flowering stage. Regarding the associated yield loss of tomato in 1995, farmers of different sites had variable estimates ranging from 25 to 40% at Risingpatan, 20 to 25% at Dhanubase and even higher at Kudule. The farmers of Kudule explained that if the disease appears at an early stage (at pre-flowering), the plants do not bear any fruit and when the disease appears at early bearing stage it reduces the yield up to 25%.

Rainy season tomato cultivation at those sites was started just 4 to 5 years before this monitoring began, but the spring cultivation might have been started about 10 years ago. Both spring and rainy season tomatoes are grown on a commercial scale in those sites. Since the disease is transmitted by whitefly, the intensification of the crop might have favored build-up of whitefly population over the years and subsequently increased incidence of the disease in tomato in

the western hills. The farmers' experiences of no fruit yield in an early attack to the crop and a reduced yield in late attack in the western hills of Nepal are in agreement with the findings of Green and Kalloo (1994). They reported that the yield reduction in tomato from TYLCV depends on the stages of crop development at which the virus attacks.

The results of field monitoring for TYLCV in tomato in different tomato growing pockets of the western hills in 1996 is summarized in Table 1. Tomato variety in the monitoring site was NCL 1. The highest percentage of TYLCV incidence was recorded at Yampaphant (27.8%) and Risingpatan (27.5%), followed by Kudule (22.5%) and Baumara (16.8%). The low level of TYLCV incidence at Dhanubase and Phorse might be due to an excessive use of pesticides in the tomato crop. It might have affected whitefly population and subsequently the TYLCV incidence.

Table 1. Incidence of tomato yellow leaf curl virus (TYLCV) in tomato in the western hills of Nepal in 1996

Site	No of plants observed	TYLCV infection, %
Yampaphant	4080	27.8
Risingpatan	4650	27.5
Baumara	20970	16.8
Dhanubase	10355	2.1
Phosre	10290	5.4
Kudule	2405	22.5

Laboratory results

The laboratory analysis of samples having clear TYLCV symptoms from the above sites are summarized in Table 2. The laboratory analysis showed the presence of both Banglore I and Banglore II strains of TYLCV in the western hills and the former was the dominating strain. A mixed infection of these strains was speculated, though it occurred less frequently. Since none of the samples was found to be infected with Banglore II strain alone, this might raise some possibilities of Banglore II strain being a satellite of Banglore I, as in the case of tobacco necrosis virus (TNV) and small satellite virus (STNV). However, it may not be the case between these two strains.

Laboratory analysis of the TYLCV infected leaf samples showed prevalence of Banglore I and Banglore II strains of TYLCV which ranged from 0 to 88% at the sites studied with a mean of 42%. It is important to note that if sample tests show negative to the above strains, it does not necessarily mean that it is free from TYLCV infection. Because other tomato samples from Nepal are reported positive to Thai strains (Joshi et al., 1997). Other strains of TYLCV not detectable by the probes used in this study may also prevail in the samples.

The laboratory analysis of TYLCV infected tomato samples in 1997 are presented in Table 3. It clearly shows that most samples (82%) were found with both strains and the frequency of infection with a single strain was minimal. The result in 1997 showed a very high incidence of the disease at Kudule (90%) and Arghaun (80%). It also shows presence of both Indian and Sri Lankan strains of TYLCV in the western hills of Nepal.

Table 2. Laboratory results of tomato yellow leaf curl virus strains in the diseased samples of tomato in the western hills of Nepal in 1996

Site	No of samples examined	No of samples positive to tomato yellow leaf curl virus (TYLCV) strain			No of infected sample
		Banglore I	Banglore II	Both	
Yampaphant	8	6	0	1	7
Risingpatan	8	4	0	0	4
Baumara	8	2	0	1	3
Dhanubase	8	5	0	0	5
Phosre	8	0	0	0	0
Kudule	8	1		0	1
Total	48	18	0	2	20

Table 3. Strains of tomato yellow leaf curl virus infected samples of tomato in the western hills of Nepal in 1997

Site	No of samples	No of samples positive to TYLCV strain/s			No of infected samples
		Sri Lanka	Banglore I	Both	
Yampaphant	10	0	1	6	7
Risingpatan	5	0	0	1	1
Baumara	9	1	1	1	3
Dhanubase	6	0	0	2	2
Phosre	15	0	1	0	1
Kudule	10	0	0	9	9
Arghaun	5	1	0	3	4
Malepatan	5	0	0	1	1
Total	65	2	3	23	28

Dynamics of TYLCV vector

The whitefly population dynamics at Yampaphant and Risingpatan for the period of July to Oct are presented in Fig. 1.

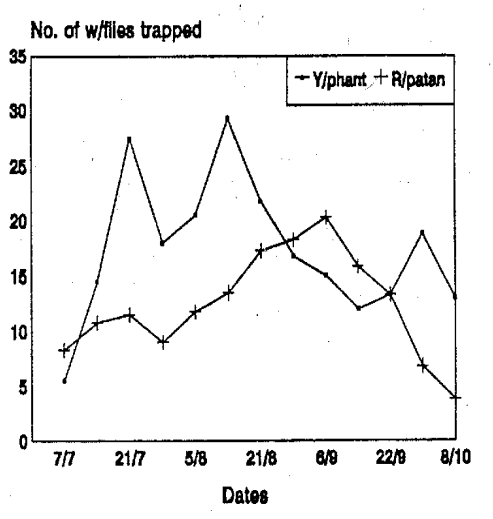

Fig. 1. Population dynamics of Bemisia tabaci at Yampaphant and Risingpatan from July to Oct 1996.

Whitefly was observed throughout the monitoring period. Three different peaks were observed at Yampaphant, in the third week of July, the second week of Aug and the fourth week of Sept. At Risingpatan, however, the whitefly population steadily increased till the first week of Sept to give a peak and then declined gradually to the first week of Oct. These results show that whitefly was active throughout the crop period at both sites.

The monitoring of TYLCV incidence in 1996 and laboratory results in 1996 and 1997 indicate a high incidence of TYLCV in the western hills of Nepal. The presence of at least three different strains Banglore I, Banglore II and Sri Lankan were known in the western hills from this study. An increased incidence of the disease, the appearance of TYLCV infected plants in high frequency over years and an active vector throughout the crop growing period in some commercial tomato growing pockets of the western hills suggest a tremendous importance of the disease in rainy season tomato. Therefore, research on the development of effective TYLCV management technology is urgently needed to sustain rainy season tomato cultivation in the area.

Acknowledgements

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Heterosis for Yield and Yield Components in Rice

Bal K Joshi*

Institute of Agriculture and Animal Science, Rampur, Chitwan

Abstract

It is important to know the degree and direction of hybrid vigor for its commercial exploitation. Heterobeltiosis and standard heterosis were studied in 14 crosses between rice (Oryza sativa L.) cultivars (improved and landraces) and three wild aborted male sterile parents. These crosses showed marked variations in the expression of heterobeltiosis and standard heterosis for yield and yield components. Grain yield manifested highly significant heterobeltiosis and standard heterosis in five crosses. Heterobeltiosis ranging from –55 to 139% and standard heterosis from –11 to 369% were observed. Highest heterotic effect among the yield components was for panicle number plant^{-1} followed by spikelet number and panicle length. With appropriate choice of parental lines, it is possible to develop F_1 rice hybrid possessing distinct yield superiority over the best-inbred lines.

Key words: F_1, heterobeltiosis, hybrid vigor, Oryza sativa, standard heterosis

Introduction

Rice is the most important crop in Nepal accounting for 50% of the total cropped area and production of the country (Upadhyaya, 1996). Efforts to improve rice productivity in Nepal have resulted in the introduction of a large number of improved cultivars with varying yield potentials. During the last 20 years, the productivity of rice remained nearly constant in spite of first priority given to the agricultural sector development during the same period by the HMG, Nepal (NARC, 1998). To meet the demand created by increasing population and rising incomes, increasing the yield potential of rice beyond that of semi dwarfing cultivars is an important strategy. Experience in China, India and Vietnam had established that hybrid rice offers an economically viable option to increase cultivars yield beyond the level of semi dwarf rice cultivars. Heterosis (also called hybrid vigor) in rice was exploited commercially in China, India, Vietnam and the Philippines. Davis and Rutger (1976), Virmani et al. (1981) reviewed on heterosis in various agronomic traits of rice. Virmani et al. (1981) reported a significant positive mid and high parent heterosis for yield ranging from 1.9 to 369% in rice. Standard heterosis for yield ranging from 16 to 63% was reported by Rutger and Shinjyo (1980) and from 29 to 45% by Yuan et al. (1994). Virmani et al. (1982) observed 54 and 34% heterosis for better parent and standard heterosis, respectively. In China yield under the large-scale production exceeded the best conventionally bred cultivars by 20 to 30% (Lin and Yuan, 1980).

It is important to know the performance of F_1 hybrids before exploitation in commercial scale. For practical exploitation of hybrid vigor in rice, emasculation is a major constraint, however the use of male sterile lines increases the chance of identifying more heterotic hybrids. In addition, parents should be locally adopted and should perform well in hybrid combinations. Hence, male sterile lines were used for the estimates of heterobeltiosis and standard heterosis for yield and yield components.

Materials and Methods

This experiment was conducted in a screen house and in experiment farm at the Institute of Agriculture and Animal Sciences (IAAS),

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Rampur, during the dry and wet seasons of 1998. IAAS is located at 84°29' E and 27°37' N and 224 masl. Eight improved Nepalese cultivars, six landraces and three wild aborted cytoplasmic-genetic male sterile (CMS) lines (Table 1) were used in this study. The improved cultivars were obtained from National Rice Research Program (NRRP), Hardinath, CMS lines from International Rice Research Institute (IRRI), the Philippines and the landraces were kindly provided by RC Sharma, IAAS, Rmapur.

Table 1. Improved rice cultivars, landraces and CMS lines used in the study†

A. Improved cultivars

Cultivar	Parentage	Origin	Grain type	Reaction to diseases‡	
				Bl	BB
Bindesowari	TN1/Co29	India	Medium	MR	MS
Chaite-6	NR6-5-46-50/IR28	Nepal	Medium	R	R
Janaki	Peta 3/TN//Renadja	Sri Lanka	Coarse	R	MR
Kanchan	CR 126-42-5/IR 2061-21-3	IRRI	Medium	MR	-
Khumal-7	Chaina1039DEFMUT/Ka18-361-1-8-6-10	IRRI	Coarse	R	-
Khumal-4	IR 28/Pokhreli Masino	Nepal	Fine	R	-
Masuli	Mayang Ebos80*2/ Taichumg65	Malaysia	Fine	S	MR
Radha-11	Local selection	India	Medium	S	MR
Sabitri	IR 1561/IR1737// CR94-13	IRRI	Coarse	MR	MR

B. Landraces

Landrace	Origin	Remarks
Chiunde	Nepal	All landraces are popular local cultivars with intermediate statured of hilly area of Nepal. They mature earlier than Terai local cultivars and are field resistant to blast and bacterial leaf blight
Deharadune	Nepal	
Gogi	Nepal	
IAR-97-34	Nepal	
Kature	Nepal	
Ratodhan	Nepal	

C. CMS lines of wild aborted type§

CMS line	Origin	Parentage	Remarks
IR58025A	IRRI	IR4843A/8* Pusa167-120	Stable in sterility, best combiner for yield, has aromatic long slender grains, developed more than 50 hybrids using this line in India.
IR62829A	IRRI	IR46828A/8* IR29744-94	Stable in sterility, has functional male sterility, very good combiner, developed more than 20 hybrids using this line in India
IR68888A	IRRI	IR62829A/6*IR62844-15//IR629744-94	Stable in sterility, good combiner

† Source: NRRP, 1997. ‡ Bl, Blast; BB, Bacterial blight; MR, Moderately resistant; R, Resistant; S, Susceptible.
§ Source: DRR, 1996.

Screen house experiment

F_1 seeds were produced in the screen house. Seeding interval (SI) for making synchronized flowering was determined from the days to 50% flowering. After determining SI between the parental lines, the seeding sequence was worked out. Three seeds of each parental line were placed over the moistened filter paper in petri dishes and were incubated for two days at 37°C. The CMS seeds were fumigated with Phostoxin whereas seeds of other cultivars were used without treatment. Three pre-germinated seeds were seeded in each five liter capacity plastic bucket filled two-third with soils and farmyard manure (FYM) in the ratio of 2:1. One seedling was removed after a month. The pollen parents were seeded thrice to ensure continuous supply of pollen to the female parent during the period of flowering whereas the CMS lines were seeded only once. There were altogether 22 plastic buckets including 18 buckets for male parent and four for seed parent (Fig. 1). Buckets were filled

with water after a week of transplanting. This condition was maintained up to the time of grain filling stage. Weeds were removed when observed in the buckets.

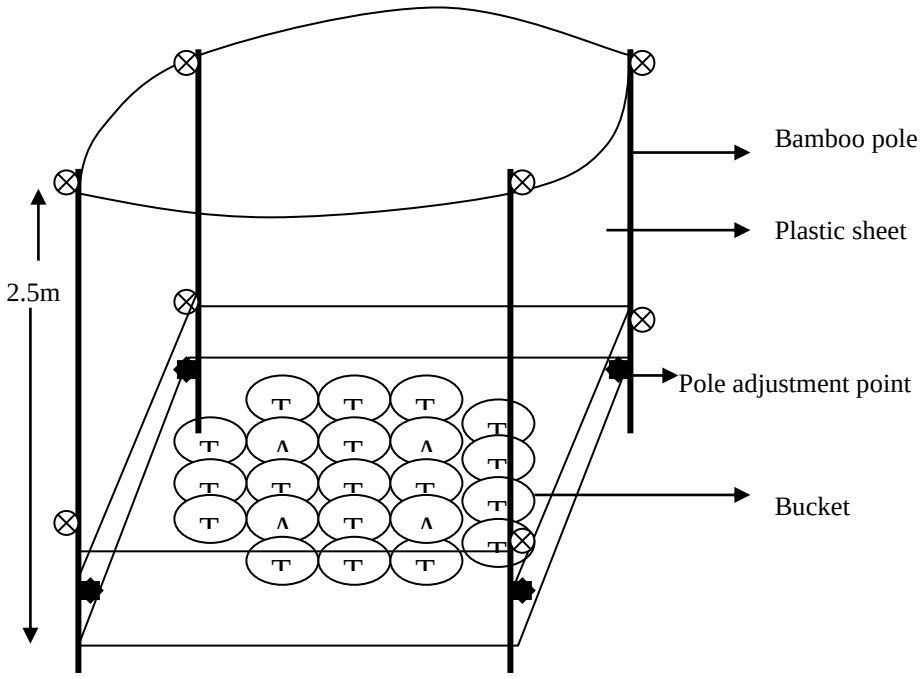

Fig. 1. Crossing chamber for crossing seed parent (A) and pollen parent (T).

Construction of crossing chamber: Four bamboo poles each of 3 m long were pegged in four corner of the crossing block in which 22 plastic buckets were easily accommodated (Fig. 1). Plastic sheet of 2.5 m width was used to make cylinder shaped crossing chamber. Top portion of chamber was left open. Fourteen crossing chambers were constructed before the extrusion of panicles.

Crossing: Pollen sterility was tested on each plant of CMS lines before crossing. This was determined by staining of pollen grains in 1% potassium iodide-iodine (I-KI) solution (Chaudhary et al., 1981). At heading, about 10 spikelets from each plant were collected in the morning just before their blooming and fixed in 70% alcohol. All the anthers from six spikelets were taken out with the help of forceps and placed in the stain and observed in microscope (10x). The CMS plants that showed complete sterility were used in crossing program. Approach method (Erickson, 1970) was used for pollinating the seed parents. Spikelets of CMS lines were cut one third from the tip of the floret to facilitate crossing and bagged with label at the time when the upper florets begin to exert anthers. The cutting procedure was carried out before 10.00 hrs and/or after 15.00 hrs. The panicles of female and pollen parents were bagged with a narrow glassine bag. Panicles with similar height were maintained by placing bricks under respective pots. The panicles of parental lines were left for 2 to 3 days and tapped gently to disperse the pollen. Shaking of the pollen parent was repeated 3 to 4 times during the day at an interval of 30 minutes. The process was repeated for 3 to 5 days as a supplementary pollination. The seed parents were harvested separately at 21 to 25 days after pollination. The

seeds were sun-dried for 2 to 3 days, bagged and labeled.

Field experiment

Field experiment, consisting of 14 F_1's, 14 pollen parents, three CMS lines and one check, Masuli was conducted to estimate the heterobeltiosis and standard heterosis. Dormancy of F_1 seeds was broken by keeping them at 50ºC for four days to grow in the field during the wet season of 1999. After breaking the dormancy, seeds were germinated in incubator as described earlier. For raising seedlings, trays of 30- × 20-cm size were filled with soil and FYM in the ratio of 2:1. The pre-germinated seeds were seeded in a 30 cm row spaced at 10 cm apart. Irrigation and weeding in nursery were done as needed.

Field layout and analytical procedures

The field was laid out in a randomized complete block with three replications. The F_1 was planted in the middle of the pollen parent and CMS line. Masuli was planted as a check variety in six replications. Field was fertilized at the rate of 120:60:60 kg $NP_2O_5K_2O$ ha^{-1}. Half of the nitrogen fertilizer was applied as a basal dose and half was top-dressed at one month after transplanting. Twenty-one day old seedlings were transplanted in the field in four rows of each plot with 10 hills per row at a spacing of 20- × 20-cm. Single seedling was planted in each hill. Irrigation was applied as necessary. Field was weeded twice at one-month interval after transplanting. Rouging was carried out at both vegetative and flowering stages. Gundhibug (Leptocorisa oratorius F.) was controlled by sprayings insecticide (Roger). All other standard agronomical practices were followed.

Following characters from the middle two rows of each plot were recorded according to IRRI (1980), Ba Bang and Swaminathan (1995).

- Panicle number $plant^{-1}$: average from 20 hills $plot^{-1}$ at maturity.
- Number of filled grains $panicle^{-1}$: average from five panicles $plot^{-1}$.
- 1000-grain weight: randomly collected from seed lot of each plot.
- Grain yield: weight of clean and dry grains, g m^{-2}.
- Panicle length: average from five panicles $plot^{-1}$, measured from panicle base to tip.
- Spikelet fertility: number of seed set divided by total spikelet.

Grain yield and 1000-grain weight were adjusted at 14% moisture as suggested by Gomez (1972). F_1 hybrid performance was evaluated on the basis of the estimates of heterobeltiosis (Fonseca and Patterson, 1968) and standard heterosis (Virmani et al., 1982; Subedi, 1982) as follows:

Heterobeltiosis = $\{(F_1\text{-BP/BP})\} \times 100$
Standard heterosis = $\{(F_1\text{-CC})/CC\} \times 100$

Heterosis is expressed as percent increase of the F_1 hybrids above the BP and CC, where F_1 is the average performance of the F_1; BP, the average performance of better parent and CC, average performance of commercial cultivar.

The analysis of the variance was performed following Gomez and Gomez (1984). The square for the interaction of blocks by entries was used to test the significance of the mean square for entries. The F test was used to test the significance of mean squares. Least significant difference (LSD) was used to compare the means. MSTATC (1986) software was used to analyze the data.

Results and Discussion

Significant positive heterobeltiosis and standard heterosis for yield were noticed in IR68888A/Radha-11, IR62829A/Ratodhan, IR62829A/Kature, IR58025A/Kanchan and IR58025A/Sabitri (Table 2). Five hybrids showed highly significant increase in yield with a standard heterosis from 67.92 to 369.27%. Three hybrids showed negative standard heterosis but the values were not significant. On an average hybrid showed superiority over inbred line in yield and yield components (data not shown). In four crosses, most of the spikelets were sterile. Therefore, heterobeltiosis and standard heterosis

for yield, grain number panicle^{-1}, spikelet fertility and 1000-grain weight were not estimated. It indicated that pollen parents of these sterile hybrids might not have restorer gene(s).

Table 2. Heterobeltiosis and standard heterosis for yield and yield components in 14 crosses of rice

SN	Hybrid	Yield, g m^{-2}	Panicle no. plant^{-1}	Spikelet no. panicle^{-1}	Grain no. panicle^{-1}	Spikelet fertility, %	1000-grain wt, g	Panicle length, cm
1	IR68888A/Radha-11							
	Heterobeltiosis	139.20**	80.53**	30.85*	35.31**	3.41	-2.04	10.5
	Standard heterosis	369.27**	98.10**	42.48**	46.86**	3.07	25.05*	14.91*
2	IR58025A/Janaki							
	Heterobeltiosis	-54.99**	0.00	13.1	-50.48**	-56.22**	-20.36**	4.15
	Standard heterosis	-20.87	-7.32	-4.6	-62.64**	-60.84**	45.55**	15.47*
3	IR58025A/Kanchan							
	Heterobeltiosis	55.64*	0	14.06	5.53	-7.48	3.41	9.88
	Standard heterosis	67.92**	7.32	16.66	-6.29	-19.67**	25.70*	12.03
4	IR58025A/Khumal-4							
	Heterobeltiosis	-37.13**	-10.87	36.12*	-12.34	-35.60**	0.29	-5
	Standard heterosis	56.68*	7.32	41.01**	-4.13	-32.01**	22.74*	14.31*
5	IR58025A/Sabitri							
	Heterobeltiosis	37.29**	-6.52	36.17*	33.15**	-2.22	0.35	9.47
	Standard heterosis	144.62**	4.83	17.16	17.44	0.23	34.53**	19.17**
6	IR58025A/Chaite-6							
	Heterobeltiosis	-42.50**	-9.82	38.71*	-7.21	-33.11**	-3.24	15.34*
	Standard heterosis	-3.15	12.14	13.14	-22.72*	-31.69**	23.81*	18.61**
7	IR68888A/Bindesowri							
	Heterobeltiosis	!	2.41	11.35	!	!	!	4.04
	Standard heterosis	!	2.41	4.67	!	!	!	17.45*
8	IR68888A/Khumal-7							
	Heterobeltiosis	!	17.65	21.43	!	!	!	5.69
	Standard heterosis	!	-2.49	2.74	!	!	!	11.73
9	IR62829A/Deharadune							
	Heterobeltiosis	!	31.32*	6.3	!	!	!	11.15
	Standard heterosis	!	2.41	11.56	!	!	!	5.41
10	IR62829A/Ratodhan							
	Heterobeltiosis	57.39**	40.55**	11.14	11.96	0.74	6.75	-1.02
	Standard heterosis	127.30**	26.77*	0.66	-5.92	-6.53	38.62**	13.15
11	IR68888A/Gogi							
	Heterobeltiosis	-25.92	48.02*	9.93	-57.09**	-60.97**	-7.88	7.77
	Standard heterosis	-8.93	-9.8	23.58*	-61.64**	-68.96**	58.64**	38.93**
12	IR62829A/Kature							
	Heterobeltiosis	42.39*	44.42**	29.08*	-0.34	-22.80**	4.67	-8.64
	Standard heterosis	92.25**	26.77*	14.96	-7.85	-19.84**	39.39**	27.20**
13	IR68888A/Chiunde							
	Heterobeltiosis	!	32.05	18.82*	!	!	!	12.27
	Standard heterosis	!	-19.53	45.69**	!	!	!	25.78**
14	IR58025A/IAR-97-34							
	Heterobeltiosis	-33.91*	17.9	9.30**	-40.45**	-45.52**	5.28	31.57**
	Standard heterosis	11.49	-19.53	48.55**	-12.99	-41.43**	27.36**	19.43**

Table 2. Continued…

SN	Hybrid	Yield, g m^{-2}	Panicle no. $plant^{-1}$	Spikelet no. $panicle^{-1}$	Grain no. $panicle^{-1}$	Spikelet fertility, %	1000-grain wt, g	Panicle length, cm
Range								
	Heterobeltiosis	-54.9 to 139	-10.8 to 80.5	6.3 to 38.7	-57 to 35.3	-60.9 to 3.4	-20.4 to 6.8	-8.6 to 31.6
	Standard heterosis	-10.9 to 369	-19.5 to 98.1	-4.7 to 48.6	-63 to 46.9	-68.9 to 3.07	22.7 to 59	5.4 to 38.9
Mean								
	Heterobeltiosis	13.75	20.54	20.45	-8.14	-25.98	-1.28	7.65
	Standard heterosis	83.66	7.79	19.88	-11.99	-27.77	34.14	18.12
SE								
	Heterobeltiosis	19.75	7.19	3.07	10.27	7.57	2.54	2.58
	Standard heterosis	36.59	7.79	4.75	10.34	7.69	3.67	2.19

* Statistically significant at 5%; ** Significant at 1%. ! All sterile spikelets. SE, Standard error.

Nine hybrids showed higher tillering capacity than check cultivar, but only three crosses manifested significant heterobeltiosis and standard heterosis for panicle number. Increase in panicle number was earlier observed by Singh et al. (1980), Anandakumar and Sree Rangasamy (1986) whereas Virmani et al. (1981, 1982), Jennings (1967) reported the negative heterosis for panicle number in the hybrids.

Hybrid vigor for panicle length was noticed in 14 crosses but only ten crosses showed significant standard heterosis. Singh et al. (1980) reported similar results. There were no positive significant heterobeltiosis and standard heterosis for spikelet fertility percentage. Six crosses showed highly negative significant heterobeltiosis and standard heterosis for spikelet fertility. Non significant positive or negative heterosis for this trait was reported by Virmani et al. (1981).

It appeared that hybrid vigor in yield were due to significantly high yield components eg tiller number, panicle length, spikelet number and 1000-grain weight. Grafius (1959) suggested that there is no separate gene system for yield per se and that the yield is an end product of the multiplication interaction between the yield components. This was confirmed by the present investigation where none showed hybrid vigor for yield alone. In five crosses, the heterotic effect in yield was along with hybrid vigor for panicle number, 1000-grain weight, spikelet number and panicle length thus, it is obvious that hybrid vigor for yield is the result of interaction of simultaneous increase in the expression of yield components. Among the yield components highest heterosis effect was for panicle number followed by spikelet number and panicle length. Similar result was observed by Mandal (1982). The major yield components in rice are number of panicles $plant^{-1}$, spikelet number $panicle^{-1}$, spikelet fertility percentage and 1000-grain weight (Virmani and Edwards, 1983). There are many reports showing evidence of significant positive high parent heterosis and standard heterosis for yield and yield components. Although the hybrids had fewer effective panicles per square meter, they had significantly more filled grains per panicles and larger seeds (Virmani et al., 1981). Significant positive mid parent, high parent and standard heterosis were observed for one or more of yield components in a number of crosses (Carnahan et al., 1972; Mohanty and Mohapatra, 1973; Saini and Kumar 1973; Mallick et al., 1978; Virmani et al., 1982; Luat et al., 1985; Peng and Virmani, 1994). Virmani et al. (1981) observed negative heterosis for panicle number per square meter. Most crosses showing significant standard heterosis for yield were found to be possessing heterosis for more than one component (Maurya and Sing, 1978; Virmani et al., 1982). Results obtained in China and at IRRI indicate that heterotic F_1 combinations usually show an increased sink size through an increase in spikelet per panicle, spikelet fertility percentage and 1000-grain weight (Virmani and Edwards, 1983).

According to Swaminathan et al. (1972) heterobeltiosis of more than 20% over better parent could offset the cost of hybrid seed. Thus,

the crosses showing more than 20% of heterobeltiosis viz., IR68888A/Radha-11, IR62829A/Ratodhan, IR62829A/Kature, IR58025A/Kanchan and IR58025A/Sabitri may be exploited for hybrid rice production.

Maximum variation was observed in heterobeltiosis and standard heterosis for yield among hybrids followed by grain number panicle^{-1}. F_1 rice hybrids are useful not only for their high grain yield per cropping season. The results indicated the possibility of obtaining more heterotic hybrids only in specific cross combinations. With appropriate choice of parental lines it appears possible to develop F_1 rice hybrid possessing distinct yield superiority over the best-inbred lines. Yield components should be considered to increase the yield through selections.

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Dedicated to my parents, Late Setu Lal Joshi and Gauri Maya Joshi.

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Response of Rice Varieties to Age of Seedlings and Transplanting Dates

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Abstract

A field experiment was conducted at Regional Agricultural Research Station, Parwanipur in 1998/99 and 1999/00 with an aim to find out the alternate management practices to compensate the loss in the grain yield due to flood. Irrespective of the varieties and transplanting dates, age of seedlings had no effect on grain yield of rice. But transplanting dates had significant effect on grain and grain contributing characters. The yield of rice transplanted at 1 Sept was 25.6 and 37.5% less in 1998/99 and 1999/00 respectively as compared to rice grain yield of 14 July transplanting. Radha 11 registered the highest grain yield of 4086 kg ha^{-1} in 1999/00 and 2662 kg ha^{-1} in 1998/99, which was at par to the yield obtained by Sabitri at the same year. The interaction effect of the age of seedlings, transplanting dates and varieties were found significant in both the years. 25 days old seedlings transplanted on 14 July in 1999/00 of rice varieties Masuli and Radha 11, produced statistically the similar yield. Radha 11 was the best among the tested varieties. 25 days old seedlings of Masuli, Basmati, Sabitri and Radha 11 can be recommended to transplant as late as Sept 1.

Key words: Age of seedlings, rice, transplanting dates

Introduction

Rice acreage in the country is 1.55 million ha with an average production of 2.59 t ha^{-1}, which is much less as compared to potential yield of the newly developed varieties (MoAC, 1999/00). The major factor of low productivity is due to rainfed condition. Nearly 79% of the rice growing area are under rainfed condition which faces prolonged drought or submergence due to excessive rain because of erratic rainfall pattern in the certain years (NARC, 1996). About 7 to 10% area of total rice growing acreage was found under submerged conditions for 2 to 10 days or even more days. Prevailing of such situations did considerable damage to the rice crop stand. And re-raising of rice seedlings and retransplanting under delayed conditions might not favor for optimum production. The information to tackle the problem caused by flood is not available. Therefore, alternate management strategy needs to be explored. Hence a study was under taken to study the effect of the age of seedling of rice varieties in relation to transplanting dates under rainfed ecosystem.

Materials and Methods

A field experiment was laid out in 2 × 5 × 3 factorial randomized block design with 3 replications at Regional Agricultural Research Station, Parwanipur (27°04' N and 84°58' E), 115 m above sea level. Seedlings of 25 and 50 days old of five rice varieties (Basamati, Sabitri, Chaite 4, Masuli and Radha 11) were transplanted on 14 July, 7 Aug, 1 and 26 Sept 1998/99 and 1999/00 wet season. The plot size was kept 4- × 3-m and with spacings of 20 cm hill to hill and row to row. Two seedlings $hill^{-1}$ were maintained in both the years. Fertilizer dose of 50:40:30 kg $N:P_2O_5:K_2O$ ha^{-1} were applied as basal and remaining nitrogen was top dressed 25 days after transplanting. The cultural practices were done as per need of the crop. Data were recorded on heading, maturity days, plant stand m^{-2}, filled grains $panicle^{-1}$, 1000-grain weight and grain yield. The last date of transplanting could not produce the grain, therefore, this treatment had been deleted from the analysis of the variance. The statistical analysis was done using MSTATC program.

Results and Discussions

Effect of the age of seedlings

The age of seedling had significant effect on heading and maturity days. The 50 days old seedling headed and matured earlier as compared to 25 days old seedlings (Table 1). The number of filled grains panicle^{-1}, 1000-grain weight, plant stand m^{-2} and even grain yield were found insignificant. Such results are in agreement with the findings of Shrestha (1975), Mallik and Singh (1976). The differences in heading and maturity days due to seedlings age could not significantly influence the grain yield in both the years.

Table 1. Average yield and yield contributing characters of 5 rice cultivars as influenced by the age of seedlings at transplanting during 1998/99 and 1999/2000

Seedling age	Heading days		Maturity days		Plant stand m^{-2}		Filled grains panicle^{-1}		1000-grain wt, g		Grain yield, kg ha^{-1}	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
25 day old	124	119	154	147	169	236	94	101	19.4	21.8	2203	3643
50 day old	102	104	133	132	174	232	97	98	19.2	21.1	2356	3320
F test	**	**	**	**	ns	ns	ns	ns	ns	ns	ns	ns
LSD (0.05)	1.7	1.2	1.9	1.8	-	-	-	-	-	-	-	-

** Significant at 0.1; ns, Non significant.

Effect of transplanting dates

The yield contributing characters and grain yield exhibited the poor performances due to heavy attack of brown plant hopper, despite of all measure taken to control in 1998/99 whereas crop was free from brown plant hopper in 1999/00. Transplanting dates had significant or highly significant effects on all characters except 1000-grain weight in both the years. The number of days from seeding to heading and maturity was recorded in the decline trend, as transplantation was delayed. The number of filled grains panicle^{-1} and plant stand m^{-2} followed the same pattern. Grain yield of rice transplanted on 14 July and 7 Aug did not differ significantly in 1998/99. The grain yield of 1 Sept transplanting was 21.7 and 37.8% less in 1998/99 and 1999/00 respectively as compared to 14 July transplanting. The results are in agreement with the findings of Koirala (1983), Kunwar and Shrestha (1979), Bhurer et al. (1990) who reported that early planting of rice gave higher yields and gradual decline in the grain yields in delayed transplanting. The reason for declining the grain yield might be due to delayed panicle formation and grain filling in the season where temperature and solar radiation was less (IRRI, 1993). It is natural process that the crop which had taken more number of days from seeding to maturity might have a more vigorous and extensive root system, increased growth rate during vegetative growth, more efficient sink formation and greater sink size, greater carbohydrate translocation from vegetative plant parts to the spikelets and longer leaf area index during grain filling period. So, this might be the possible reason to have high yields in earlier transplanting.

Table 2. Effect of the transplanting dates on agronomic characters and grain yield (average of 5 rice cultivars) during 1998/99 and 1999/2000

Transplanting date	Heading days		Maturity days		Plant stand m^{-2}		Filled grains panicle^{-1}		1000-grain wt, g		Grain yield, kg ha^{-1}	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
14 July	120	114	149	141	176	257	110	103	19.2	21.9	2574	4149
7 Aug	112	112	141	139	163	227	91	106	19.4	21.4	2349	3706

1 Sept	107	110	140	139	150	200	85	90	19.5	21.1	1914	2590
F test	**	**	**	**	**	**	*	*	ns	ns	**	**
LSD (0.05)	2.1	1.5	2.3	1.93	11	14	11.3	11.6	-	-	225	184

** Significant at 0.1; ns, Non significant.

Effect of varieties

All characters were found statistically highly significant different among rice varieties (Table 3). Chaite 4 took minimum days to heading and maturity and vice versa in Basmati in both the years. Radha 11 registered the maximum number of grains panicle^{-1} and reverse trend was reflected in Chaite 4 in both the years. Radha 11 produced the highest grain yield of 4086 kg ha^{-1} in 1999/00 and 2662 kg ha^{-1} in 1998/99 which was at par to the grain yield of Sabitri in the same year. The highest grain yield of Radha 11 might be due to more number of filled grains panicle^{-1} and plant stand m^{-2}.

Table 3. Agronomic characters of 5 rice cultivars during 1998/99 and 1999/2000

Cultivar	Heading days		Maturity days		Plant stand m^{-2}		Filled grains panicle^{-1}		1000-grain wt, g		Grain yield, kg ha^{-1}	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
Basamati	122	120	156	152	142	223	74	97	22	24.2	1930	3259
Sabitri	118	118	150	148	194	241	105	103	19.1	21.1	2693	3421
Chaite 4	90	89	119	116	202	260	65	75	20.2	23.8	1874	3020
Masuli	120	115	148	142	151	219	125	122	17	17.6	2238	3623
Radha 11	116	114	144	141	170	226	107	101	18.4	20.7	2662	4086
F test	**	**	**	**	**	**	**	**	**	**	**	**
LSD (0.05)	2.7	1.9	2.9	2.5	14	18.3	14.6	14.9	0.83	0.6	290	238

** Significant at 0.1.

Interaction effect of grain yield

Three level interaction of age of seedlings, transplanting dates and varieties of two years is presented in Table 4. Two level interactions of three factors are not included, however, the LSD values can be used to compare any two means within a year.

Twenty five days old seedlings of Basamati rice transplanted either on 14 July or 1 Sept could not bring statistical differences in the grain yield and the same case was observed in 50 days old seedlings in 1999 (Table 4). However, 25 days old seedlings of Masuli and Radha 11 produced the grain yield more or less similar to transplanted on 14 July and 7 Aug and significant decline in the grain yields was observed on 1 Sept transplanting in both the years. 50 days old seedlings of Sabitri in first year and all varieties in second year gave significantly high yield transplanted on 14 July as compared to 1 Sept 1 transplanting. 25 days old seedlings of Sabitri, Masuli and Radha 11 produced significantly higher grain yield as compared to 50 days old seedlings transplanted on 1 Sept.

Radha 11 was considered as the best among the test varieties in respect of producing the grain yield. Though, reduction in the grain yield had been noticed to a greater extent on 1 Sept transplanting as compared to 14 July and 7 August transplanting. However, 25 days old seedlings of Basmati, Sabitri, Masuli and Radha 11 might be recommended to transplant as late as 1 Sept. Transplanting of rice irrespective of age of seedlings and varieties should be adjusted up to first week of Aug. Otherwise, decline trend in grain yield might be registered in delayed transplanting.

Table 4. The grain yield, kg ha^{-1} as affected by the interaction effect of age of seedlings, transplanting dates and rice cultivars

	25 days old seedlings									
	1998/99					1999/00				
Transplanting date	Basmati	Sabitri	Chaite 4	Masuli	Radha 11	Basmati	Sabitri	Chaite 4	Masuli	Radha 11
14 July	1997	2427	2278	2672	2758	3303	3738	3387	4352	4506
7 Aug	1650	3165	1480	2509	2762	4026	3894	2905	4116	4187
1 Sept	1530	2504	1807	1657	1843	2663	3473	2941	3444	3702
	50 days old seedlings									

14 July	2423	3002	2309	2586	3288	3577	4945	3822	4576	5278
7 Aug	2115	3233	1738	2069	2771	3562	3627	3190	3609	3942
1 Sept	1865	1825	1634	1933	2549	2420	846	1874	1639	2898
F test			**					**		
LSD (0.05)			712					580		

** Significant at 1%.

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Honeybee Flora at Kabre, Dolakha District

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Abstract

Adequate knowledge about bee flora is the prerequisite to initiate bee keeping. A study was conducted at Kabre area of Dolakha district during 1997-1999 to identify existing bee flora and develop a floral calendar. Based on the interview with bee farmers and visual observations, 119 important plant species were recorded, out of which 47 species were found major sources for honeybees. Spring season (mid-March to mid-June) and autumn season (mid-Sept to Oct) were identified as honey flow periods having a number of floral plants such as Guizotia abyssinica, Fraxinus floribunda, Prunus cerasoides, Pyrus communis, Castanopsis indica, Brassica spp., Citrus spp., Berberis spp., Rubus spp., Rhododendron spp. and Trifolium spp. Winter season (mid-Nov to Feb) is the critical dearth period with a few flowering plants like Reinwardtia indica, Pogestemon glaber, Caesalpinia spp. and Eupatorium spp. Depending upon the climatic conditions, possibility of planting multipurpose plants has been discussed. Based on available flora, major characteristics of these plant species, utility status and flowering duration a bee floral calendar was developed for Kabre. To conserve these floras, attention must be made to maintain and multiply the existing flora.

Key words: Apis cerana, bee flora, bee keeping, dearth period, honey flow

Introduction

Bee keeping is one of the important farming activities in Nepal since ancient times. Being a non-land based enterprise with multipurpose output, the demand of bee keeping has been increased tremendously in Nepal. Success in bee keeping depends upon many factors, among them availability of bee flora is the fundamental one. Bees obtain nectar, pollen, or both from flowers, which are the mainstay of honeybee's life. The value of flora in bee keeping has been observed in many parts of the world. For instance, the directory of world honey sources (Crane et al., 1984), honey plant resources of Hindu Kush-Himalayan region (Verma, 1990; Partap, 1997) and bee flora of India (Kaur and Sihag, 1994) are some existing examples of such efforts. In the context of Nepal, Kafle (1984), Thapa and Dangol (1990) reported one hundred fifty six and over one hundred bee floras available respectively in Kathmandu valley and Rampur, Chitawan.

However, plant types and their flowering duration differ from one place to other due to variation in topography, climate and other cultural and farming practices. The extensive knowledge on type, density and quality of bee flora in a region are prerequisites for successful bee keeping. Such information enable beekeepers to utilize them at the maximum level, so that, they can harvest a good yield of honey and other bee products in addition to effective pollination which enhances crop yields. Every region has its own honey flow and floral dearth period(s) of short or long duration. Such knowledge on bee flora help in the effective management of bee colonies during such periods. Considering these facts, the present study was made to prepare an inventory of existing bee flora and develop floral calendar for Kabre region of Dolakha district.

Materials and Methods

This study was undertaken during 1996/97 to 98/99 around Kabre Village Development Committee in Dolakha district. Geographically, Kabre is located at 27.8º N latitude and 86.3º E longitude. The average altitude of this area is 1740 meter above sea level (m asl). However, the foraging area of honeybee ranges approximately

from 1250 to 2200 masl. The mean minimum temperature during 1993-1997 was 10.8 ± 0.99ºC but it dropped down to 0ºC during winter. Dec and Jan are the coldest months with average minimum and maximum temperature of 4.51 ± 0.09ºC and 16.22 ± 0.54ºC respectively. The hottest days of the year are during mid-April to mid-Sept, when the mean maximum temperature during 1993-1997 reached up to 22.4 ± 0.18ºC. The average annual rainfall was 2192.5 ± 87.5 mm. Over 90% of total rainfall was received during the months of June-Sept (HCRP, 1999).

A survey questionnaire was prepared comprising mainly of common and local names of different flowering plants of that area, their flowering season and duration, habit, nectar or pollen yielding ability and their abundance in the area. A total of 28 questionnaires were prepared, out of which 24 were interviewed with farmers, two with agriculture and two with forestry personnel to gather information on honey plant resources, available honeybee species and bee keeping practices around Kabre area.

The information was focused mainly on the farmers' statement in the questionnaires. However, the major bee foraging plants were further verified by visual observation. The foraging plants were marked and two observations were made in each flowering season. Such observations were made for three seasons (three years). The observation on nectar and pollen source was based on activities performed by honeybees on different flowers. Honeybees with their activity of extending their proboscis into the flowers are considered as nectar source and bees carrying pollen on their hind legs were determined as pollen source. The status of flowering plants, whether they are major or minor, was determined by the frequency and the number of honeybees' visits. The density of those plants found around the region determined the abundance of bee plants. Finally, the plants visited by honeybees were later on collected, identified and then compared with the published reports (Partap, 1997, Polunin and Stainton, 1997, Shrestha, 1998) for their uses by honeybees.

Results and Discussion

Honeybee species and bee keeping practices

Three different honeybee species were found at Kabre. They were little honeybee (Apis florea F.), the common hive bee (Apis cerana F.) and the giant bee (Apis dorsata F.). According to farmers' experience, the predominant species is A. cerana (78.6%), followed by A. dorsata (17.9%) and A. florea (3.6%).

A. cerana was the predominantly cultivated species and almost all the farmers maintained it on traditional fixed hives as wall hive (Khopa Ghar) or log hive (Mude Ghar). A few farmers (21.4%) kept modern hives but the production of honey was not satisfactory due to lack of appropriate management practices. Swarming and absconding were the major problems. Cutting off of the drone brood and cleaning up of the hive during autumn season to minimize swarming were the main management practices followed by farmers. These activities were not enough. April-May is the annual honey-extracting period with average 5-6 kg of honey per colony. However, some experienced farmers (32.1%) also extract during Oct getting a total extract of 10-15 kg honey per colony per year.

Honeybee flora

Various plants were blossoming in different seasons and honeybees visited these plants for nectar and pollen. Based on the source status and abundance, altogether 119 plant species were identified as important bee flora at Kabre area. Based on frequency, number of bee visits and abundance, they were further classified into three groups. Forty-seven plant species were recognized as major source, forty-five species as medium source and the remaining twenty-seven species as minor source for honeybees (Annex 1). Among major plant species, Guizotia abyssinica, Pyrus communis, and Brassica spp. as cultivated plants and Prunus cerasoides, Fraxinus floribunda, Berberis spp., Rubus spp. and Rhododendron spp. among wild plants were identified as extremely important bee floras of Kabre area. Some of the medium and minor

source plant species blossomed for long periods about 5-6 months or more were Ageratum conyzoides, Colebrookea oppositifolia, Inula cappa, Nicandra physaloides, Osbeckia stellata, Oxalis corniculata, Persicaria capitata, Sapium insigne, Vitex negundo, Cynoglossum spp., Polygonum spp., Plectranthus spp. and considered them as important floral species. Some ornamental plants Euphorbia pulcherrima, Malvaviscus arboreus, Salvia splendens and Tagetes erecta though in less area, blossomed also for longer period. The honeybees utilized these plant species during colony development and dearth periods. Likewise, plant species Aesandra butyracea, Callistemon citrinus and Grevillea robusta were found in a few number but these plants were referred to as good nectar and pollen source for honeybees (Partap, 1997).

Number of honey plant species found at different altitudes around Kabre area are presented in Fig. 1. 81 and 104 species were found in Lekh high hill (above 1500 masl), Besi foot hill (below (1500 masl) respectively. Among them 66 were common in both sides. Some plants like Zea mays and Juglans regia were found in abundance at both areas and the bees utilized these plants as the source of pollen. Apart from these two above species, honeybees utilized almost all identified bee floras as the source of both pollen and nectar. The source status of different identified plant species are presented in Annex 1. Some traditional bee farmers informed that the honey from Lyonia ovalifolia, Prinsepia utilis and some species of Rhododendron as well as Cannabis sativa yielded toxic nectar, which are non-poisonous to honeybees but poisonous to human health. This was also reported earlier by Kafle (1992).

Likewise various vegetables as Abelmoschus esculentus, Coriandrum sativum and different gourds have been grown at every homestead garden for kitchen purposes and some vegetables such as Allium cepa and Brassica spp. are grown for seed purposes. All these plants were regularly visited by honeybees. Some farmers (10.71%) were found using pesticides such as dichlorovos (Nuvan) and methyl parathion (Metacid) in some vegetables in foot hill areas, but other were using wood ashes to control pests causing no harm to bees. It was observed that some bee floras like Melastoma melabathricum, Grevillea robusta, Grewia optiva and Bauhinia spp. were used as fodder and the farmers cut them before or at the time of flowering. So these plant species were of less value to honey production in that area.

Honeybee foraging activity, honey flow and dearth periods

At Kabre area, the peak periods of honeybee foraging activity were recorded during mid-Feb and May (spring season) and mid-Aug and Oct (autumn season) (Fig. 2). During the seasons, abundant bee floral plants were found blossoming with mild temperature and little or no rainfall. Eight plant species (Brassica spp., Citrus spp., Pyrus communis, Berberis spp., Fraxinus floriblunda, Rubus spp., Rhododendron spp. and Trifolium spp.) during the spring season and five plant species (Guizotia abyssinica, Prunus cerasoides, Brassica spp., Castanopsis indica and Mirre jhar) during the autumn season were recorded as the major source of honey production around Kabre area. Honeybees visited these plants extensively for honey production and colony multiplication. Other medium and minor floras during these periods also supported the honey production.

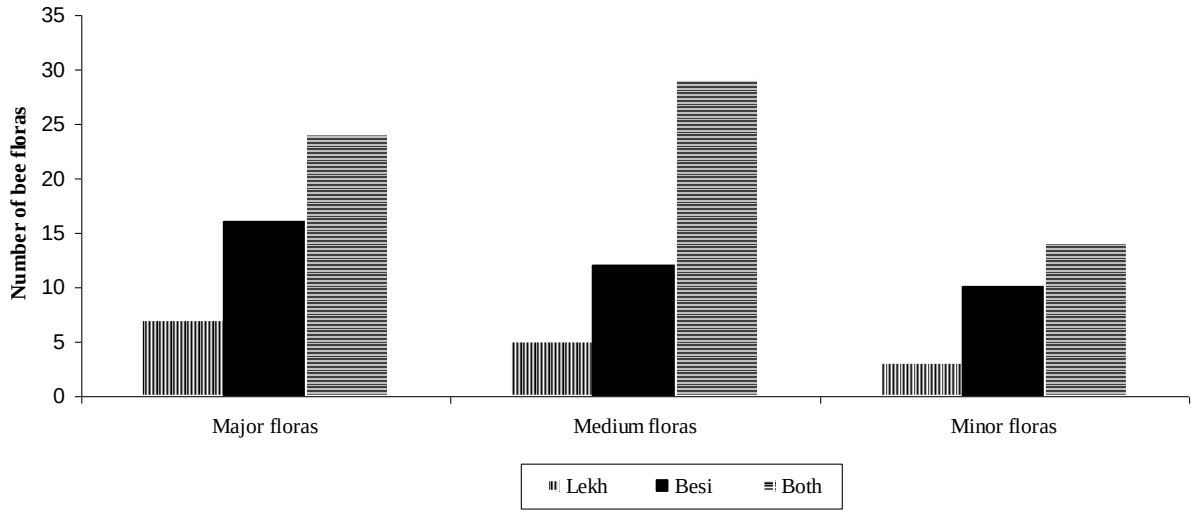

Fig. 1. Number of available major, medium and minor bee floras at different altitudes in Kabre area of Dolakha district.

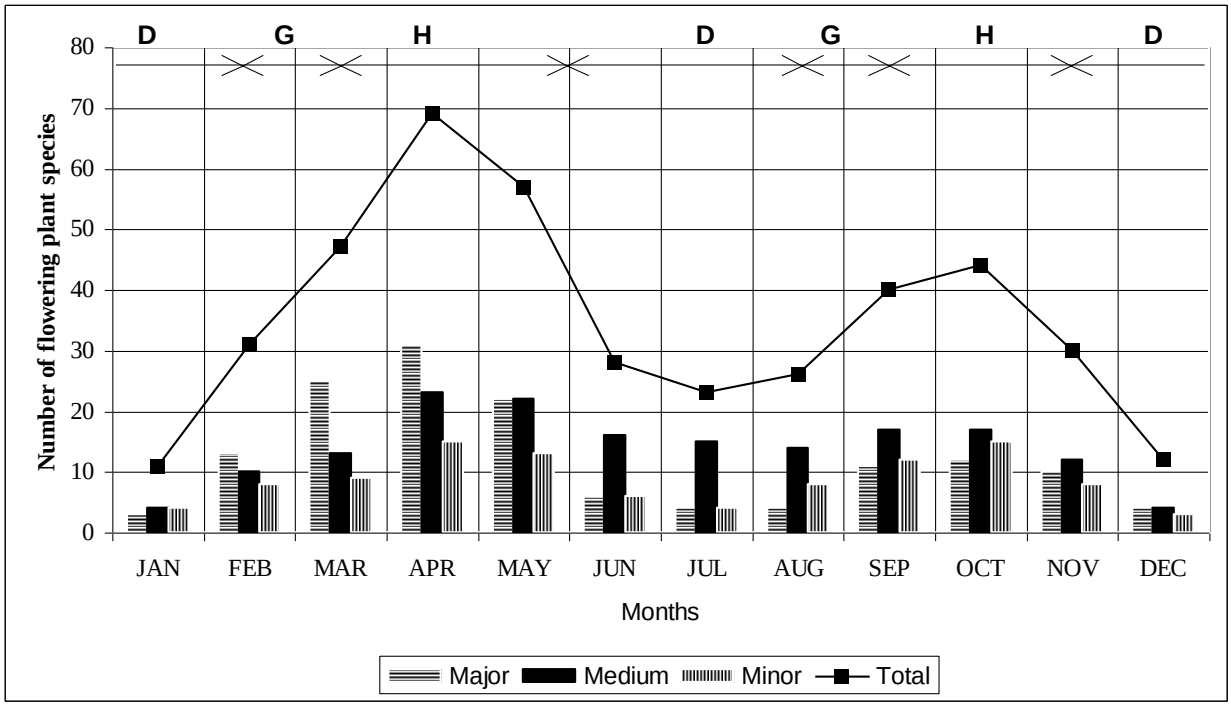

Figure 2. Number of major, medium and minor bee floras flowering in different months and colony growth (G), honey flow (H) and dearth period (D) at Kabre area of Dolakha district

Early spring (from mid-Feb to mid-March) and autumn season (from mid-Aug to mid-Sept) were observed to be the colony development period for honeybees at Kabre. The climate gradually become favourable for bees and the plant species Caryopteris odorata, Leucosceptrum canum, Buddleia spp., Prunus domestica, Prunus persica and Eupatorium spp. during early spring season and Rhus spp., Porana grandiflora, Glycine max, Osbeckia stellata and Rubus spp. during early autumn season help in the colony development.

Mid-Nov - Feb (winter season) and June - Aug (rainy season) were identified as the dearth periods for honeybee at Kabre area. Winter season is the critical dearth period with low temperature (minimum temperature often goes below 0°C), short sunshine period and very few flowering plants like, Reinwardtia indica, Pogestemon glaber, Caesalpinia spp., Eupatorium spp. Although some honey floras, Zea mays, Phaseolus spp., Ranunculus spp., Vitex negundo, Crinum amoenum, Mussaenda roxburghii, Lagerstromia spp., Curcuma aromatica and some vegetables blossomed during the rainy season, they were not found sufficient to sustain for the honeybee colonies in that area. Because of continuous rain and thereby fluctuation in temperature, this period was also found unfavorable for honeybee foraging. However, the pollen requirement during the rainy season was found to be fulfilled by Zea mays, Phaseolus spp. and Glycine max. Major and minor plants, dearth period, colony growth and honey flow period at different months are shown in Fig. 2.

Bee floral calendar

Based on the availability of different plants along with their flowering time, a bee floral calendar was developed for Kabre area (Table 1).

Suggestions for plantation of bee floral plants

Due to high variation in altitude and climatic condition, this region is suitable for growing various multipurpose plants such as Aesandra butyracea, Cedrela toona, Azadirachta indica, Melia azedarach, Grevillea robusta, Grewia optiva, Morus alba, Albizzia spp., Bauhinia spp., Eucalyptus spp., Eurya spp. and different Trifolium spp., which have been growing in the region but in limited number. Horticultural trees such as Citrus spp., Prunus domestica, Prunus persica, Pyrus communis, Phyllanthus emblica, Choerospondias axillaris, Musa paradisiaca, Diospyros virginiana, Syzygium spp. and Psidium guajava could be replanted to increase the number. This view was expressed also by Partap (1992).

The success of bee keeping depends not only on honeybee strains, its management and hive structures, but also on the abundance and availability of bee floral plants around bee farming area. Based on the study and available bee floras, Kabre is a suitable area to initiate bee farming. However, attention must be given to maintain the existing bee flora and multiplication of multipurpose plant species in order to make it sustainable. To initiate bee keeping, one must give attention to provide artificial feeding during winter and rainy months and other management practices when necessary. Such studies need to be carried out in other ecological regions of the country as well.

Table 1. Different available honeybee plants and floral calendar in different months of the year in Kabre area of Dolakha district

Plant name	Jan	Feb	Mar	April	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Musa paradisiaca												
Prunus persica												
Pyrus communis												
Prunus domestica												
Brassica spp.												
Buddleia spp.												
Rhododendron spp.												
Rubus spp.												
Trifolium spp.												
Melia azedarach												
Pinus spp.												
Citrus spp.												
Woodfordia fruticosa												
Erythrina stricta												
Coriaria nepalensis												
Maesa macrophylla												
Shorea robusta												
Pyrus pashia												
Engelhardtia spicata												
Berberis spp.												
Fagopyrum spp.												
Holboellia latifolia.												
Trichilia connaroides												
Juglans regia												
Cordia obliqua												
Fraxinus floribunda												
Pyracantha crenulata												
Grewia optiva												
Psidium guajava												
Choerospondias axillaris												
Phyllanthus emblica												
Ampelocissus rugosa												
Zizyphus spp.												
Schima wallichii												
Zea mays												
Phaseolus spp.												
Sechium edule												
Elsholtzia spp.												
Mirrre jhar												
Guizotia abyssinica												
Castanopsis indica												
Prunus cerasoides												
Myrica esculenta												
Pogestemon glaber												
Caesalpinia spp.												
Eupatorium spp.												
Reinwardtia indica												

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agriculture office and other concerned persons around Kabre VDC for their support. Also the suggestions and deep interest provided by other senior scientists and technical officers of Entomology Division are highly appreciated. Our sincere appreciation and thanks go to Mr T Pokherel, Mr SP Nepal and Mr JB Tamang for their sincere help to collect information during the course of study.

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Annex 1. Different plant species of honeybee flora identified in Kabre area of Dolakha district

SN	Scientific name	Common name	Local name	Family	Habit	Flowering period	Status†
a. Major bee flora							
1	Ampelocissus rugosa	Ampelocissus	Pureni	Vitaceae	Climber	May-June	N–P
2	Berberis spp.	Barbery	Chutro	Berberidaceae	Shrub	Mar-May	N–P
3	Brassica spp.	Mustard	Tori	Cruciferae	Herb	Feb-Mar, Sep-Nov	N–P
4	Buddleia spp.	Butterfly bush	Bhimsenpathi	Loganiaceae	Tree	Feb-Apr	N–P
5	Caesalpinia spp.	Molucca bean	Bhaisen kanda	Leguminosae	Shrub	Nov-May	N–P
6	Castanopsis indica	Chestnut	Dhale katus	Fagaceae	Tree	Sept-Nov	N–P
7	Choerospondias axillaris	Hog plum	Lapsi	Anacardiaceae	Tree	Apr-May	N–P
8	Citrus spp.	Citrus	Kagati	Rutaceae	Tree	Mar-Apr	N–P
9	Cordia obliqua.	Cordia	Bohari	Cordiaceae	Tree	Apr-May	N–P
10	Coriaria nepalensis	Coriaria	Machino	Coriariaceae	Tree	Mar-Apr	N–P
11	Elsholtzia spp.	Elsholtzia	Ban silam	Labiateae	Herb	Sep-Oct	N–P
12	Engelhardia spicata	Engelhardtia	Mauwa	Juglandaceae	Tree	Mar-May	N–P
13	Erythrina stricta	Coral bean	Phaledo	Leguminosae	Tree	Mar-Apr	N–P
14	Eupatorium spp.	Throughwort	Banmara	Compositae	Herb	Nov-May	N–P
15	Fagopyrum spp.	Buckwheat	Fapar	Polygonaceae	Herb	Mar-Apr, Oct-Nov	N–P
16	Fraxinus floribunda	Ash tree	Lakunri	Oleaceae	Tree	Apr-May	N–P
17	Grewia optiva	Grewia	Syal phusro	Tiliaceae	Tree	Apr-May	N–P
18	Guizotia abyssinica	Niger	Jhuse til	Compositae	Herb	Sep-Nov	N–P
19	Holboellia latifolia	Holboellia	Gulfo	Lardizabalaceae	Climber	Apr-May	N–P
20	Juglans regia	Walnut	Ookhar	Juglandaceae	Tree	Apr-May	P
21	Maesa macrophylla	Maesa	Bhagate	Myrsinaceae	Shrub	Mar-Apr	N–P
22	Melia azedarach	China berry	Bakaino	Meliaceae	Tree	Mar-Apr	N
23	-	-	Mirre jhar	-	Herb	Sep-Nov	N-P
24	Musa paradisiaca	Banana	Kera	Musaceae	Tree	All year	N–P
25	Myrica esculenta	Bayberry	Kafla	Myricaceae	Tree	Oct-Nov	N–P
26	Phaseolus spp.	Beans	Simi	Leguminosae	Herb	July-Aug	N–P
27	Phyllanthus emblica	Goose berry	Aamala	Euphorbiaceae	Tree	Apr-Jun	N–P
28	Pinus spp.	Chir pine	Khote salla	Pinaceae	Tree	Mar-Apr	N–P
29	Pogostemon glaber	Pogostemon	Rudilo	Lamiaceae	Shrub	Nov-Feb	N–P
30	Prunus cerasoides	Wild cherry	Painyu	Rosaceae	Tree	Oct-Nov	N–P
31	Prunus domestica	Plum	Arubakhada	Rosaceae	Tree	Feb-Mar	N–P
32	Prunus persica	Peach	Aru	Rosaceae	Tree	Feb-Mar	N–P
33	Psidium guajava	Guava	Aamba	Myrtaceae	Tree	Apr-May	N–P
34	Pyracantha crenulata	Fire thorn	Ghangharu	Rosaceae	Shrub	Apr-May	N–P
35	Pyrus communis	Pear	Naspati	Rosaceae	Tree	Feb-Mar	N–P
36	Pyrus pashia	Wild pear	Mayal	Rosaceae	Tree	Mar-Apr	N–P
37	Reinwardtia indica	Winter flax	Pyawuli	Linaceae	Shrub	Nov-May	N–P
38	Rhododendron spp.	Rhododendron	Lali gurans	Ericaceae	Tree	Feb-Apr	N–P
39	Rubus spp.	Raspberry	Ainselu	Rosaceae	Shrub	Feb-May, Oct-Nov	N–P
40	Schima wallichii	Needle wood	Chilaune	Theaceae	Tree	May-Jun	N–P
41	Sechium edule	Chayote	Iskush	Cucurbitaceae	Climber	July-Nov	N–P
42	Shorea robusta	Sal	Sal	Dipterocarpaceae	Tree	Mar-Apr	N–P
43	Trichilia connaroides	Trichilia	Aankha taruwa	Meliaceae	Tree	Apr-May	N–P
44	Trifolium spp.	Clover	Pyawali	Leguminosae	Herb	Feb-Jun	N–P
45	Woodfordia fruticosa	Fire flame bush	Dhaiyaro	Lythraceae	Shrub	Mar-Apr	N–P
46	Zea mays	Maize	Maikai	Gramineae	Herb	July-Aug	P
47	Zizyphus spp.	Bead plum	Hade bayar	Rhamnaceae	Tree	May-Jun	N–P
b. Medium bee flora							
1	Ageratum conyzoides	Goat weed	Gandhe jhar	Compositae	Herb	Feb-Nov	N–P
2	Albizia spp.	Albizia	Shiris	Leguminosae	Tree	Apr-May	N–P
3	Alnus nepalensis	Alder	Utttis	Betulaceae	Tree	Oct-Nov	N–P
4	Amaranthus spp.	Pigweed	Lunde	Amaranthaceae	Herb	Jun-Aug	N–P
5	Arisaema spp.	Cobra lily	Sarpa makai	Araceae	Herb	May-Jun	N–P
6	Artemisia spp.	Mugwort	Titepati	Compositae	Herb	Aug-Oct	N–P
7	Bauhinia spp.	Bauhinia	Koiralo/tanki	Leguminosae	Tree	Mar-May, Sep-Oct	N–P
8	Butea minor	Forest flame	Bhuletro	Leguminosae	Shrub	Apr-May	N–P
9	Cannabis sativa	Hemp	Bhang	Cannabaceae	Herb	Feb-Apr	N–P
10	Caryopteris odorata	Caryopteris	Ghusere	Verbenaceae	Shrub	Feb-Apr	N–P
11	Cedrela toona	Cedrela	Tooni	Meliaceae	Tree	Apr-May	N–P
12	Chenopodium album	Lamb's quarter	Bethe	Chenopodiaceae	Herb	Mar-May	N–P
13	Colebrookea oppositifolia	Colebrookea	Ghursul	Labiateae	Shrub	Dec-Apr	N–P
14	Crinum amoenum	Crinum	Hade lasun	Amaryllidaceae	Herb	May-Jul	N–P
15	Curcuma aromatica	Zedoary	Ban haledo	Zingiberaceae	Herb	June-Jul	N–P
16	Cynoglossum spp.	Hounds tongue	Kanike kuro	Boraginaceae	Herb	May-Aug	N–P

Annex 1. Continued…

SN	Scientific name	Common name	Local name	Family	Habit	Flowering period	Status†
17	Diospyros virginiana	Persimmon	Haluwabed	Ebenaceae	Tree	Mar-Apr	N–P
18	Eurya acumiata	Osmanthus	Jhigani	Theaceae	Tree	Sep-Nov	N–P
19	Glycine max	Soyabean	Bhatmas	Leguminosae	Herb	July-Sep	N–P
20	Ilex excelsa	Holy tree	Puwanle	Aquifoliaceae	Tree	Apr-May	N–P
21	Inula cappa	Samphire	Kan pake	Compositae	Shrub	Sept-Feb	N–P
22	Ipomoea batatas	Sweet potato	Sakharkhand	Convolvulaceae	Climber	Aug-Nov	N–P
23	Justicia adhatoda	Malabar nut	Aasuro	Acanthaceae	Herb	Sept–Oct	N–P
24	Lagerstroemia spp.	Crape myrtle	Aasare	Lythraceae	Tree	Jun-July	N–P
25	Leucosceptrum canum	Leucopscepturm	Bhusure	Labiateae	Tree	Feb-Apr	N–P
26	Lyonia ovalifolia	Lyonia	Angeri	Ericaceae	Tree	Mar-May	N
27	Mahonia napaulensis	Mahonia	Jaman mandro	Berberidaceae	Shrub	Nov-Feb	N–P
28	Mussaenda roxburghii	Paper chase	Dhobini	Rubiaceae	Shrub	May-Aug	N–P
29	Nicandra physalodes	Peru apple	Poke chinek	Solanaceae	Herb	Mar-Nov	N–P
30	Osbeckia stellata	Osbeckia	Chuleshi	Melastomataceae	Shrub	July-Nov	N–P
31	Oxalis corniculata.	Crepping sorrel	Chari aamilo	Oxalidaceae	Herb	Feb-July	N–P
32	Persicaria capitata	Smart weed	Pire	Polygonaceae	Herb	Mar-Nov	N–P
33	Phaseolus calcaralus	Red bean	Masyang	Leguminosae	Herb	Oct-Nov	N–P
34	Phlogacanthus thyrsiflorus	Phologacanthus	Choyua	Acanthaceae	Shrub	Sep-Nov	N–P
35	Porana grandiflora	Porana	Aakash beli	Convolvulaceae	Climber	Aug-Oct	N–P
36	Ranunculus spp.	Butter cup	Nak kure	Ranunculaceae	Herb	Apr-Jul	N–P
37	Rhus spp.	Nepal sumac	Bhalayo	Anacardiaceae	Tree	May-Jun, Aug-Sep	N–P
38	Rosa spp.	Wild rose	Jangali gulaf	Rosaceae	Shrub	Apr-Jun	N–P
39	Sapium insigne	Tallow	Khirro	Euphorbiaceae	Tree	Nov-May	N–P
40	Saurauia nepaulensis	Saurauia	Gogan	Saurauiaceae	Tree	Sept-Oct	N–P
41	Smilax spp.	Green briers	Kukur daino	Liliaceae	Climber	Apr-May	N–P
42	Swertia spp.	Chiretia	Chiraito	Gentianaceae	Shrub	Aug-Oct	N–P
43	Symplocos spp.	Symplocos	Kholme	Symplocaceae	Tree	Apr-May	N–P
44	Vegetables					All year	
45	Vitex negundo	Privet	Simali	Verbenaceae	Shrub	Apr-Oct	N–P
46	Zanthoxylum armatum	Nepal pepper	Timbur	Rutaceae	Shrub	Apr-May	N–P
c. Minor bee flora							
1	Aesandra butyracea	Butter tree	Chiuri	Sapotaceae	Tree	Sep-Feb	N–P
2	Cajanus cajan	Piegon pea	Rahar	Leguminosae	Herb	Oct-Nov	N–P
3	Callistemon citrinus	Bottle brush	Kalki phul	Myrtaceae	Tree	Mar-Apr, Sep-Oct	N–P
4	Chrysanthemum segetum	Chrysanthemum	Godavari	Asteraceae	Herb	Aug-Sept	N–P
5	Cirsium spp.	Field thistle	Dhade kande	Compositae	Herb	Feb-Jun	N–P
6	Cosmos sulphureus	Cosmos	Cosmos	Asteraceae	Herb	Oct-Nov	N–P
7	Eriobotrya dubia	Medlar	Jure kaphal	Rosaceae	Tree	Feb-Mar, Sep-Oct	N–P
8	Euphorbia pulcherrima	Poinsettia	Lalupate	Euphorbiaceae	Shrub	Oct-Feb	N–P
9	Ficus spp.	Fig	Ber	Moraceae	Tree	Feb-Apr, Oct-Nov	N–P
10	Fragaria nubicola	Alpine strawberry	Bhun ainselu	Rosaceae	Herb	Apr-Jun	N–P
11	Grevillea robusta	Silky oak	Kangiyo	Proteaceae	Tree	Apr-May	N–P
12	Impatiens spp.	Balsam	Tiwuri	Balsaminaceae	Herb	Jul-Sep	N–P
13	Malvaviscus arboreus	Chinese lantern	Ghante phul	Malvaceae	Shrub	All year	N–P
14	Melastoma melabathricum	Indian rhododendron	Angeri	Melastomataceae	Shrub	Mar-Jun	N–P
15	Mimosa spp.	Sensitive plant	Lazzavati	Leguminosae	Herb	Oct-Dec	N–P
16	Perilla frutescens	Perilla	Silam	Labiateae	Herb	Sep-Oct	N–P
17	Pisum sativum	Pea	Kerau	Leguminosae	Herb	Mar-Apr	N–P
18	Plectranthus spp.	Shain		Labiateae	Shrub	Aug-Nov	N–P
19	Polygonum spp.	Polygonum	Thotne	Polygonaceae	Herb	May-Sep	N–P
20	Prinsepia utilis	Prinsepia	Dhatelo	Rosaceae	Herb	Oct-Nov, Apr-May	N–P
21	Punica granatum	Pomogranate	Aanar	Punicaceae	Tree	Apr-May	N–P
22	Punica nana	Wild Pomegranate	Darim	Punicaceae	Tree	Apr-May	N–P
23	Salvia splendens	Scarlet sage	Lwang phul	Labiateae	Herb	All year	N–P
24	Sesamum indicum	Sesame	Kalo til	Pedaliaceae	Herb	Sep-Oct	N–P
25	Syzygium jambos	Rose apple	Ban jamum	Myrtaceae	Tree	Apr-May	N–P
26	Tagetes erecta	Marigold	Sayapatri	Compositae	Herb	Feb-May, Aug-Nov	N–P
27	Tamarindus indica	Tamarind	Imili	Leguminosae	Tree	Apr-May	N–P

† N, Nectar source; P, Pollen source.

Use of Botanicals for the Management of Pulse Beetle (Callosobruchus maculatus F.) in Lentil

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Abstract

Some plant materials, sweet flag (Acorus calamus), goat weed (Ageratum conyzoids), lantana (Lantana camara), Indian privet (Vitex negundo), mug-wort (Artimisia vulgaris), chinaberry (Melia azederach), rice husk ash, mustard (Brassica spp.) oil and neem (Azadirechta indica) oil were evaluated for their effects against pulse beetle (Callosobruchus maculatus F.). The powder or oil from the above plant materials were thoroughly admixed at the rate of 0.5, 1 and 2% w/w or v/w with lentil grains. Randomly selected 25 pulse beetle adults were exposed to the grains for each concentration of the main treatment. Rhizome powder of sweet flag, rice husk ash and mustard oil showed a significant effect in killing the pulse beetle within a week at 0.5, 1 and 2% concentrations. Neem oil was found very effective with 100% mortality of the beetle within two days. Other tested materials also showed insect killing properties but the effect was comparatively lower than that of sweet flag rhizome powder, mustard oil, neem oil and rice husk ash.

Key words: Botanicals, Callosobruchus maculates, lentil, pulse beetle

Introduction

Lentil (Lens esculenta M.) is the number one crop among pulses in terms of production and area in Nepal. It is considered as the major source of protein in the daily diet of Nepalese people, because protein from other sources has become scarce in our day to day food. The lentil has covered 50% of the total pulse area and production in Nepal (Pandey, 1994). Production potential of the crop has been increasing in Nepal because of its commercial value. It is grown both under irrigated and rainfed land and can fit under both inter and mixed cropping systems. There is a good marketing facility of the pulse and it is easy to cook. It has been observed that the pulse is highly susceptible to pulse beetles (Callosobruchus maculatus, C. chinensis) during storage. Due to invasion of the beetle, deterioration in quality and quantity of lentil grains is high in Terai and foothill region of Nepal. Room sanitation and fumigation are the commonly used and recommended control practices for management of stored grain pests. But these practices are limited to a number of farm households of Nepal, as majority of them do not have separate grain stores to ensure efficient sanitation. Many farmers have very little knowledge about the use of toxic fumigants. In the present context, use of botanicals is considered as eco-friendly and effective materials against stored grain pests.

Some indigenous plant materials have been known for their effectiveness to reduce oviposition, egg hatchability and adult emergence of pulse beetle. Chinwada and Giga (1993) reported that commercial vegetable oil and neem oil were very effective against pulse beetles till sixteen weeks to reduce oviposition, percent eggs hatching, progeny emergence and seed damage when applied at the rate of 2.5 ml kg^{-1} seeds. They also reported that mortality of these pulse beetles was more than 90%. The cinnamon oil (Cinnamomum camphora N.) was toxic to the bean weevil having lethal concentration (LC) 50 < 200 mg per ml. It has fumigant action, which caused 100% mortality with the application of 50 mg of 100% oil per 40 cubic centimeter space (Garcia, 1990). Rojesus et al. (1989) reported that the oil of goat weed (Ageratum conyzoids), Pyrethrum (Chrysenthemum indicum), Indian privet (Vitex negundo) and neem tree (Azadirachta indica) had contact toxicity, which caused mortality ranging from 43 to 90% at 100 mg ml^{-1} for 48 hours exposure when applied by

filter paper impregnation method. At the same concentration, the oil form of the above materials were more toxic than the powder form when mixed with the seeds. Oil treatment had 100% mortality within 24 hours. The neem oil and pyrethrum flower powder were reported the best control of pulse beetle when these products were used at the rate of 50 mg per ml per 100 g of faba bean seeds (Bayeh and Tadese, 1996). Neem seed powder, pepper (Piper longum L.) and persian lilac (Melia azederach) seed powder were reported to protect the bean seeds from bruchid beetle's attack for up to 120 days (Negasi and Abage, 1992). The objectives of this study was to know the effectiveness of formulation, doses and methods of application of some botanicals that are found in Nepal.

Materials and Methods

The performance of eight different plant materials was evaluated against pulse beetle in the room condition at Entomology Division, Khumaltar, where the range of minimum and maximum temperatures during the experiment was 25-27ºC and 26-27ºC, respectively for the first year (1998/99) and 25-27ºC and 26-29ºC, respectively in the second year (1999/00). Coarse powder of some botanicals were prepared out of shade dried rhizomes of sweet flag (Bojho) (Acorus calamus), leaves and stem of goat weeds (Ageratum conyzoids), lantana (Lantana camara), Indian privet (Vitex negundo) and Mug-wort (Artemisia vulgaris) and seeds of chinaberry (Melia azederach). Rice husk ash was obtained by burning rice husk. The commercially available mustard oil was also included in the test. The MargoSom (Azadirachtin, 0.15% of Azadirachta indica) was included in the first year, while mug-wort (Artemisia vulgaris) was used in the second year. Altogether there were eight main treatments. Each main treatment had three different concentrations of powder form 0.5, 1and 2% w/w and liquid form at 0.5, 1, 2% v/w) as sub treatments. The lentil grains without any treatment served as the control treatment. Pulse beetle, which was originally collected from Mugitar, Ramechhap was multiplied and maintained in the laboratory of Entomology Division.

One hundred grams of lentil grains were thoroughly mixed with botanicals at rate of above concentrations for each sub treatment. Effects of these materials were evaluated against bruchids in lentil in a plastic container of 250 ml capacity. Randomly selected 25 bruchid adults were exposed to each concentration of main treatments. The experiment was set up in a completely randomized factorial design in three replications. The number of dead and morbid adults was counted at an interval of 48 hours and such observations were made for up to 12 days for actual effects of each plant material. The data wherever necessary (ie mortality observed in the control treatment) were corrected (Abott, 1925). The percent data were transformed into arc sine values and analysis of variance (ANOVA) was used to analyze the data. Interaction effect was also observed so as to differentiate superior combinations for higher mortality of the pulse beetle. The mean separation was done by using Duncan's Multiple Range Test (DMRT) at 0.05 level.

Results and Discussion

Mean mortality of the pulse beetles in each treatment is presented in Table 1 for the year 1998/99 and 1999/'00. The data reveal that the mortality of the adult beetles increased with the increment of exposure time to botanical extracts.

The first year experiment indicated that neem oil (@ 0.5, 1 and 2% v/w concentrations) was very effective to cause 100% mortality of pulse beetle within 2 days. This was followed by sweet flag rhizome powder and mustard oil (@ 1% and 2% v/w) within 8 days. However, the mustard oil @ 0.5% took 12 days to kill 100% of beetles adult. Similarly, > 90% beetles were killed with rice husk ash on the 12^{th} day. Rest of the botanicals were comparatively less toxic to the beetles but were better than control. Similarly, the second year experiment indicated that sweet flag rhizome powder, rice husk ash and mustard oil (@ 0.5, 1 and 2% concentrations) were effective to cause 100% mortality of the beetle within six days. The mortality in the second year experiment was comparatively faster than that of the first year. It could probably be due to

temperature, which was slightly higher at the time of conducting the experiment during the second year. The analysis made on the data recorded in the first year was found significantly different at $P \leq 0.01$ on all dates except 12th day, while it was significantly different at $P \leq 0.01$ on 2nd, 4th and 6th day but no significant different on 8th, 10th and 12th day in the second year.

Table 1. Mean mortality of pulse beetle (Callosobruchus maculates) on lentil grains treated with different extracts of botanicals at three concentrations (conc) up to 12 days of exposure, 1998/99 and 1999/00

Treatment	Mean mortality, % and days of exposure											
	Conc,	2nd Day		4th Day		6th Day		8th Day		10th Day		12th Day	
	% w/w, v/w	1998/99	1999/00	1998/99	1999/00	1998/99	1999/00	1998/99	1999/00	1998/99	1999/00	1998/99	1999/00
Sweet flag	0.0	0e†	0g	0f	0d	1.3i	-	-	-		-		-
	0.5%	8.0d	44cde	53.3b	88b	96.0ab	-	-	-	-	-	-	-
	1.0%	10.7d	68abc	96.0a	100a	100a	-	-	-	-	-	-	-
	2.0%	12.7cd	68abc	90.7a	100a	100a	-	-	-	-	-	-	-
Goat weed	0.0	0e	0g	0f	0d	1.3i	0e	5.3g	0	9.3e	4	9.3	4
	0.5%	1.3e	4fg	1.3b	20c	9.3fghi	32d	28.0ef	44	32.0bcd	48	56.0	56
	1.0%	0e	12efg	9.3def	32c	10.7efgh	48bcd	20.0efg	64	22.7d	72	65.3	72
	2.0%	6.7d	20def	5.3ef	36c	6.7ghi	56bc	21.3ef	68	48.0bcd	80	73.3	80
Lantana	0.0	0e	0g	0f	0d	1.3i	0e	5.3g	0	9.3e	4	9.3	4
	0.5%	0e	8fg	4.0ef	20c	9.3fgh	52bcd	30.7ef	60	46.7bcd	60	77.3	64
	1.0%	0e	12defg	5.3ef	20c	9.3fgh	64b	20.0ef	68	32.0bcd	72	52.0	76
	2.0%	0e	12fg	6.7def	24c	8.0fghi	68b	24.0ef	72	48.0bcd	76	62.7	80
Dadelno	0.0	0e	0g	0f	0d	1.3i	0e	5.3g	-	9.3e	-	9.3	-
ko Kharani	0.5%	0e	68bc	9.3ef	100ab	22.7def	100a	44.0de	-	57.3b	-	93.3	-
	1.0%	1.3e	80ab	16.0de	100ab	28.7de	100a	61.3cd	-	89.3a	-	98.7	-
	2.0%	0e	88ab	18.0de	100a	66.7c	100a	92.0ab	-	100a	-	100	-
Indian	0.0	0e	0g	0f	0d	1.3i	0e	5.3g	0	9.3e	4	9.3	4
privet	0.5%	0e	8fg	1.6f	32c	1.6hi	44bcd	20.0efg	60	37.3bcd	64	60.0	68
	1.0%	0e	20def	2.7ef	36c	8.0fghi	52bcd	22.7ef	68	33.3bcd	72	60.0	76
	2.0%	1.3e	8defg	1.3f	36c	14.7defgh	60bc	30.7ef	72	50.7bc	76	72.0	80
Chinaberry	0.0	0e	0g	0f	0d	1.3i	0e	5.3g	0	9.3e	4	9.3	4
	0.5%	0e	4fg	5.3ef	16c	20.0defg	32d	24.0ef	44	33.3bcd	52	50.7	56
	1.0%	0e	8fg	4.0ef	16c	8.0fghi	36cd	10.7fg	44	24.0cd	60	36.0	64
	2.0%	0e	16defg	6.7ef	32c	29.3d	48bcd	36.0de	60	42.7bcd	76	56.0	76
Mustard	0.0	0e	0g	0f	0d	1.3i	-	5.3g	-	-	-		-
oil	0.5%	13.3d	48cd	28.0cd	92b	62.7c	-	74.7bc	-	-	-	-	-
	1.0%	21.3c	88ab	46.7bc	100a	93.3b	-	100a	-	-	-	-	-
	2.0%	93.3b	96a	100a	100a	100a	-	100a	-	-	-	-	-
Neem oil	0.0	0e	0g	-	0d	-	0e	-	0	-	4	-	4
(1st Year)	0.5%	100a	12fg	-	24c	-	44bcd	-	64	-	68	-	68
Mug wort	1.0%	100a	16defg	-	32c	-	60bc	-	68	-	68	-	72
(2nd Year)	2.0%	100a	20def	-	36c	-	60bc	-	76	-	80	-	84
F value													
Conc.		**	**	**	**	**	**	**	**	**	**	**	**
Treatment		**	**	**	**	**	**	**	ns	**	ns	**	ns
Interaction		**	**	**	**	**	**	**	ns	**	ns	ns	ns

** Significant difference at $P \leq 0.01$; ns, Non-significant difference. † Means followed by the same letter are not difference at 5%.

During the first year, neem oil (@ 0.5%, 1% and 2% v/w) was found superior to other treatment combinations to cause 100% mortality of the beetles within 2 days. The mustard oil (2%) caused > 93% mortality of the beetles within 2 days in the first and second year. On the 4th day, sweet flag rhizome powder (@ 1% and 2%) and mustard oil (@ 2%) were superior to other treatments to cause high mortality of pulse beetles in the 1st year, while the sweet flag rhizome powder (@ 1% and 2%), mustard oil (@ 1% and 2%) and rice husk ash (@ 0.5%, 1% and 2%) were superior in the second year.

On the 6th day, sweet flag powder (@ 1%, 2%) and mustard oil (2%) were found superior to cause 100% mortality of beetles in 1998/99, while 100% mortality was found within 4 days in 1999/00. On the 8th day, mustard oil (1% and 2%) caused 100% mortality of beetles in the 1st year, while 100% mortality was recorded at same concentrations within 4 days. On 10th and 12th day, rice husk ash (@ 1% and 2%) caused > 89% mortality of beetles in the first year, while 100% mortality was observed even at 0.5% concentration within 6 days in the second year.

The treatments, which caused 100% mortality of the beetles at all concentrations except in respective control were excluded for further analysis. The mortality caused by remaining treatments on later dates was found no-significant different at $P \leq 0.05$ indicating the similar response in killing the beetles.

Based on two years results, the sweet flag rhizome powder, rice husk ash and mustard oil have shown high bruchid killing properties at 0.5, 1 and 2% concentrations. The neem oil was also observed very effective to kill the insect within 2 days. Other tested materials were found to have some effects in killing the pulse beetle but the rate of killing in the given time was comparatively lower than those of neem oil, mustard oil and sweet flag rhizome powder. The other evidences indicated similar effects of botanicals to stored grain beetle as well. Sweet flag rhizome powder mixed with lentil may not have any side effect on seed germination as in the case of powder mixed with wheat grains did not have any side effects on germination (Paneru et al., 1997). However, the effects of mustard oil and rice husk ash on germination of treated seeds is yet to be known. Farmers are very familiar to sweet flag rhizome powder and rice husk ash. These materials are easier to prepare and apply than other products. Thus, rice husk ash and sweet flag rhizome powder at 0.5% concentration would be the best choice among the botanicals for management of pulse beetle.

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Sustaining Wheat Productivity and Maintaining Soil Fertility in Maize-Wheat System

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Abstract

Field experiments on maize-wheat system were carried out in rainfed upland (bari) at Agricultural Research Station, Lumle for three years (1994/95-1996/97) to determine the effect of different quality organic materials on maize and wheat yields and soil properties. Wheat grain yield significantly differed over years and the highest mean grain yield (1.98 t ha^{-1}) was recorded in the treatment of mixture of low and high quality organic materials (maize + leaf litter, farmyard manure). Maize and wheat grain as well as straw yield significantly differed over years. In all the three years, the mixture of low and high quality organic materials produced the highest grain yield of maize plus wheat ranging from 6.72 to 2.20 t ha^{-1} with mean yield of 4.43 t ha^{-1}. Mean N uptake by wheat grain and straw ranged from 32.2 to 40.4 kg ha^{-1} and 13.8 to 16.0 kg ha^{-1}, respectively in different treatments. Similarly, mean P uptake by wheat grain was the highest (25.4 kg ha^{-1}) in the mixture treatment while mean P uptake by wheat straw was the highest (26.5 kg ha^{-1}) in low quality organic materials. Mean soil pH after wheat harvest was the lowest (5.1) in the low quality organic material. Organic carbon and available P ranged from 3.4 to 3.7% and from 329 to 370 ppm, respectively in different treatments while total N and exchangeable K increased to 0.31% and 0.2 me/100g, respectively with the application of low quality organic material. The three years result showed that semi-decomposed organic materials were more efficient in improving and stabilizing production of wheat and maize yields in maize-wheat system as well as in maintaining N fertility than high quality organic materials.

Key words: Maize-wheat system, organic materials, soil fertility, sustainability

Introduction

Nitrogen, essential to plant growth is taken up by plant from the soil in simple inorganic form, ie as nitrate and ammonium. These are released from complex organic compounds by the action of decomposing microorganisms, a process known as mineralization. The amount of nitrogen supplied to a crop after application of manure depends on the interaction of several processes (immobilization, leaching, mineralisation, nitrification, volatilization, denitrification), which are markedly affected by manure type, time and rate of application and management (Shepherd, 1993). However, during decomposition of organic matter, organic acids and carbon-dioxide are formed (Tisdale et al., 1985) and added organic materials appear to have a solubilizing effect on Fe, Al and Ca phosphate due to the increase in biological activities (RAPA, 1986). Nutrient recycling is a dynamic process and it is not easy to assess fertility decline, as it requires long-term monitoring of the soils.

For farmyard manure (FYM) compost to be considered as an effective N source for maize and wheat in the maize-wheat system, it must supply sufficient N and there must be a synchrony between FYM/compost N release and maize and wheat demand. If the mineral N pool in soil is produced too early, it can potentially be lost through leaching and/or denitrification. If released too late, N application will not benefit the crop and possesses a potential threat to groundwater quality via leaching. Wilson and Hargrove (1986), Wagger (1989) used fine-pore (53 µm) litterbag to monitor the rate of residue biomass disappearance and Varco et al. (1989) used 15-N deplete residue to monitor decomposition dynamics. They found that green

manures decomposed rapidly (a 50% loss of biomass within a month) in warm soils and could be a significant source of N to the following maize crop.

In the western hills, farmers traditionally apply a lot of semi-decomposed FYM/compost to upland (bari) soils for growing different crops. The crop cut survey and associated data suggested that bari soils are relatively fertile because they receive most of the available FYM/compost (typically 20 to 50 t ha^{-1} yr^{-1}) (Tuladhar, 1995).

It is essential to know the effect of different levels of decomposed organic materials on wheat grain in maize-wheat system. Therefore, this experiment was designed to determine the potential extent and method by which crop production can be altered by optimizing efficiency of nutrient use through the management of manuring of contrasting chemical composition.

Materials and Methods

The experiment was initiated in summer maize growing season of 1994 in maize-wheat system. The field experiment was conducted continuously for three years (1994/95, 1995/96 and 1996/97) in randomized complete block design (RCBD) with four replications in the rainfed terrace land (1620 masl) at Agricultural Research Station (ARS), Lumle. The plot size was 8- × 4-m, and the net harvested area was 6.0- × 2.5-m. There were four treatments (control, low quality organic materials ie maize stover + forest leaf litters to supply 40 kg N ha^{-1}, high quality organic materials ie FYM/compost to supply 40 kg N ha^{-1} and mixture of low and high quality organic materials to supply 40 kg N ha^{-1}. To supply 40 kg N ha^{-1} through low quality organic materials, 16 kg maize stover and 4 kg forest litters $plot^{-1}$ were applied. Similarly, to supply 40 kg N ha^{-1} through high quality organic materials, 6.5 kg FYM/compost $plot^{-1}$ were applied. The mixture of low and high quality organic materials were supplied through 8 kg maize stover + 2 kg forest litter + 3.2 kg FYM/compost $plot^{-1}$. All the organic materials were incorporated during the land preparation for wheat sowing. In case of maize, similar quantity of 40 kg N ha^{-1} were supplied through wheat straw, leaf litter and FYM/compost in the above treatments. Wheat variety Annapurna 3 was planted in 16 lines $plot^{-1}$ at 25 cm row spacing with continuous seeding. Wheat seeds were sown during the second and the fourth week of Oct. The wheat crop was harvested in the last week of April for all three years.

Composite soil samples were collected before planting the first crop of wheat and analyzed for pH, organic carbon, total N, available P and exchangeable K. Measurements on organic carbon and the total N were made in the sampling plots by collecting soil samples at three weeks interval by mesh litter bag method. Measurements of N mineralisation rate in the sample plots were also made at three weeks interval by field incubation method. There were 5 litter bags in each sample plot. The N and P uptake of the above ground biomass (grain and straw) were determined by collecting the above ground wheat plants at the time of harvest. After the harvest of each wheat crop, soil samples from individual plots were collected and analyzed for different chemical properties. The data were analyzed by MSTATC.

Results and Discussion

Wheat yield

Wheat grain yields over years (1994/95, 1995/96 and 1996/97) decreased significantly for all treatments (Table 1). Differences among treatments were not evident. However, the mean wheat grain yield was the highest in the treatment of mixture of low and high quality organic materials in all three years with the mean of 1.98 t ha^{-1} (Table 1). The declining trend of wheat grain yield in all treatments in later years could be due to insufficient quantity of nutrients particularly N (40 kg ha^{-1}) supplied through different types of organic materials, which could not meet the crop requirements. Moreover, the residual effect of organic fertilizers was not enough in the second and the third year in addition to 40 kg N ha^{-1} applied in each maize and wheat crop.

Wheat straw yields significantly differed over the years but significant treatment effects were obtained only in 1994/95 (Table 2). The mean wheat straw yield of three years showed that the application of 40 kg N ha^{-1} through the low quality organic materials alone or the mixture of low and high quality organic materials gave similar responses, while control treatment produced the lowest straw yield (3.11 t ha^{-1}). The wheat straw yield was drastically reduced in all treatments over the years. The reason of declining straw yield in later years is probably due to low quantity N (40 kg ha^{-1}) applied through organic materials and also negligible residual effect of previous years organic materials.

Combined maize and wheat yield

Analysis revealed that combined mean maize and wheat grain yields significantly differed over years and treatments (Table 1). The mixture of low and high quality organic materials gave the highest grain yield in all years. The total maize and wheat grain yields in all treatments gradually declined in subsequent years indicating that 40 kg N ha^{-1} in each maize and wheat crop supplied through different organic materials were not sufficient to meet the crop requirements in high rainfall area of Lumle.

The combined maize and wheat straw yields also differed significantly among years and treatments (Table 2). The mean straw yield of control treatment was the lowest (7.92 t ha^{-1}) among treatments. Interaction effects were also significant because the trend of declining straw yield was different in different treatments. The straw yields of maize and wheat significantly reduced over years in all treatments showing that 40 kg N ha^{-1} supplied in each maize and wheat crop was not enough to meet crop requirements.

Table 1. Wheat grain yield and combined maize + wheat yield at 12% moisture in 1994/95, 1995/96 and 1996/97 at Agricultural Research Station, Lumle

Treatment	Wheat grain yield, t ha^{-1}				Maize + wheat grain yield, t ha^{-1}			
	1994/95	1995/96	1996/97	Mean	1994/95	1995/96	1996/97	Mean
Control	2.30	1.84	0.44	1.53	5.75	2.85	1.17	3.26
Maize stover + leaf litter (2)	2.50	2.04	0.55	1.70	6.06	3.92	2.07	4.02
FYM (3)	2.65	1.75	0.43	1.61	6.33	3.17	1.18	3.56
Mixture (2 + 3)	2.78	2.43	0.75	1.98	6.72	4.38	2.20	4.43
Mean	2.56	2.02	0.54	1.71	6.22	3.58	1.66	3.82
F (year)	-	-	-	**	-	-	-	**
F (treatment)	ns	ns	ns	ns	ns	ns	ns	**
F (year × treatment)	-	-	-	ns	-	-	-	ns

** Highly significant ($P \leq 0.01$); ns, Non significant.

Table 2. Wheat straw yield and combined maize stalk + wheat straw yield at Agricultural Research Station, Lumle

Treatment	Wheat straw, t ha^{-1}				Maize stalk + wheat straw, t ha^{-1}			
	1994/95	1995/96	1996/97	Mean	1994/95	1995/96	1996/97	Mean
Control	4.88	3.48	0.99	3.11	12.75	7.93	3.09	7.92
Maize stover + leaf litter (2)	6.40	4.20	1.35	3.98	16.15	11.71	4.41	10.76
FYM (3)	5.63	3.28	1.29	3.40	14.90	8.68	3.71	9.10
Mixture (2 + 3)	5.30	4.32	1.66	3.76	14.90	12.18	4.25	10.44
Mean	5.55	3.82	1.32	3.56	14.67	10.12	3.87	9.55
F (year)	-	-	-	**	-	-	-	**
F (treatment)	*	ns	ns	**	*	**	ns	**
F (year × treatment)	-	-	-	ns	-	-	-	*

** Highly significant ($P \leq 0.01$); * Significant ($P \leq 0.05$); ns, Non significant.

Nitrogen and phosphorus uptake

Nitrogen uptake by wheat grain and straw differed significantly over years (Table 3) because both wheat grain and straw yields decreased in the second year (1995/96) as compared to the first year (1994/95). A

significant effect of treatment on N uptake by wheat straw was evident only in 1994/95. Interaction effects between year and treatment existed only in N uptake by straw, most probably due to high N uptake (19.7 kg ha^{-1}) by wheat straw in the control treatment (10.4 kg ha^{-1}) in 1995/96 as compared to 1994/95. Mean N uptake by wheat grain and straw varied from 32.2 to 40.4 kg ha^{-1} and 13.8 to 16.0 kg ha^{-1}, respectively.

Effects of year and treatment on phosphorus uptake by wheat grain were not observed. However, mean P uptake was the highest (25.4 kg ha^{-1}) in the mixture of low and high quality organic material (Table 3) indicating that semi-decomposed organic materials are better source for P uptake by wheat grain.

Phosphorus uptake by wheat straw did not differ significantly over years but the treatments were different (Table 3). P uptake by straw was higher in 1995/96 in all treatments than in 1994/95 and low quality organic material had the highest P uptake (30.8 kg ha^{-1}) of all other treatments. This is probably due to slow release of nutrients from low quality organic materials, which contributed to P uptake by straw. The mean P uptake by straw over years was the highest (26.5 kg ha^{-1}) again in the low quality organic material treatment.

Table 3. Nitrogen and phosphorus uptake by wheat grain and straw during 1994/95 and 1995/96 at Agricultural Research Station, Lumle

Treatment	N uptake by wheat grain, kg ha^{-1}			N uptake by wheat straw, kg ha^{-1}			P uptake by wheat grain, kg ha^{-1}			P uptake by wheat straw, kg ha^{-1}		
	1994/95	1995/96	Mean	1994/95	1995/96	Mean	1994/95	1995/96	Mean	1994/95	1995/96	Mean
Control	36.1	28.8	32.2	10.4	19.7	15.0	21.0	19.4	20.2	16.2	20.9	18.5
Maize stover + leaf litter (2)	46.2	24.7	35.5	14.8	17.1	16.0	23.6	18.6	21.2	22.3	30.8	26.5
FYM (3)	46.1	25.1	35.6	13.6	14.3	13.9	25.3	17.1	21.2	19.6	22.3	20.9
Mixture (2 + 3)	45.7	35.2	40.4	12.5	15.1	13.9	23.5	27.4	25.4	18.9	27.1	23.0
Mean	43.5	28.3	35.9	12.8	16.6	14.7	23.4	20.6	22.0	19.3	26.3	22.3
F (year)	-	-	**	-	-	*	-	-	ns	-	-	-
F (treatment)	ns	ns	ns	*	ns	ns	ns	ns	ns	*	ns	**
F (year × treatment)	-	-	ns	-	-	*	-	-	ns	-	-	ns

** Highly significant (P ≤ 0.01); * Significant (P ≤ 0.05); ns, Non significant.

Effect on soil chemical properties

The bench mark soil analysis before planting the first wheat crop in Oct 1994 indicated that the soil pH was about neutral (6.3), organic carbon was high (3.4%), total N (0.33%) and exchangeable K (0.43 me/100 g) were medium, and available P was very high (416 ppm). Monitoring of soil pH, organic carbon and total N at 3, 9 and 15 weeks after wheat germination in 1994/95, 1995/96, 1996/97 revealed that soil pH remained low (5.5 to 5.6) upto 6 weeks of wheat germination and increased to 5.6-5.7 and 5.8-5.9 after 9 weeks and 12 weeks of germination, respectively in all treatments. After 15 weeks of germination, soil pH decreased to 5.5 in all treatments (data not shown). The mean (over years) showed that soil organic carbon remained more or less the same (3.2 to 3.3%) upto 9 weeks after germination. After 12 weeks of germination, the organic carbon increased in all the treatments but this increment was greater in the treatment of low quality organic matter and in the mixture of low and high quality organic materials. Again, the organic carbon decreased in all treatments after 15 weeks of germination.

Total N content of the soil increased slowly after 3 weeks of germination up to 8 weeks of germination indicating that the uptake of N by wheat crop was lower most probably due to low soil temperature and low demand of small plants. Sharp decline in total N was obtained after 12 weeks of sampling showing uptake of N between 9-12 weeks after germination. Total N content of the soil remained low in control treatment in all five samples.

In 1995/1996 and 1996/97, mineral-N differed significantly over sampling time (3 to 15 weeks) and treatments differed significantly in the samples collected after 3 weeks of germination only in 1996/97 (Table 4). In 1995/96, mineral N

declined slowly after 3 weeks upto 15 weeks, whereas reverse trend was observed in 1996/97. This is probably due to interaction of soil and environment. Further study is required to confirm mineral N variations in different years, as Wilson and Hargrove (1986) reported that N release was highly variable when residues were left on the soil surface. Mean mineral N of two years varied from 7.05 to 9.37 kg ha^{-1} in different treatments.

The soil chemical properties of each individual year and combined analysis of variance over years are presented in Table 5. Organic carbon, available P and exchangeable K significantly differed over years. Organic carbon increased in all treatments in 1997 as compared to that in 1996 and this increase was the highest (5.1%) in the mixture treatment. Though available P was high, it decreased over years in all treatments most probably due to low P supplied through different types of organic materials. Treatments differed for total N and exchangeable K as indicated by the combined analysis of variance. Mean total N was the highest (0.31%) in low quality organic material and the lowest (0.24%) in high quality organic material indicating rapid use of N by wheat crop or loss of N with the use of high quality (well decomposed) organic materials. Exchangeable K was also significantly higher (0.23 me/100 g) in the same low quality organic material treatment showing less use of this nutrient by wheat crop. Interaction effect between year and treatment existed only on exchangeable K. The soil analysis after harvest of the wheat crop showed that, a large amount of total N and exchangeable K was in the soil of low quality organic material treatment indicating residual effect of these two nutrients.

Table 4. Effect of time on mineral N (kg ha^{-1}) release and accumulation in bag soil during wheat growing season during 1994/95 and 1995/96

Treatment	3 weeks after germination			9 weeks after germination			15 weeks after germination		
	1994/95	1995/96	Mean	1994/95	1995/96	Mean	1994/95	1995/96	Mean
Control	15.7	1.8	8.8	10.8	3.9	7.4	6.0	8.2	7.1
Maize stover + leaf litter (2)	15.1	1.9	8.8	10.4	4.0	7.2	7.4	6.9	7.1
FYM (3)	15.6	3.1	9.4	10.6	3.5	7.1	6.8	9.3	8.1
Mixture (2 + 3)	14.2	2.4	8.3	10.4	4.0	7.2	7.8	9.1	8.5
Mean	15.2	2.3	8.8	10.6	3.8	7.2	7.0	8.4	7.7
F (year)	**	**	-	SED (Y)	-	-	1.2	0.6	-
F (treatment)	ns	ns	-	SED (T)	-	-	0.7	0.4	-
F (year × treatment)	ns	ns	-	SED (Y × T)	-	-	1.2	0.6	-

** Highly significant (P ≤ 0.01); ns, Non significant; SED, Standard error of difference.

Table 5. Soil Chemical properties after wheat harvest in 1995, 1996 and 1997†

Treatment	N, %				P, ppm				K (me/100g)				Organic carbon, %				pH			
	1995	1996	1997	Mean	1995	1996	1997	Mean	1995	1996	1997	Mean	1995	1996	1997	Mean	1995	1996	1997	Mean
Control	0.3	0.3	0.2	0.3	467	389	221	370	0.4	0.3	1.2	0.3	-	2.5	4.0	3.6	5.7	5.5	5.4	5.5
Maize stover + leaf litter (2)	0.4	0.3	0.2	0.3	336	307	173	341	0.5	0.2	0.1	0.3	-	2.6	4.4	3.5	5.6	4.1	5.4	5.1
FYM (3)	0.3	0.2	0.2	0.2	508	442	265	340	0.4	0.3	0.2	0.2	-	2.5	3.6	3.4	5.7	5.4	5.3	5.5
Mixture (2 + 3)	0.3	0.3	0.3	0.3	411	330	295	329	0.4	0.3	0.1	0.3	-	3.6	5.1	3.7	5.7	5.4	5.4	5.5
Mean	0.3	0.3	0.2	0.3	431	367	239	345	0.4	0.3	0.2	0.3	-	2.8	4.3	3.6	5.7	5.1	5.4	5.4
F (year)	-	-	-	ns	-	-	-	*	-	-	-	**	-	-	-	*	-	-	-	ns
F (treatment)	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	**	-	ns	ns	ns	ns	ns	ns	ns
F (year × treatment)	-	-	-	ns	-	-	-	ns	-	-	-	ns	-	-	-	*	-	-	-	ns

** Highly significant (P ≤ 0.01); * Significant (P ≤ 0.05); ns, Non significant; † Part per million; me, mili equivalent.

The wheat grain and straw yields as well as N uptake by grain and straw decreased over years due to low N application (40 kg N ha^{-1}) through organic materials. Combined maize and wheat grain yield also decreased over years in all treatments but this decrease was the lowest in the mixture of low and high quality organic material treatment indicating that semi-decomposed organic material is good for maize and wheat crop in high rainfall area of Lumle. Research should be diverted using higher dose of N through semi-decomposed organic materials and split application of high quality FYM for continuous supply of N for later part of crop growth under high rainfall area.

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Management of Botrytis Gray Mold (Botrytis cinerea Pers. Ex. Fr.) of Chickpea at Tarahara, Nepal

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Abstract

An experiment was conducted in randomized complete block design with six treatments and four replications at loamy soil of Regional Agriculture Research Station, Tarahara, Nepal during 1997/98 and1998/99 seasons to find out the effect of bioagent Trichoderma viride as compared to fungicide Bavistin (carbendazim) for management of Botrytis gray mold (Botrytis cinerea) of chickpea (Cicer arietinum). Treatments were use of water spray (check), three sprays of T. viride (10^{7}-10^{8} spores/ml of water), three sprays of Bavistin @ 0.2%, three sprays of Bavistin @ 0.1% + T. viride, two sprays of Bavistin @ 0.2% and two sprays of T.viride. The area under the disease progress curve (AUDPC) was the least (193.6) with three sprays of Bavistin (0.2%) followed by two sprays of Bavistin (0.2%) and three sprays of Bavistin + T. viride (216.9). The highest mean grain yield of 267.3 kg ha^{-1} was produced by three sprays of Bavistin + T. viride followed by three sprays of Bavistin. Three sprays of T. viride was inferior to three and two sprays of Bavistin but it was at par with two sprays of T. viride with respect to grain yield production and reduction of Botrytis gray mold severity. When AUDPC was regressed against grain yield, a negative correlation was obtained between the disease and the grain yield. The correlation coefficients for the disease and yields during1997/98 and 1998/99 respectively were -0.583, and - 0.490.

Key words: Botrytis gray mold, chickpea, Trichoderma

Introduction

Chickpea (Cicer arietinum L.) is the second most important pulse crop in Nepal. The crop occupies 19,510 ha and produces 13,990 tons with an average productivity of 717 kg ha^{-1} (CBS, 1998). Its acreage in eastern terai has declined due to flower drop problem in which no pod formation occurs. Flower drop is mainly attributed to attack by Botrytis gray mold (Botrytis cinerea Pers. Ex. Fr.). However, some workers indicated that flower drop is due to deficiency of boron, which causes flower abortion in chickpea (Srivastav et al., 1996). Botrytis gray mold (BGM) is the most important disease of chickpea in the eastern terai region of Nepal and is responsible for complete failure of the crop. The disease occurs almost every year and the estimated loss of 66% in the experimental plots and about 15% in farmers fields have been reported (Joshi, 1992). However, more than 80% crop loss was reported due to the disease in Bangladesh, Nepal and north-western India in recent years (Pande et al., 1998). BGM causes severe damage to the crop and yield loss up to 95% can be experienced in some fields, if the conditions are favorable during vegetative and reproductive growth stages of the crop (Pande and Rao, 2000). Some works have been done for its management in India and other parts of chickpea growing countries of this region (Grewal and Laha, 1983; Meeta et al., 1986; Pandey, 1988; Haware et al., 1997; Pande et al., 1998,). In India, spray of Trichoderma viride has been recommended for its management (Pande et al., 1998). In Nepal, however, its effect on BGM has not been studied. This study was undertaken to assess the effectiveness of T. viride in managing BGM of chickpea as compared to available fungicide, Bavistin.

Materials and Methods

The experiment was carried out at the loamy soil of Regional Agricultural Research Station, Tarahara, Sunsari, Nepal in a randomized complete block design with six treatments and

four replications for two consecutive chickpea growing seasons 1997/98 and 1998/99. The chickpea variety Sita was planted in 3.5- × 2.4-m plot for treatment. The plot was fertilized at the rate of 20:40:20 kg $N_2P_2O_5K_2$ ha^{-1} and the fertilizers used were diammonium phosphate, urea and muriate of potash. Endosulfan was used whenever pod borer (Helicoverpa armigera) was problem. The chickpea was planted in Nov and harvested in April. The treatments were check (water spraying only), three sprays of T. viride (10^7-10^8 spores ml^{-1} of water) mixed with carboxyl methyl cellulose, three sprays of Bavistin (carbendazim 50 WP) @ 0.2%, three sprays of Bavistin (0.1%) + T. viride, two sprays of Bavistin @ 0.2% and two sprays of T. viride. Treatments began when plants showed symptoms of BGM and they were repeated at 10 days interval.

The disease was scored using 1-9, scale as used by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Pande and Rao, 2000) in which 1 means no symptom and 9 means more than 75 percent leaves necrotic. The disease was scored four times on whole plot basis. The latest (last week of March) disease scores were presented for interpretation. Area under the disease progress curve (AUDPC) was calculated following the method used by earlier workers (Shaner and Finney, 1977). Grain yield data and terminal disease scores were analyzed using MSTAT-C computer program and AUDPC was regressed against grain yield (Gomez and Gomez, 1983).

Results and Discussion

Effect of spray on BGM severity

The terminal BGM score was the lowest when three sprays of Bavistin (0.2%) were applied (Table 1). The second lowest terminal score was in case of three sprays of Bavistin (0.1%) + T. viride in 1997/98. In 1998/99, however, the lowest disease score was recorded in three sprays of Bavistin (0.2%) followed by two sprays of Bavistin (0.2%). Means of two BGM scores of two seasons indicated that three sprays of Bavistin (0.2%) was the most effective in reducing the severity of BGM followed by two sprays of Bavistin (0.2%) and three sprays of Bavistin (0.1%) + T. viride. There were slight differences in the disease scores in the first year and the second one. In India, it was reported that Bavistin was found effective in reducing BGM severity and increasing the grain yield of chickpea (Grewal and Laha, 1983; Meeta et al., 1986; Rawal, 1987; Pandey, 1988). Our finding is that Bavistin was effective in reducing BGM severity. Studies carried out at Pantnagar, India indicated that three sprays of T. viride was as good as three sprays of Ronalin in reducing BGM severity. In our experiment, however, three sprays of T. viride was found inferior to three sprays of Bavistin in reducing the severity of BGM.

Table 1. Effect of bioagent Trichoderma viride and fungicide Bavistin on Botrytis gray mold (BGM) scores, grain yield and area under the disease progress curve (AUDPC) at Regional Agricultural Research Station, Tarahara during 1997/98 and 1998/99 seasons

SN	Treatment/s	BGM score, 1-9		AUDPC		Grain yield, kg ha^{-1}	
		1997/98	1998/99	1997/98	1998/99	1997/98	1998/99
1	Check	7.8a†	6.5a	256.6a	248.3a	29.4	251.1 b
2	3 sprays of T. viride	7.8a	6.3 b	247.9ab	245.5ab	16.3	315.5ab
3	3 sprays of Bavistin @ 0.2%	4.5c	5.0 b	194.1c	193.6c	52.3	409.5a
4	3 sprays of Bavistin @ 0.1% + T. viride	6.0b	6.8a	217.7bc	216.0b	65.7	468.8a
5	2 sprays of Bavistin @ 0.2%	6.0b	6.0a	215.6c	215.6bc	103.7	286.7 b
6	2 sprays of T. viride	7.8a	6.3a	248.5a	244.6a	18.6	296.4ab

† Means followed by the same letters are not significantly different ($P \leq 0.05$).

Effect of sprays on AUDPC

During 1997/98, AUDPC varied from 194.1 to 256.6. It was the highest (256.6) in the control plot followed by two sprays of T. viride (248.5) and three sprays of T. viride (247.9). It was the lowest (194.1) in three sprays of Bavistin. In 1998/99, the AUDPC values again varied from 193 to 248.3. It was the highest in the control plot

followed by two and three sprays of T. viride respectively (Table 1).

Use of three sprays of Bavistin (0.1%) + T. viride had lower AUDPC (216.0) in both the seasons. Although the grain yields varied from one year to the next, AUDPC values did not vary. Bavistin was effective in reducing BGM severity significantly as compared to T. viride. This is in contrast to the finding of Pantnagar, India, where the effect of three sprays of T. viride was as good as three sprays of Ronalin (Haware et al. 1997). The effect of three sprays of Bavistin (0.01) + T.viride was better in reducing the severity of BGM than three or two sprays of T. viride as T. viride was less sensitive to Bavistin (Akbari and Parakhia, 2000).

Effect of spray on grain yield

In 1997/98 grain yield was very poor and it ranged from 16.3 to 103.7 kg ha^{-1}. It was mainly due to the effect of incidence of wilt (Fusarium spp.) for which no control measure was applied.

During 1998/99 season, grain yield was better and it varied from 251.1 to 468.8 kg ha^{-1}. The highest grain yield (468.8 kg ha^{-1}) was produced by three sprays of Bavistin (0.01) + T. viride. Three sprays of T. viride and three sprays of Bavistin individually produced 315.5 and 409.5 kg ha^{-1} respectively of grain yield of chickpea (Table 1).

Relationship between AUDPC and grain yield

When AUDPC of BGM was regressed against grain yield, negative correlations were observed in both the seasons (Fig. 1 and 2).

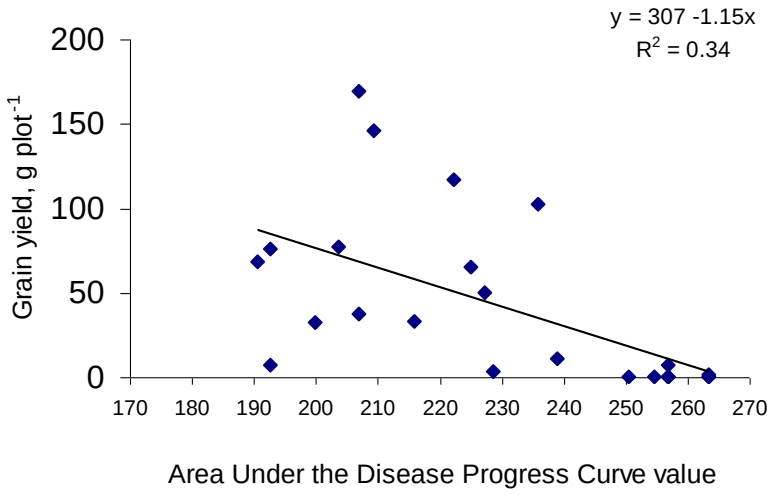

Fig. 1. Relationship between Botrytis gray mold and grain yield of chickpea in 1997/98.

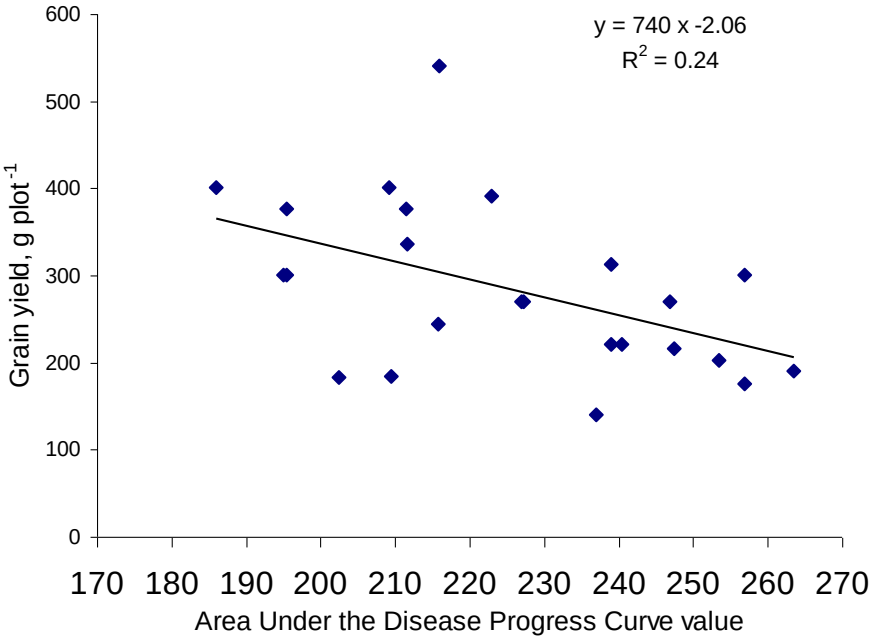

Fig. 2. Relationship between Botrytis gray mold and grain yield of chickpea in 1998/99.

Both the regression equations were the same statistically. It indicted that a unit increase in disease will result in two units decrease of grain yield of chickpea. However, only 30% variation in the grain yield of chickpea was due to variation in AUDPC under Tarahara condition. Rest of the variation in grain yield was attributed to other causes. The correlation coefficients were –0.583 and –0.490 and these were significant at 5% level.

Based on the data of two seasons, it can be concluded that three sprays of Bavistin (@ 0.2%) is effective in reducing the severity of BGM and increasing the grain yield followed by three sprays of Bavistin (0.01) + T. viride. T. viride was not found superior to Bavistin. Therefore, three sprays of Bavistin is recommended for the management of BGM of chickpea in eastern terai.

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Characteristics of Ralstonia Solanacearum Strains of Potato Wilt Disease from Nepal and Thailand[1]

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Abstract

Characterization of strains of Ralstonia solanacearum, the causal agent of potato bacterial wilt disease from Nepal and Thailand was performed based on pathogenicity, biochemical/physiological and serological tests. Fifteen R. solanacearum strains isolated from wilt infected potato plants and tubers grown in Nepal were characterized as race 3, biovar II based on the pathogenicity on different host plants, hypersensitive reaction on tobacco leaf and utilization of some sugars. Results of pathogenicity test show that all strains from Nepal had limited host range. Degree of virulence of all strains varied from high to medium in potato and tomato and medium to low in eggplant. They did not cause wilting in tobacco, pepper and peanut plants. Six strains from Thailand were characterized as biovar II and III. Additionally, comparisons on the physiological, biological and serological characters of seven strains from Nepal and six from Thailand revealed similar characters. Race 3 and biovar II of the pathogen was widely spread over potato growing areas of mid and high hills of Nepal. Both biovars II and III were prevalent in the potato growing areas of Thailand but biovar III was the most dominating one.

Key words: Bacterial wilt, potato, Pseudomonas solanacearum, Ralstonia solanacearum

Introduction

Ralstonia solanacearum (Yabuuchi et al., 1995), wilt of potato and solanaceous crops including other host plants is formerly known as Pseudomonas solanacearum EF Smith. The pathogen is also identified as Burkholderia solanacearum (Yabuuchi et al., 1992). Bacterial wilt is one of the most important and widespread diseases of solanaceous plants in the world. In Nepal, the disease is considered as the most important one that causes a considerable yield loss every year (Pradhanang et al., 1993).

Buddenhagen et al. (1962) divided the pathogen into three races. Race 1 infects many solanaceous plants such as tomato, tobacco, pepper and other plants including some weeds. However, race 2 causes wilt of triploid banana (Musa spp.) and Heliconia spp. Race 3 affects potato and tomato but is weakly virulent on other solanaceous crops. Later, Aragaki and Quinon (1965) reported race 4 from infected ginger in the Philippines. He et al. (1983) reported race 5 from mulberry in China. Therefore, five races have been described so far, but they differ in host range, geographical distribution and ability to survive under different environmental conditions (French, 1986).

The four biovars of R. solanacearum have been characterized on the basis of utilizing and/or oxidizing three hexoses mannitol, dulcitol and sorbitol and three disaccharides lactose, maltose and cellobiose. Biovar I oxidizes hexose alcohols but not disaccharides, whereas biovar II oxidizes only disaccharides. Biovar III oxidizes both disaccharides and hexose alcohols, but biovar IV oxidizes only alcohols (Hayward, 1964). However, races and biovars are poorly correlated except for race 3, which is more or less similar to biovar II (French, 1986). In Nepal, Shrestha (1977) and Adhikari (1993) reported the race 3 and the biovar II in the potato from mid to high

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hill region and the race 1 and biovar III from eggplant, pepper, tomato and marigold from lowland areas. Titatarn (1986) classified the bacterial wilt pathogen of potato as biovar III and IV from mid hills and biovar II from high hills of Thailand. The objective of this study was to characterize the potato bacterial wilt strain of R. solanacearum from Nepal and Thailand based on pathogenicity, biochemical/physiological and serological tests.

Materials and Methods

Bacteria isolation and cultures

Infected potato stems or tubers collected from different sources and locations in Nepal and Thailand (Table 1) were cut into small pieces and placed in test tubes containing 5 ml of sterile distilled water for standard isolation (Hildebrand et al., 1988). Bacteria were allowed to flow from the vascular bundles for 5 to 10 minutes. One loopful of the bacterial suspension was streaked onto tetrazolium chloride (TZC) agar medium (Kelman, 1954) and incubated at 28°C for 48 h. A single colony of R. solanacearum showing virulent, fluidal, irregular and creamy white with pink at the center was selected and multiplied in a TTC (without adding TZC) medium. After 24-48 h of incubation, virulent cultures were maintained in sterile distilled water in screw-capped tubes at room temperature.

Table 1. Biovar characterization of Ralstonia solanacearum strains isolated from bacterial wilt infected potato plants in Nepal and Thailand

Strain	Location	Saccharides reactions†						Biovar classification
		Maltose	Lactose	Cellobiose	Mannitol	Sorbitol	Dulcitol	
Nepal								
NSPC 1	Nucleus Seed Potato Center, Sindhupalcok	+	+	+	-	-	-	II
NSPC 3	Nucleus Seed Potato Center, Sindhupalcok	+	+	+	-	-	-	II
NF 5	Nigale, Sindhupalcok	+	+	+	-	-	-	II
NF 6	Nigale, Sindhupalcok	+	+	+	-	-	-	II
MB 9	Mude, Sindhupalcok	+	+	+	-	-	-	II
MB 10	Mude, Sindhupalcok	+	+	+	-	-	-	II
MB 12	Mude, Sindhupalcok	+	+	+	-	-	-	II
KD 17	Kharidhunga, Dolakha	+	+	+	-	-	-	II
BA 2	Balaju, Kathmandu	+	+	+	-	-	-	II
BA 4	Balaju, Kathmandu	+	+	+	-	-	-	II
BA 5	Balaju, Kathmandu	+	+	+	-	-	-	II
SA 1	Sankhu, Kathmandu	+	+	+	-	-	-	II
SA 2	Sankhu, Kathmandu	+	+	+	-	-	-	II
NA 4	Nala, Kavrepalchok	+	+	+	-	-	-	II
NA 5	Nala, Kavrepalchok	+	+	+	-	-	-	II
Thailand								
1073	Doi Poo Muan, Fang, Chiang Mai‡	+	+	+	+	+	+	III
1089	Hort. Res. Station, Kaoko, Petchabun‡	+	+	+	+	+	+	III
1155	Hueysithon, Fang, Chiang Mai‡	+	+	+	-	-	-	II
1252	Noungmaloa, , Lumpun‡	+	+	+	-	-	-	II
1255	J.D. Kok, Mae Sot Tak‡	+	+	+	+	+	+	III
KUT 1	Kasetsart market, Bangkok	+	+	+	+	+	+	III

† + Positive reaction (color of medium was changed from green to yellow); - Negative reaction (color of medium was not changed); ‡ Received from Mr. W. Boenjuebsaku, Bacteriology Section, Department of Agriculture, Bangkok.

Hypersensitive reaction and pathogenicity test

All fifteen bacterial wilt strains of potato from Nepal were tested for hypersensitive reaction (HR) on tobacco leaf (Table 2). The bacterial suspension was prepared and adjusted to 0.2 OD (optical density) at 600 nm by Spectonic 20 (Bausch and Lomb, Co. Ltd.), which was about 10^8 colony forming unit (cfu) per ml. One side of completely expanded tobacco leaves was infiltrated with 1.0 ml of bacterial suspension and the opposite sides with water as a control. The HR was observed daily for 5 days after infiltration of bacterial suspension.

Table 2. Pathogenicity test on potato, tomato and egg plant and hypersensitive reaction (HR) on tobacco leaves and classification of Ralstonia solanacearum strain in Nepal

Strain	Pathogenicity reaction†			HR‡
	Potato	Tomato	Egg plant	
NSPC 1	M	H	L	+
NSPC 3	H	H	M	+
NF 5	M	H	M	+
NF 6	M	M	L	+
MB 9	M	M	L	+
MB 10	M	M	L	+
MB 12	H	H	L	+
KD 17	M	M	L	-
BA 2	M	H	L	+
BA 4	H	M	M	+
BA 5	M	M	L	+
SA 1	M	M	L	-
SA 2	M	M	M	+
NA 4	M	M	M	+
NA 5	M	M	L	+

† Average disease indices of 5 plants at 28 days after inoculation and rating scales (He et al., 1983) were as followed: H, High (disease index 4.1 to 5.0); M, Moderate (2.6 to 4.0); L, Low (1.1 to 2.5); and, 0, None (1.0).
‡ + Infiltrated area become necrosis; - No reaction.

A mixture of substrates eg sand, compost and soil (1:1:1) treated with formalin was prepared and filled in clay pots of 20 cm diameter. Six different host plants, such as tobacco (Nicotiana tabacum L. cv Local), tomato (Lycopersicon esculentum Mill. cv Pussa Ruby), eggplant (Solanum melongena L. cv Nurkee), pepper (Capsicum annuum L. cv California Wonder), potato (Solanum tuberosum L. cv Kufri Jyoti) and peanut (Arachis hypogaea L. cv Local) were planted in the pots and placed in glasshouse at Khumaltar, Nepal. All test plants were allowed to grow for 6-8 weeks or until they were 15-20 cm high. Five plants of each host were inoculated with each strain of the bacterium by inserting a sterile micropipette tip containing 100 µl at the axil of a fully expanded leaf from the top. The micropipette tips were left in position until the inoculum was absorbed. Inoculated plants were observed daily for evaluation of pathogenicity and severity. Disease severity was assessed at weekly interval for four weeks following the scale of He et al. (1983) (1, no symptoms; 2, two leaves wilted; 3, three leaves wilted; 4, four or more leaves wilted and 5, plant dead).

Physiological and biochemical test

Seven bacterial strains of potato wilt from Nepal and six from Thailand (Table 1) were characterized by using the following tests: oxidation/fermentation, starch hydrolysis, indole production and nitrate (NO_3) reduction (Hayward, 1964; Lelliott and Stead, 1987; Hildebrand et al., 1988). Additionally, the tests such as oxygen relation, levan production, urease test, gelatin liquefaction, tween 80 hydrolysis, catalase production, sodium chloride (5 and 7%) tolerance, oxidase test and growth on potato slice were also performed according to Lelliott and Stead (1987), Hildebrand et al. (1988). Furthermore, some tests were made on arginine dihydrolase, motility, citrate utilization and ammonia production following the method of Hildebrand et al. (1988).

Biovar characterization was carried out based on the ability of strains to oxidize certain disaccharides and sugar alcohols as described by Hayward (1964). Dulcitol, mannitol, sorbitol, cellobiose, lactose and maltose were prepared at 10% solution in distilled water and added separately into Hayward's basal medium modified by He et al. (1983) in order to make a final concentration 0.1%. Each medium was inoculated separately with one loopful of 48 h old bacterium culture of each strain and the tubes containing such cultivars were incubated at room temperature up to 30 days.

Serological test

Seven potato strains of R. solanacearum from Nepal and six from Thailand, were tested by using immunofluorescence (IF) test (Schaad, 1978) against an antiserum produced from whole cells of R. solanacearum from ginger. Suspension of one loopful of each culture was made in 1.0 ml NaCl (0.85%) with 100 µl of sterile formalin (40%) solution. After mixing, 5 µl of each suspension was placed on each well of a multiwells slide and replicated two times. The slides were air-dried and flooded with Kirkpatrick's fixative (ethanol 60%, chloroform 30% and formalin 10%) and kept in a petri dish with moist filter paper for 5 minutes. The slides were rinsed with fixative and allowed to dry. After drying, the slides were stained with R. solanacearum antiserum and the control wells were treated with 0.01 M phosphate buffered saline (PBS) and then incubated in a moist

chamber in the dark for 30 minutes. The slides were rinsed with sterile NaCl solution, followed by PBS buffer and sterile distilled water and then dried. Again slides were stained with a second antiserum (goat antirabbit IgG conjugated FITC fluorescent dye) and placed in a moist chamber in the dark for 20 minutes. The slides were rinsed and dried as described above. Finally, the slides were mounted in a carbonate buffered solution (pH 9.0) with glycerin and examined under the epifluorescent microscope.

Results and Discussion

Pathogenicity test and hypersensitive reaction

All fifteen potato strains of R. solanacearum from Nepal produced fluidal and irregular colonies with pink or light red at centers on TZC medium at 30°C after 48 h of incubation. Thirteen strains out of fifteen caused necrosis in tobacco leaves within three days of infiltration (Table 2). Two strains, SA-1 and KD-17 showed slow collapses after five days of infiltration. Among fifteen strains tested on differential hosts, NSPC-3 and MB-12 were highly virulent on potato and tomato plants but were moderate to slight virulent on eggplant after four weeks of inoculation. Similarly, the degree of virulence of the strain BA-4 was high in potato and moderate in both tomato and eggplant. Other strains NSPC-1, NF-5 and BA-2 were highly virulent on tomato and moderate to low on both potato and eggplant. The rest of the strains showed moderate to low virulence on potato, tomato and eggplant. None of the strains expressed wilting symptom in tobacco, pepper and peanut. Therefore, all of them had characteristic of race 3 with a limited host range on potato, tomato and a few other hosts (Table 2).

In a host range study, all strains were pathogenic (low to high) on potato, tomato and eggplant. But other hosts such as tobacco, pepper and peanut did not show wilting symptoms. The limited host range is the characteristic of race 3 of R. solanacearum (Buddenhagen et al., 1962; He et al., 1983; French, 1986).

Physiological and biochemical tests

All thirteen strains were arginine dihydrolase negative and oxidase, catalase and urease positive. All of them oxidized citrate within 4-5 days of inoculation by changing blue media into green. On the other hand, none of the strains either hydrolyzed starch or produced indole and liquefied gelatin. Strains from Nepal and Thailand were highly sensitive to NaCl at 5% but not at 7%. All the strains produced nitrate and ammonia after 2-3 days of inoculation and they showed positive reactions in levan production, motility, lipolytic and oxygen relation. These strains also hydrolyzed tween 80 and produced black color in potato slants (Table 3).

Biochemical test of all 15 bacterial wilt strains from Nepal oxidized disaccharides, maltose, lactose and cellobiose by changing color of the medium from green to yellow. On the other hand, the strains failed to oxidize hexose sugar alcohols; mannitol, sorbitol and dulcitol, even after 28 days of inoculation (Table 1). Therefore, they were classified as biovar II. Four out of six strains from Thailand oxidized three disaccharides and three hexose sugar alcohols, which were the characteristics of biovar III. However, two strains of R. solanacearum 1155 and 1252, oxidized disaccharides but failed to oxidize hexose sugar alcohol even after 28 days of incubation. Such were the characteristic of biovar II (Table 1).

Serological test

Seven strains from Nepal and six strains from Thailand showed positive fluorescent staining by IF test but the strain BA 4 from Nepal showed moderate fluorescence, whereas the control, Bacillus spp. (non pathogen) was negative (Table 4). Similarly, strains 1073, 1089 and KUT 1 from Thailand produced moderate fluorescence. From the IF test, it was concluded that all strains were positive to the antiserum from ginger but Bacillus sp. was negative. This confirmed the serological character of R. solanacearum.

Table 3. Biochemical and physiological characteristics of Ralstonia solanacearum from wilted potato plant in Nepal and Thailand compared with strains from China

Biochemical/ Physiological test	Reactions†													
	Nepal strains							Thailand strains						China strains‡
	NSPC3	NF6	MB10	KD17	BA4	SA1	NA4	1073	1089	1155	1252	1255	KUT1	
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole production	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate production	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Levan production	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uerase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidative	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fermentative	-	-	-	-	-	-	-	-	-	-	-	-	-	NA
Arginine dihydrolase	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth on potato	+	+	+	+	+	+	+	+	+	+	+	+	+	NA
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	NA
Ammonia production	+	+	+	+	+	+	+	+	+	+	+	+	+	NA
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	NA
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salt tolerance at 5%	+	+	+	+	+	+	+	+	+	+	+	+	+	NA
Salt tolerance at 7%	-	-	-	-	-	-	-	-	-	-	-	-	-	NA
Oxygen relationship	+	+	+	+	+	+	+	+	+	+	+	+	+	NA
Hydrolysis of Tween 80	+	+	+	+	+	+	+	+	+	+	+	+	+	NA

† + Positive reaction or growth; - Negative reaction or no growth; +/- Reaction not defined.
‡ NA, Not available.

Table 4. Immunoflurescent (IF) test of Ralstonia solanacearum strains from wilted potato plant in Nepal and Thailand by using antiserum of Ralstonia solanacearum from diseased plant of ginger

Strain	IF reaction†
NSPC 3	+++
NF 6	+++
MB 10	+++
KD 17	+++
BA 4	++
SA 1	+++
NA 4	+++
1073	++
1089	++
1155	++
1252	+++
1255	+++
KUT 1	+++
Bacillus sp.	++

† +++ Positive reaction (strong fluorescent); ++ Positive reaction (weak fluorescent); - Negative reaction (no fluorescent).

On the basis of the cultural characters, pathogenicity, physiological/biochemical and serological tests, all strains of R. solanacearum from mid and high hills of Sindhupalchok and Kathmandu valley, Nepal and mid hills of Thailand were similar to the strains from other parts of the world (Buddenhagen et al., 1962; Hayward, 1964; He et al., 1983). All strains of R. solanacearum from Nepal were confirmed to be race 3 and biovar II. This result also supports the findings of Shrestha (1977) and Adhikari (1994). They also found that biovar II and race 3 were widely distributed to the mid and high altitude of Nepal.

Similarly, out of six strains tested from Thailand, two were characterized as biovar II and four were biovar III. This result also supports the findings of Titatarn (1986). It was reported that biovar II, III and IV were spread over potato growing areas of Thailand (Titatarn, 1986).

The isolated R. solanacearum pathogen from Nepal and Thailand was confirmed as the causal agent of bacterial wilt of potato by performing hypersensitivity, physiological/biochemical, pathogenicity, cultural and serological tests. Race 3 and biovar II of the pathogen were widely spread over potato growing areas of mid and high hills of Nepal. It was concluded that pathotypes and biotypes of bacterial wilt pathogens of potato were remained the same in Nepal from the last two decades. Both biovar II and biovar III of R. solanacearum were prevalent in Thailand but biovar III was the most dominating one in the potato growing areas of the country.

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Plant Spacing: A Key Husbandry Practice for Rainy Season Cabbage Production

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Abstract

A field experiment was accomplished to establish appropriate plant spacing for summer season cabbage production in the rain fed condition of the high hills of eastern Nepal. The experiment was conducted during the summer of 1997, 1998 and 1999. Five different plant spacings (45- × 60-cm, 45- × 50-cm, 45- × 40-cm, 45- × 30-cm and 45- × 20-cm) and two varieties, Green Stone and Green Coronet were tested. The interactive effect between variety and spacing was non-significant. However, among the tested spacings, head yields were statistically higher at 45- × 30-cm and 45- × 20-cm plant spacings. There was a positive linear correlation between closer plant spacing and cabbage head yield. The number of unmarketable heads and the lowest head compactness was recorded in 45- × 20-cm spacing. Spacing of 45- × 30-cm was found more economical. Plant population can be maintained as high as 74,074 number ha^{-1} by decreasing the spacing from 75- × 60-cm to 45- × 30-cm for the rainy season cabbage cultivation in high hills. By using this spacing, as high as 35 t ha^{-1} cabbage head can be produced in the rainfed condition of the high hills.

Key words: Cabbage, hybrid, plant spacing, rainy season

Introduction

The potential yield of cabbage (Brassica oleraceae var. capitata) is determined by appropriate husbandry practices and the surrounding environment provided to the crop. Among the husbandry practices, a direct effect can be observed due to an increase or a decrease in plant population because a cabbage plant bears a single head. However, serious thoughts have not been given in this aspect in Nepal (VDD, 1987; Rai and Gauchan, 1987). Vegetable Development Division (VDD) of Department of Agriculture has recommended 45- × 45-cm and 75- × 60-cm between and within row plant spacings respectively for early and late season cabbage production (VDD, 1987). In a cultivar evaluation trial in May sowing under Marpha condition, Rai and Gauchan (1987) used a spacing of 45-× 30-cm for a variety Pride of India. However, they used 60-× 30-cm for Late Large Drum Head, September and Danish Ball without giving the basis of selecting different spacings for different tested varieties. A spacing trial on cabbage indicated the highest head yield of cultivar Pride of India at 40- × 30-cm plant spacing (Gurung, 1985). However, Agriculture Research Station, Pakhribas (then PAC) recommended 60- × 45-cm plant spacing for cabbage cultivation for winter as well as rainy season (PAC, 1990).

As in Nepal, different plant spacings have been tried in other countries (Berard, 1990; Subba, 1991). In the Sikkim hills, which has similar climatic conditions to that of the eastern hills of Nepal, spacings, 45- × 45-cm and 60- × 45-cm have been recommended for early as well as mid and late season, respectively (Subba, 1991). In a comparison between 60- × 60-cm and 60- × 45-cm spacings, the higher cabbage head yield (22.9 t ha^{-1}) was obtained from the closer spacing than that from the wider one (20.7 t ha^{-1}) with the cultivar Pusa Drum Head (Mallik and Bhattacharya, 1996). Another study conducted by Lal (1996) showed that cabbage variety Golden Acre yielded 24.5 t ha^{-1} at a spacing of 30- × 60-cm and 18.47 t ha^{-1} at a spacing of 60- × 60- cm. In contrast, Berard (1990) used 90- × 45-cm plant

spacing in Canada, but the Canadian situation does not resemble ours.

Under farmers' field condition, even the plant spacing (60- × 45-cm) recommended by PAC was found inappropriate, especially with the hybrids leading to an improper utilization of available land, light and nutrition. Farmers have been maintaining a wide range of plant population ranging between 50,000 and 100,000 plants ha^{-1} (Khatiwada and Gupta, 1997). The above mentioned plant population is far higher than the recommended plant population (37,037 plants ha^{-1}) for normal and late season planting (VDD, 1987).

As cabbage is an important commercial vegetable in Nepal (APROSC and JMA, 1995), an extensive research has been carried out at Pakhribas for all year-round production. Hybrid varieties have been found suitable for rainy season production (PAC, 1990). As a result, a large number of hybrids is extensively cultivated, particularly during the seasons other than that of winter in the eastern hills. The previously recommended hybrid, KK Cross (Thapa et al., 1997) has been replaced by new hybrids, Green Stone and Green Coronet due to their small and tight heads and they have relatively better storability and easy to market (Tiwari et al., 1996). Informal discussion with semi-commercial cabbage growers revealed that closer plant spacing leads to produce relatively smaller heads, despite having genetic potential for large head size. Owing these considerations, this experiment was conducted to determine the effect of plant spacings on the head yield of cabbage hybrids.

Materials and Methods

Two popular hybrid varieties, Green Coronet and Green Stone were selected for this experiment. The experiment was conducted at Sindhuwa (2200 masl) of Dhankuta district during the summer in 1997, 1998 and 1999. After analyzing the first year's research results five different plant spacings (45- × 60-cm, 45- × 50-cm, 45- × 40-cm, 45- × 30-cm and 45- × 20-cm) and two varieties (Green Stone and Green Coronet) were tested using two factors factorial randomized complete block design with three replications during the spell later two consecutive years. The experimental area was east facing with sandy loam soil.

Six metres long and 1 metre wide individual nursery was made for each variety. Compost at the rate of 5 kg m^{-2} and Malathion dust at the rate of 1 g m^{-2} were applied in the nursery. Seeds were sown in the second week of May under shade. In the first year, seed was sown in the third week of May. Mulching was done immediately after sowing followed by watering. One weeding and one spraying of Indofil M-45 (Mancozeb) at the rate of 2 g l^{-1} were performed in the nurseries.

Farm yard manure was applied at the rate of 33 t ha^{-1} and it was incorporated into the soil during land preparation. At the final stage of land preparation, Malathion (5% dust) @ 20 kg ha^{-1} and chemical fertilizers at the rate of 60:60:30 kg N P_2O_5 K_2O ha^{-1} were applied. Thirty to thirty-one day old seedlings were transplanted onto the experimental field in the second week of June (in the first year, where 34-day old seedlings were transplanted in the last week of June). The moisture level was sufficient in the field, hence, irrigation was not applied. To maintain the required plant population, gaps (plants killed by cutworms and red ants) were filled. To minimize the damage, Cyperin-10 (cypermethrine) at the rate of 1.5 ml l^{-1} of water was drenched 2-3 times in the experimental plots. Seedling mortality was compensated by gap filling even at the later stages in order to provide adequate competition using plants from the boarder rows. Plants transplanted at later stages (within 15 days after transplanting) were marked and excluded for the analysis.

Nitrogen was first top-dressed at the rate of 30 kg ha^{-1} after 30 days of transplanting. Weeding was done prior to the second top-dressing. The second top-dressing was done 45 days after transplanting applying the same dose of nitrogen as in the first top-dressing. Weeding was further carried out at a month intervals. During the whole growing season, 1-2 sprayings of insecticides (metacid-a

methyl parathion, at the rate of 2 ml l^{-1}) and fungicide (Indofil M-45 at the rate of 2 g l^{-1}) were applied. Cabbage heads were harvested when they matured physiologically. Physiological maturity was assessed on the basis of head firmness. In addition to the marketable head weight, other parameters like unmarketable head weight, total upper ground biomass, outer leaf number, head polar as well as equatorial diameter, leaf length, leaf diameter and disease as well as insect incidences were recorded. Leaf length was measured by taking average of five leaves (outer, middle and inner leaves) of 10 plants. As in leaf length, leaf breadth was measured by taking average of five leaves (outer, middle and inner leaf) of 10 plants. After completion of the crop harvest, representative soil samples were collected from each plot. Soil samples were analyzed for nitrogen, phosphorus, potash and organic matter for the crop season, 1999. Calculation was done to know the gross income from cabbage cultivation in the rainy season in the high hills. Market price at the time of harvest was recorded based on Sindhuwa Co-operative. The data collected from the experiment were analyzed using GENSTAT 5 statistical package.

Results and Discussion

Marketable head weight

During the first year of the experiment, the effect of spacing was highly significant ($P < 0.001$). At the spacing of 45- × 30-cm, cabbage variety Green Coronet produced the highest yield (61.2 t ha^{-1}), which was significantly higher than those obtained from rest of the spacings. The widest spacing (60- × 60-cm) produced the lowest yield (30.2 t ha^{-1}), which was significantly lower than those of other tested spacings. The combined analysis of the second and the third year results revealed that the effect of spacing ($P < 0.01$), varieties ($P < 0.05$) and year ($P < 0.05$) were significant (Table 1). However, their interaction was non-significant. Hence, only the mean values have been presented.

The results clearly demonstrated that the increased yield was obtained with the decreasing plant spacing. There was linear relationship between plant spacing and marketable head yield (Fig. 1).

Table 1. Mean marketable head yield of cabbage in association with plant spacing, variety and year

Plant spacing, cm	Head yield, t ha^{-1}	Variety	Head yield, t ha^{-1}	Year	Head yield, t ha^{-1}
45 × 20	39.90	Green Coronet	33.98	1997/98	29.75
45 × 30	35.89	Green Stone	29.76	1998/99	33.99
45 × 40	31.14				
45 × 50	29.15				
45 × 60	23.27				
LSD	5.43				
P value	< 0.001		0.023		0.014

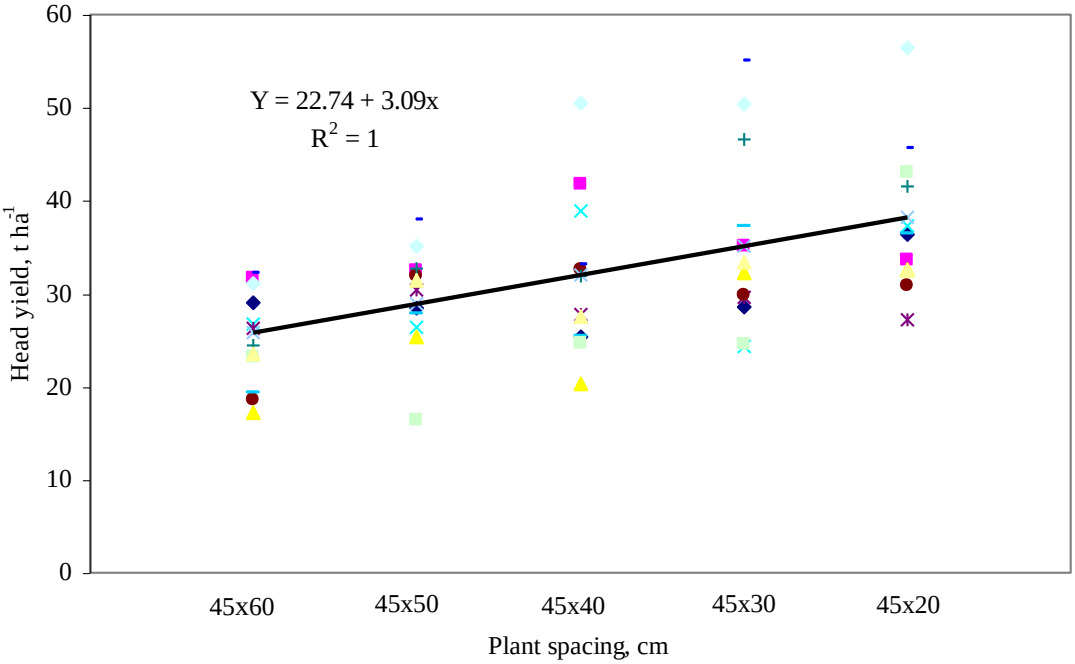

Fig 1. Relationship between cabbage head yield with the spatial plant spacing.

The linear regression analysis of head yield against plant spacing showed a very strong relationship (Fig. 1). Past results have shown that cabbage can be produced throughout the year in the upper mid-hills and high hills of the eastern Nepal (PAC, 1990). As a result the use of hybrids in the summer season has been increased dramatically in the road access areas of the eastern hills (Khatiwada, 1998). The higher yield from the closest spacing proved that the recommended spacing (60- × 45-cm) was too wide for summer season production. Marketable head yield was found higher than the national average (9.7 t ha^{-1}) as reported by Shrestha and Ghimire (1996) even in summer season with recommended spacing. This could be due to cultivation of cabbage hybrids. More than 35 t ha^{-1} marketable head yields were recorded from 45- × 20-cm and 45- × 30-cm spacings. A non-significant yield difference between 45- × 20-cm and 45- × 30-cm, and significantly higher number of unmarketable head number (primarily due to loose heads) along with total upper ground biomass with rest of the spacings suggest that 45- × 30-cm spacing should be recommended to farmers. It also eases intercultural operations and needs lower amount of seed. Therefore, it can be recommended to double plant population (74,074 ha^{-1}) as compared to that of previously recommended plant population (37,037 ha^{-1}). This result supports the findings made on cabbage spacing by other workers (Gurung, 1985; Rai and Gauchan, 1987).

Head compactness

Interaction effects of spacing, variety and year were not significantly different. Similarly, head compactness was not significant due to varieties. However, the effect of spacing was significant ($P < 0.01$) on head compactness (Table 2). Head compactness was the highest in 60- × 45-cm and the lowest in 45 × 20-cm spacings. The difference was not statistically detectable between 60- × 45-cm and 45- × 40-cm at the upper limit and 45- × 30-cm and 45- × 20-cm at the lower side. Results further showed that increasing the plant population also decreased the head compactness up to a certain level. The head compactness from the close spacing was, however, quite acceptable to the local market.

Head weight

Effects of plant spacing and varieties on the individual head weight of cabbage as a single factor were found different ($P < 0.01$). In contrast to that, the interaction effects of spacing and variety were not reckoned significantly different.

An average head weight of the variety Green Stone was lower than that of Green Coronet (Table 2). Similarly, it is apparent from the Table 1 that the head weight was different between 45- × 20-cm, 45- × 30-cm and 45- × 40-cm spacings but was not different in 45- × 50-cm and 45- × 60-cm spacings. Among these spacings, desirable head weight of 408 g in 45- × 20-cm and 566 g in 45- × 30-cm were obtained. Experience has shown that vegetable consumers like cabbage heads having an average weight of around 500 g. The result also shows that increasing plant population per unit area decreases the head weight simultaneously.

Table 2. Individual cabbage head weight as affected by plant spacings and varieties

Plant spacing, cm		Head weight, g	Variety	Head weight, g
45 × 20		408.0	Green Stone	611.0
45 × 30		566.0	Green Coronet	713.0
45 × 40		672.0		
45 × 50		824.0		
45 × 60		839.0		
	LSD	95.3		
	P value	< 0 .001	> 0.05	≤ 0.01

Cropping duration

Green Stone was ready for the first harvest after 67 days of transplanting and was harvested till 134 days after transplanting (DAT). However, Green Coronet took 92 days for the first harvest and lasted for 139 DAT. Effects of spacings were pronounced in Green Stone, in which harvesting could be done up to 83 DAT in the widest spacing and 134 DAT in the closest spacing. The effect was very little in Green Coronet, in which difference between close and wide spacing on DAT was only 9 days.

Unproductive plant number

Non-headed and stalk rot disease affected plants were regarded as the unproductive ones. There were very few stalk rot affected heads in the experimental plots. The analysis of variance showed significantly ($P < 0.001$) higher number (7.25 in number from the area of 5.04 m^2) of unproductive plants at 45- × 20-cm and this was followed by 45- × 30-cm (4.21 in number from 5.05 m^2). The unproductive plants were not significantly different among the 45- × 40-cm, 45- × 50-cm and 45- × 60-cm plant spacings.

Plant height

Plant height was not significantly different among different plant spacings, varieties and years. Similarly, the interaction effects on spacing and variety were not different. Furthermore, different plant spacings failed to show statistical differences in plant height. However, Green Coronet was found taller (27.52 cm) than that of Green Stone (23.44 cm).

Plant spreading

Plant spreading was significantly ($P < 0.001$) influenced by spacings and varieties. The result showed that closer spacing was not enough for the proper plant spreading (Table 3). However, the interaction effect between spacing and variety was not different. Similarly, the combined interaction effects among spacings, varieties and years were not significantly different.

Table 3. Cabbage plant spreading as affected by plant spacings and varieties

Plant spacing, cm	Spreading, cm	Variety	Spreading, cm
45 × 20	32.53	Green Stone	41.23
45 × 30	36.06	Green Coronet	35.10
45 × 40	37.90		
45 × 50	42.12		
45 × 60	42.20		
LSD	2.946		
P value	< 0.01		< 0.001

Leaf length

Leaf length was not influenced significantly by plant spacing, varieties and their interactions. The mean leaf length irrespective of varieties and spacings was 22.8 cm.

Leaf breadth

Statistical difference ($P < 0.001$) was noted in the leaf breadth at different plant spacings. Furthermore, weaker evidence ($P < 0.05$) was shown by varieties and interaction between varieties and spacings (Table 4). However, interaction effect of year, variety and spacing was not prominent.

Outer leaf number

Strong evidence was shown that the effect of spacing and variety was prominent on outer leaf number. The widest spacing (60- × 45-cm) produced the highest number (9.71) of outer leaves and was significantly different ($P < 0.01$) from 45- × 40-cm, 45- × 30-cm and 45- × 20-cm but not from 45- × 50-cm. The rest of the spacings were not different. Green Stone produced significantly higher number (9.43) of leaves than that of Green Coronet (8.79).

Upper ground biomass

The interaction effects between spacing and variety was not different on the total production of upper ground biomass. However, the effect of spacing was significant at $P < 0.01$ level. The total upper ground biomass production from 45- × 20-cm, 45- × 30-cm, 45- × 40-cm, 45- × 50-cm and 45- × 60-cm were 53.9 t ha^{-1}, 48.1 t ha^{-1}, 44.2 t ha^{-1}, 42.2 t ha^{-1} and 34.1 t ha^{-1}, respectively. Productions from all spacings except 45- × 40-cm and 45- × 50-cm, were significantly different.

Diseases and insects

Alterneria leaf spot (Alternaria brassicae) and stalk rot (Screrotinia sclerotiorum) were two diseases recorded in the experimental plots. Alterneria leaf spot disease severity was scored 12-21% on lower leaves. The lower level of the disease was recorded in the wider spacing and higher level in the closer spacing. A maximum of 6% cabbage heads were found infected by stalk rot in the experimental plots but there was no relation between number of stalk rot affected plants and plant spacings. Cutworm (Agotis segatum) attack was found severe during the establishment phase of transplanted cabbage. Similarly, red ant (Dorylus orientalis) attack was also found severe in the experimental plots.

Table 4. Effects of spacings on leaf breadth of two cabbage varieties, Green Coronet and Green Stone

Plant spacing, cm	Leaf breath, cm	
	Green Coronet	Green Stone
45 × 20	15.60	16.94
45 × 30	17.13	19.37
45 × 40	18.93	18.62
45 × 50	19.7	19.19
45 × 60	18.78	19.83
LSD		1.42
P value	> 0.05	< 0.05

Major nutrients exhaustion

Analysis of variance revealed that the nutrient depletion level was not significant due to plant spacings. Many positive attributes have been associated in the farming system due to close planting. Perusal of meteorological data recorded in the experimental site revealed that roughly one half of the annual rainfall was recorded between July and Sept. Besides, yield advantage, close planting reduced soil erosion and also suppressed weed infestation due to ground cover by cabbage plants during the period of high rainfall. The increase in 10 cm plant to plant distance without losing head yield meant, principally, there would be less nutrient mining. However, this notion was nullified by the major nutrient analysis results. Despite having different nutrient mining effects, a non-significant difference among tested plant spacings could be due to nutrient leaching.

Gross income

During summer 1998, the market price of Green Stone and Green Coronet was the same. But there was always higher market price for Green Coronet by Rs 1.0 kg^{-1} than that of Green Stone during harvesting period of 1999. In Sept, the market price was Rs6.0 kg^{-1} and at the end of Oct, the price had dropped down to Rs4.50 kg^{-1}. The reason behind the higher market price was due to longer life of Green Coronet during transportation than that of Green Stone. As a result, ha^{-1} gross income was found roughly Rs50,000.00 higher in Green Coronet than that of Green Stone while using the close spacing (Table 5).

Table 5. Gross return from cabbage cultivation using different varieties and plant spacing using the market price of summer 1999

Plant spacing, cm	Green Stone		Green Coronet	
	Yield, t ha^{-1}	Gross return, Rs'000†	Yield, t ha^{-1}	Gross return, Rs'000†
45 × 20	39.07	214.88	40.74	264.81
45 × 30	34.33	188.81	37.45	243.42
45 × 40	26.7	146.85	35.58	231.27
45 × 50	26.31	144.70	31.98	175.89
45 × 60	22.40	123.2	24.15	159.97

† Price @ Rs5.5 kg^{-1} of Green Stone and Rs6.5 kg^{-1} of Green Coronet.

The results have clearly shown that the appropriate spacing was found 45- × 30-cm for rainy season cabbage for the tested hybrid varieties. Though this study failed to establish nutrient mining due to increasing plant population, it needed further investigation. Besides, spacing trial needs to be conducted for open pollinated varieties in normal season for appropriate spacing recommendation.

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Genetic Variability and Heritability in Sugarcane

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Abstract

Thirty-two genotypes were evaluated in a replicated trial at Sugarcane Research Program, Jitpur, Bara, Nepal in 2000-2001 to estimate phenotypic and genotypic coefficients of variation, heritability and genetic advance for seven stalk characters in sugarcane (Saccharum officinarum L). Analysis of variance revealed highly significant differences between genotypes for all the characters studied. Genotypic variance was higher than environmental one for cane yield, millable cane number, single cane weight, stalk diameter and stalk length. A single cane weight, germination at 45 days after planting and millable cane number had high genotypic and phenotypic coefficients of variation. High heritability estimates were recorded for millable cane number, stalk diameter and single cane weight. Maximum genetic gain as percent of mean was observed for single cane weight and millable cane number.

Key words: Genetic advance, genetic variability, heritability, sugarcane

Introduction

Sugarcane varieties in commercial cultivation are complex polyploid. The heterozygous and polyploid nature of this crop have resulted in generation of greater genetic variability. The information on the nature and the magnitude of variability present in the genetic material is of prime importance for a breeder to initiate any effective selection program. Genotypic and phenotypic coefficients of variation along with heritability as well as genetic advance are very essential to improve any trait of sugarcane because this would help in knowing whether or not the desired objective can be achieved from the material (Tyagi and Singh, 1998). The present study was, therefore carried out to know the nature and extent of genetic variability, heritability and genetic advance in some important traits of sugarcane.

Materials and Methods

The experimental material for the present study consisted of 32 genotypes of sugarcane including four standard checks viz. BO 99, BO 102, BO 91 and CoS 767 representing early, mid and late maturing groups (Annex 1). Sugarcane was planted in ring method with three replications at the farm of Sugarcane Research Program, Jitpur, Bara, Nepal during Feb 2000. The crop received 150 kg N, 60 kg P_2O_5 and 40 kg K_20 ha^{-1}. All agronomical practices were adopted during the entire crop season. Data were recorded on germination percentage at 45 days after planting (DAP), millable cane number, stalk diameter, stalk length, single cane weight, sucrose content and cane yield (Annex 1).

Analysis of variance was used for calculating genotypic, phenotypic and environmental characters. The broad sense heritability was estimated according to the method suggested by Johnson et al. (1955) and the expected genetic advance was calculated by the method given by Robinson et al. (1949).

Results and Discussion

The analysis of variance for all the characters showed that genotypes included in the test differed significantly ($p \leq 0.01$) with respect to all characters studied (Table 1). This indicates that there was significant amount of phenotypic variability and all the genotypes differed each other with regard to the characters that opened a way to proceed for further improvement through simple selection (Punia, 1982).

Table 1. Analysis of variance for seven stalk characters in 32 sugarcane genotypes grown at the farm of Sugarcane Research Program, Jitpur, Nepal during 2000/2001

Source	df	Cane yield	Millable cane number	Single cane weight	Stalk diameter	Stalk length	Germination at 45 DAP†	Sucrose
Replications	2	19.84	16.86	0.0017	0.0108	436.02	7.868	0.811
Genotypes	31	219.04**	2817.45**	0.094**	0.025**	1918.8**	160.96**	20.087**
Error	62	33.58	117.3	0.005	0.0092	215.95	75.39	8.334

** Significant at 1% level; † DAP, Days after planting.

Mean values for cane yield varied between 30.8 t ha^{-1} in clone Co 94024 and 86.67 t ha^{-1} in clone CoSe 95422 (Annex 1). Millable cane varied from 73000 in clone BO 99 to 158000 in clone CoSe 95422. Single cane weight varied from 0.35 kg in clone Co 94024 to 0.64 kg in clone CoB 94162. The clone Co92031 was the tallest (221 cm), while the clone Co 94023 was the shortest (133 cm) in stalk length. Likewise sucrose content varied from 17.56% in the clone CoP 92181 and 21.4% in the clone CoB 94162 (Annex 1).

Genotypic and phenotypic coefficients of variation

After partitioning phenotypic variance, it was found that genotypic variance was higher than the environmental one for five characters studied (Table 2). The magnitude of variance was the highest in millable cane (σ^2_g = 900.05, σ^2_e = 117.3) followed by stalk length (σ^2_g = 567.61, σ^2_e = 215.95). These results indicate that a negligible role was played by the environmental factors in the inheritance of these characters in sugarcane. The high genotypic variance for millable cane was reported also by other researchers (Balasundarum and Bhagyalakshmi, 1978; Nair et al., 1980).

Table 2. Components of variances, coefficients of variation, heritability, genetic advance for seven stalk characters in sugarcane genotypes grown at Jitpur, Nepal in 2000/2001

Component†	Cane yield	Millable cane	Single cane weight	Stalk diameter	Germ 45 DAP‡	Stalk length	Sucrose
PCV, %	19.4	29.18	41.52	14.64	32.56	15.48	18.33
GCV, %	15.63	27.44	38.01	13.54	21.86	13.17	10.37
σ^2_p	95.4	1017.35	0.0346	0.062	137.24	783.56	12.25
σ^2_g	61.82	900.05	0.029	0.053	61.85	567.61	3.92
σ^2_e	33.58	117.3	0.005	0.009	85.39	215.95	8.334
H, %	65	88	84	85	45	72	32
GAdv, %	25.98	52.9	70.0	25.58	30.19	38.15	12.08

† PCV, Phenotypic coefficient of variation; GCV, Genotypic coefficient of variation; σ^2_p, Phenotypic variance; σ^2_g, Genotypic variance; σ^2_e, Environment variance; H, Heritability percentage; GAdv, Genetic advance.
‡ Germ 45 DAP, Germination at 45 days after planting.

The estimates for phenotypic coefficient of variation (PCV) were higher than for genotypic coefficient of variation (GCV) in all the traits, indicating greater influence of environment on genetic variation. The highest phenotypic and genotypic coefficient of variation were observed for single cane weight (PCV = 41.52%, GCV = 38.01%) followed by germination at 45 DAP (PCV = 32.56%, GCV = 21.86%) and millable cane number (PCV = 29.18% and GCV = 27.44%). High genotypic and phenotypic coefficients of variation for a single cane weight and millable cane number were reported earlier by Singh and Sangwan (1980).

Heritability

Genotypic coefficient of variation is not a correct measure to know the heritable variation present and should be considered together with heritability estimates. In the present experiment, high heritability estimates were recorded for millable cane number (88%), stalk diameter (85%) and a single cane weight (84%). This suggests that simple selection for these traits would be effective. It is

reported that a high heritability estimate for single cane weight (Nair et al., 1980; Singh et al., 1994). Moderate values for heritability estimates were found in stalk length (72%) and cane yield (65%), whereas, low heritability estimates were observed in germination at 45 days after planting (45%) and sucrose percent (32%). Similar results were obtained by Sahi et al. (1977) for juice quality characters. Selections might be considerably difficult or virtually impractical for a character with low heritability (less than 0.4) due to the masking effect of environment on genotypic effects (Singh, 1993).

Genetic advance

Heritability estimates along with expected genetic gain is more useful than the heritability value alone in predicting the resultant effect for selecting the best genotypes (Johnson et al., 1955). Maximum genetic gain (as percent of mean) was observed for a single cane weight (70%) followed by millable cane number (52.9%) indicating that there exists a scope to improve cane yield to a considerable extent by adopting suitable breeding procedures. High genetic advance (as percent of mean) for single cane weight was also reported by Sahi et al. (1977), Tyagi and Singh (1998). Stalk diameter had high heritability with moderate genetic advance. Pandey (1989) had earlier reported the low genetic advance with moderate to high amount of heritability for stalk diameter suggesting a little scope in the improvement of this character.

The results suggest that selection should be practiced on the basis of single cane weight and millable cane number for higher cane yield. Improvement in these traits would lead to a significant improvement in yield in limited selection cycles.

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Annex 1. Sugarcane yield and its attributes as affected by different genotypes

SN	Genotype	Cane yield, t ha^{-1}	Single cane weight, kg	Stalk length, cm	Stalk diameter, cm	Germination at 45 DAP†, %	Millable cane number, '000 ha^{-1}	Sucrose, %
1	Co 92030	50.72	0.41	174	1.95	34.6	129	19.50
2	Co 92031	36.50	0.40	221	1.81	23.5	85	19.14
3	Co 94022	42.35	0.39	163	1.72	40.8	117	19.40
4	Co 94023	34.45	0.37	133	1.63	41.4	89	18.72
5	Co 94024	30.83	0.35	189	1.87	25.6	97	18.93
6	CoP 92181	50.21	0.43	186	1.78	35.4	117	17.46
7	BO 130	63.56	0.58	197	1.85	46.7	113	19.83
8	CoB 94161	40.78	0.46	185	1.75	34.8	116	19.50
9	CoB 94162	55.49	0.64	192	1.86	35.7	83	21.41
10	CoSe 95421	51.26	0.36	198	1.48	49.2	137	18.98
11	CoSe 95422	86.67	0.51	204	1.83	46.4	158	18.50
12	CoSe 91232	53.30	0.53	169	1.74	34.4	89	19.50
13	CoSe 96234	59.65	0.54	167	1.67	36.8	109	18.75
14	BO 131	63.20	0.53	175	1.69	31.1	111	19.40
15	BO 132	55.15	0.48	194	1.62	32.4	103	18.01
16	CoSe 95435	62.30	0.55	179	1.71	31.3	101	17.90
17	CoP 92184	53.82	0.52	178	1.73	33.2	105	19.20
18	CoP 92186	40.05	0.44	166	1.64	42.3	93	19.15
19	CoSe 92429	42.36	0.46	157	1.84	48.1	94	19.46
20	CoSe 92423	75.53	0.61	209	2.02	36.3	108	18.98
21	CoSe 92430	68.34	0.59	195	1.85	31.2	95	18.51
22	CoSe 92437	44.80	0.42	167	1.77	41.9	90	18.70
23	CoSe 92440	48.68	0.54	178	1.78	38.4	95	19.04
24	CoSe 92032	39.40	0.53	164	1.89	43.4	84	18.64
25	CoP 95181	51.12	0.55	215	1.79	29.7	93	17.85
26	CoP 95182	50.14	0.51	214	1.76	27.5	104	17.90
27	CoSe 93234	52.50	0.53	205	1.75	33.6	106	19.13
28	BO 99	53.45	0.36	165	1.64	35.3	73	17.84
29	BO 102	55.15	0.41	176	1.63	40.5	127	17.60
30	CoS 687	38.39	0.38	168	1.70	31.1	99	18.67
31	BO 91	55.68	0.46	204	1.88	43.2	113	19.71
32	CoS 767	36.67	0.47	213	1.70	32.5	78	17.78

Effect of Different Feed Ingredients on the Growth of Caged Common Carp

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Abstract

To know the effect of four different fish feed, nine months old common carp about 47 g size were stocked at the stocking density of 12 fish m^{-3} in the cages and cultured in Lake Phewa for 175 days. Four different feed ingredients used were fish meal + soybean + oil cake + rice bran + wheat flour, fish meal + oil cake + rice bran + wheat flour, soybean + oil cake + rice bran + wheat flour and commercial cattle feed. The crude protein ranges from 23 to 32.2%. 32.2% protein content feed containing ingredients of fish meal + soybean + oil cake + rice bran + wheat flour was better for the fish growth (0.30 g day^{-1}) but was the lowest survival rate (65.4%) followed by 27.1% protein content feed containing fish meal + oil cake + rice bran + wheat flour (0.23 g day^{-1}). 26.2% protein content feed containing soybean + oil cake + rice bran + wheat flour without animal protein was the poorest growth rate (0.17 g day^{-1}) with the highest survival rate (95.7%) among the tested feed. It was observed that feed with higher protein level was better for the fish growth and the growth of the fish was different significantly among the treatments except the treatment fish meal + oil cake + rice bran + wheat flour and commercial cattle feed. The common carp fish cultured in cages through artificial feed was not satisfactory. It digs and burrows the pond embankments and sides in search of organic matter that makes pond turbid.

Key words: Cage fish culture, common carp, feed ingredients

Introduction

Cage fish culture can be done either giving or without giving supplementary feed. It is easy to handle and can get higher production from limited area. Planktivorous fish species are commonly used for cage fish culture without giving supplementary feed in Nepal but the omnivorous common carp fish has not been practiced yet. Common carp was originated from the central Asia and introduced in ancient times into China and Japan (Okada, 1960). Now it is widely spread out all over the world. It is an omnivorous fish and can eat any digestible feed item. It eats zooplankton and phytoplankton during young stage and more than 10 cm size eats insects, decayed vegetable matter and bottom dwelling organisms, notably tubificids, molluscs, chironomids, ephemerids and trichopterans. It digs and burrows the pond embankments and sides in search of organic matter that makes pond turbid.

Common carp, an exotic fish, is very popular and fetch higher price than Chinese carp (silver carp and bighead carp) in Nepal. The artificial feed especially for common carp has not been developed yet in Nepal. The growth of this carp depends upon the local environment, cultural method, stocking density, quality and quantity of feed supplied. Alikunhi (1966) has reported that under given conditions, growth of common carp is different in different countries (300g in China, 400 g in Malaysia, Thailand, and Indonesia, 35-50 g in Europe and 15 g in England). The fish eats decayed pieces of plants, the young shoots of aquatic weeds and the natural food contains basically rich in animal protein. Common carp eats artificial protein-rich foodstuffs such as fish meal, blood meal, caracass meal, dried insects, silkworm pupae, flesh of mollusks, minced flesh of fish, frog and snake (Woynarovich, 1975). The growth of common carp was satisfactory by feeding on poultry feed pellets having about 20% animal protein and 10% vegetable protein content. The carp has its maximum appetite when the water temperature remains between 20-25ºC

and under 14ºC the fish takes little food. Woynarovich (1975) reported that the carp gets daily food of about 5-6% of its body weight and grow fast (1 to 2% of the body weight per day).

Cage culture is a method of farming aquatic organisms in a particular type of rearing facility (Beveridge, 1987) and fish growth depends entirely on the external supply of high proteinous feed (> 20%). Cage fish culture is defined as the method of fish rearing from fingerlings to marketable size in enclosed cages that allow free circulation of water into and out of the cages (Kuronuma, 1968; Schmittou, 1970). This culture method is popular as it involves relatively low initial cost, easy to handle and easy to manage. It is simple technique and has simple management practice, which depends upon the feeding habit of fish species either giving supplementary feed or depending solely upon natural food available. Channel catfish production in cages is, at least, as profitable as other methods of rearing (Lowell, et al., 1982). Rearing of fish in cages is the most economical and comparatively profitable, although it depends very much upon availability of feed stuffs, feed cost and the local circumstances. Fish meal is expensive in Nepal and need to import from other countries. So, this study has been carried out to see the effect on the fish growth by feeding different feed containing different feed ingredients with and without supplemented fish meal.

Materials and Methods

Eight floating cages (4- × 4- × 2-m) of 30 mm stretch mesh size were set in Lake Phewa at Pokhara valley. The cages were fixed with bamboo frames anchored in a straight line. Treatment was replicated twice. Each cage was covered from the top to prevent fish loss and predatory. Cages were set 2 m apart to allow free circulation of water in and out of cages.

Nine months old of about 47 g of common carp fish were stocked at the rate of 12 m^{-3} and cultured for 175 days by feeding different feed ingredients having 26-32% crude protein including 23% in control commercial cattle feed. The fish were fed twice a day. The feed ingredients are given in Table 1. The feed were made pellet and fed at the rate of 4% body weight of fish. Twenty percent of fish were sampled monthly for their growth check-up. The water temperature was also measured during the fish sampling. Statistical analysis for significant differences among the experimental diets was determined by using analysis of variance.

Results and Discussion

The fish fed with fish meal + soybean + oil cake + rice bran + wheat flour had better growth (0.30 g day^{-1}) followed by fish meal + oil cake + rice bran + wheat flour (0.23 g day^{-1}), commercial cattle feed (0.20 g day^{-1}) and soybean + oil cake + rice bran +wheat flour (0.17 g day^{-1}), respectively (Table 2). The survival rates was the highest (95.7%) when the fish were fed with soybean + oil cake + rice bran + wheat flour and it was the lowest (65.4%) with fish meal + soybean + oil cake + rice bran + wheat flour.

Table 1. Composition of different feed ingredients used in preparing fish meal in different treatments (Trt)

Ingredient	Trt-I	Trt-II	Trt-III	Control
Fish meal, %	20	25	-	0
Soybean, %	20	-	25	0
Oil cake, %	22	35	45	0
Rice bran, %	20	25	25	0
Wheat flour, %	18	15	5	0
Commercial cattle feed, %	0	0	0	100
Mixed vitamin	1	1	1	0
Mineral	1	1	1	0
Crude protein	32.2	27.1	26.2	23.0
Crude fat	17.0	18.9	9.3	2.8
Ash	7.4	7.4	5.5	10.7
Moisture	7.6	9.5	11.4	9.6

Table 2. Mean weight, survival rate and growth rate of common carp fish cultured in cages and fed with different feed ingredients

Description	Trt-I	Trt-II	Trt-III	Control
Mean weight at stocking, g	47.0 ± 4.2	46.5 ± 4.1	48.0 ± 3.3	47.0 ± 4.1
Mean weight at harvest, g	99.7 ± 4.4	86.5 ± 5.8	77.4 ± 5.1	82.7 ± 4.7
Survival rate, %	65.4	94.3	95.7	91.7
Growth rate, g day^{-1}	0.30	0.23	0.17	0.20

The largest size of fish (99.7 ± 4.4 g) was obtained when fed with fish meal + soybean + oil cake + rice bran + wheat flour and the smallest size of fish (77.4 ± 5.1 g) was obtained when fed with soybean + oil cake + rice bran + wheat flour. The fish growth was higher when fed higher protein containing feed (32.2%) and lower growth when fed without having fish meal. The fish growth containing lower protein feed (23.0%) the commercial cattle feed was better than the fish fed having fish meal but contained higher protein level (26.2%).

The fish growth had no regular pattern though the growth of fish was less without fish meal than with fish meal (Fig. 1). The growth was very slow from Feb to April when water temperature was below 20°C and it started increasing after April as the water temperature increased above 20°C. The fish did not grow from May to June in Trt-I with very little growth in Trt-II (0.02 g day^{-1}) and in control (0.02 g day^{-1}). However the growth of the fish was significantly differences ($p < 0.001$) fed with different diets among the treatments except between the Trt-II and control. The growth pattern was better after June as the water temperature increased above 22°C. The highest growth rate of the fish was 0.67 g day^{-1} in June/July in Trt-I when fed with fish meal + soybean + oil cake + rice bran + wheat flour (Fig. 1).

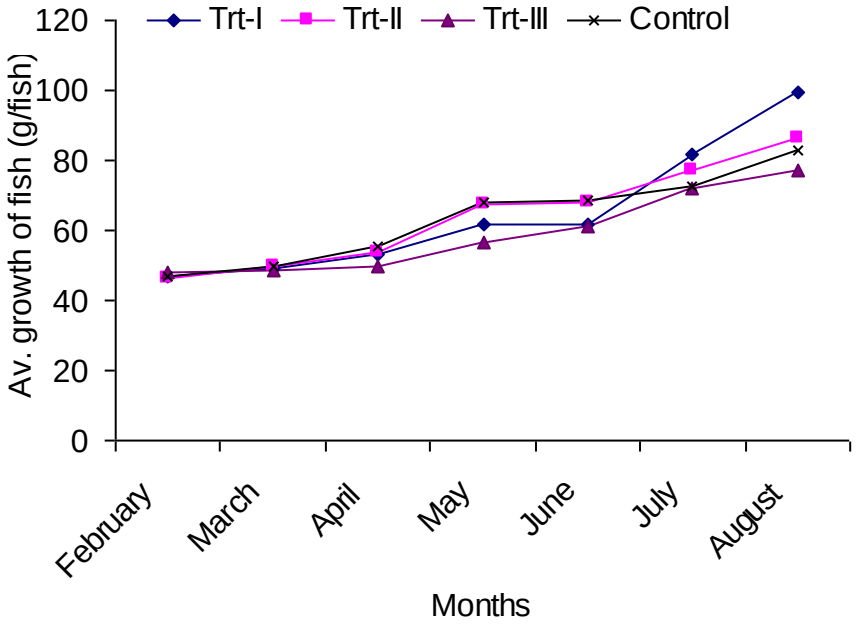

Fig. 1. Monthly growth rate of caged common carp fed with different feed ingredients at Phewa Lake.

Common carp are omnivorous fish and they eat any food, which can be digested. However, their habit are to dig and burrow into the soil in search of organic matter such as larvae of insects, worms, mollusks and decayed vegetable matters containing bottom dwelling organisms, pieces of plants, the young shoots of aquatic weeds, (Woynarovich, 1975; Jhingran and Pullin, 1985).

In case of suspended cage culture, common carp fish can't get their natural proteinous food except plankton available inside the cage. Therefore, the fish might not grow satisfactorily. It also indicates that the fish growth would be better when fed with higher protein containing feed especially animal protein. The fish growth was better when fed with cattle feed containing animal protein than the fish fed with higher protein containing feed without fish meal. It means that the fish need higher protein containing feed with necessary animal protein too. However the common carp did not grow satisfactorily in cage culture even the proteinous artificial feed is given which might be due to its scrapping nature in the bottom.

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RESEARCH COMMUNICATION

Pathogenic Variability in Pigeonpea Wilt Pathogen Fusarium udum Butler in Nepal

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Wilt caused by Fusarium udum Butler is an important disease of pigeonpea in Nepal. The disease is prevalent in all pigeonpea growing areas of Nepal, but its severity differs from place to place. In mid-western terai, the disease is more severe killing almost 90% of plants in farmers' field (RARS, 1996). Limited studies on variability in the wilt fungus F. udum have indicated that the fungus exhibits physiologic specialization (Shit and Sen Gupta, 1978; Reddy and Raju, 1993). However, information on the variation of the pathogen in isolates collected from different agro-ecological zones of Nepal is lacking. Such information will help in developing disease resistant pigeon pea varieties. Variability between 2 isolates of F. udum from Nepal are studied and discussed in this paper.

Differential pigeonpea lines from International Crop Research Institute for Semi Arid Tropics (ICRISAT) were used. These include a susceptible line ICP 2376 and other resistant and susceptible lines from different locations. Each line was seeded on 23 July 1996 on fine riverbed sand in polythene bags. Pathogenic variability in 2 isolates of F. udum collected from Khajura, Nepalgunj, Western Nepal and Nawalpur, Sarlahi, Central Nepal was studied using root dip inoculation and transplantation methods (ICRISAT, 1986). Seven to ten days old roots of ten to thirty seedlings of each differential line were inoculated on 2 August by immersing these roots for 30 minutes in spore suspension of each isolate of the fungus. The seedlings were transplanted in sterilized sand and soil (1:1) mixture in plastic pots. Inoculated plants were kept at $25^0 \pm 3^0$C in a screen house for 40 days. Disease incidence and reactions were taken 11, 21 and 31 days after transplanting. These were begun from 15 Aug. Similarly, the second experiment was begun on 26 Aug, inoculated on 5 Sept and the disease observations were made on 18 Sept, 30 Sept and 11 Oct. The lines with 1-10% wilt were categorized as resistant, 11-20% as moderately resistant, 21-40% as moderately susceptible, 41-60% as susceptible and 61-100% as highly susceptible (Reddy and Raju, 1993).

The pigeonpea differential lines showed four types of reactions 1. No apparent symptoms, 2. Chlorosis, 3. Chlorosis and early wilting (after 10-15 days) and 4. Chlorosis and late wilting (after 15-30 days). There was variation in the reactions of differential lines (Table 1). Lines ICP 8862 and ICP 8863 were resistant to Nepalganj and Sarlahi isolates. Lines ICP 9145, ICP 9174, ICP 8859 and BDN 2 showed resistant to Nepalganj isolate, but susceptible to Sarlahi isolate. The result of this experiment indicated that F. udum isolates of Khajura and Nawalpur are two distinct pathogenic races. The lines like ICP 8859 and ICP 8863 were resistant also at wilt sick plot of farmer's fields, Sanoshree, Bardia district and Dhaulagiri, Banke district (Jha and Neupane, 1998). It is also reported that lines ICP 8859 and ICP 8863 were resistant at RARS, Nepalgunj. The disease incidences and reactions of lines ICP 8863, ICP 9174, ICP 8862 and ICP 8859 were similar to that observed in ICRISAT, Patancheru (Reddy and Raju, 1993). So, before selection of a resistance line for a particular location, it is necessary to screen pigeonpea lines separately at different locations.

Table 1. Disease incidences and reactions of pigeonpea differential lines to Fusarium udum isolates of Khajura, Nepalgunj and Nawalpur, Sarlahi by root dip method in 1996

Differential line	Khaujra, Nepalgunj isolate				Nawalpur, Sarlahi isolate			
	Experiment 1		Experiment 2		Experiment 1		Experiment 2	
	Disease		Disease		Disease		Disease	
	Incidence, %	Reaction†	Incidence, %	Reaction	Incidence, %	Reaction	Incidence, %	Reaction
ICP 2376	80.0	S	90	S	100	S	100	S
ICP 8863	0.0	R	2.5	R	7.5	R	4.5	R
ICP 8858	90.9	S	83.3	S	100.0	S	65.0	S
ICP 9145	0.0	R	0.0	R	100.0	S	75.0	S
T 21	100.0	S	100.0	S	100.0	S	100.0	S
ICP 9174	0.0	R	0.0	R	100.0	S	100.0	S
ICP 8862	0.0	R	9.2	R	10.0	R	10.0	R
ICP 8859	0.0	R	0.0	R	100.0	S	66.7	S
C 11	100.0	S	83.3	S	100.0	S	66.7	S
BDN 2	0.0	R	0.0	R	100.0	S	62.5	S

† R, Resistant; S, Susceptible.

In conclusion, the pathogenic races of F. udum of Nepalgunj and Sarlahi were two distinct types. This information helps to identify pigeonpea varieties resistant to wilt at different regions.

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REVIEW

Antibiotic Resistance: A Concern to Veterinary and Human Medicine

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Abstract

Bacterial resistance to antibiotics occurs even without the use of antibiotics. Antibiotic use exerts a selective pressure to the bacterial flora that help in the emergence and development of antibiotic resistance. Antibiotics are used worldwide both in veterinary and human medicine. The wide spread use of antibiotics in human and animal has raised the concern about the development of resistant and multi resistant bacteria that possess a potential danger to animals and men, as resistance may cause treatment failure. Resistance may be natural or acquired. Acquired resistance is due to transfer of extrachromosomal genetic material (R-plasmids) and is very important. The R-plasmids are spread to other bacterial cells by transformation, transduction, conjugation and transposition. Transmitted antibiotic resistance in disease causing bacteria may cause zoonotic infections and resistant non-infectious bacteria may serve as a reservoir of R- plasmids for the pathogenic organism(s). This paper highlights the mechanism of development of resistance in bacteria and means to minimize it.

Key words: Antibiotic resistance, bacteria, extrachromosomal material, resistance, R-plasmids

Introduction

The use of antibiotics in veterinary practice started soon after it became available for the treatment of human diseases in mid 1940s. Use of penicillin was started before world war II to treat mastitis. Antibiotic resistant strain of bacteria was recognized in the late 1950s and became evident that microorganisms resistance to one or more antibiotics can transfer it to other bacteria (Stalheim, 1987). The problem of antimicrobial resistance has become common today, especially in the area of bacterial chemotherapy (Nandivada and Amyes, 1990; Davies, 1994). A large amount of drugs are being used worldwide annually to secure sufficient quantities of food to feed fast growing world population (WHO, 1985). The wide spread use of antibiotic in human and animals has been followed by the increased emergence of bacteria resistance to these antibiotics, particularly in Enterobacteriaceae (Prescott and Baggot, 1993). A casual relationship between increased use of antibiotics and increased prevalence of resistant bacteria has been demonstrated (Holmberg et al., 1987). Transmission of resistance has been reported from person to person (Linton et al., 1972), animal to animal and animal to person (Levy et al., 1976). Diseases caused by drug-resistant strains of bacteria may transfer their resistance to the drug susceptible strains of bacteria that ultimately may act as reservoir of resistance to pathogenic organisms. Thus, the occurrence of antibiotic resistant bacteria is a great concern in both human and animal medicine.

Types and mechanism of resistance

Resistance to an antibiotic may be an inherent property of the infecting organism or it may be acquired. Acquired resistance may result from mutation or from transfer of an extrachromosomal genetic material followed by selection of resistant organisms during therapy (Davis et al., 1980). Mutations that result in antibiotic resistance are spontaneous events involving changes in chromosomal nucleotide sequences. The development of mutational resistance is favored by low and intermittent drug dosage (Prescott and Baggot, 1993).

R-plasmids (R-factors) are the extrachromosomal substances responsible for antibiotic resistance. In recent years, there has been increasing recognition of the role of extra chromosomal material of heredity for antibiotic resistance (Davis et al., 1980). These R-factors or R-plasmids are spread to other bacterial cells by transformation, transduction, conjugation and transposition (Timoney et al., 1988; Prescott and Baggot, 1993). The most common and important of them is conjugation in which two organisms exchange R-plasmids by contact through sex pilus. R-factors may also be released by one bacterium and take in through the cell wall of another (transformation). R-factors, therefore, can circulate in humans, in animals and in the environment and possibly between animals and humans (Landicho, 1996). Transmitted antibiotic resistance in disease-causing bacteria may cause zoonotic infections, while resistant non-infectious bacteria may serve as a reservoir of R-plasmids for other virulent organisms. R-factors encode at least four different biochemical mechanisms. These involve either enzymatic degradation or alteration of the antibiotic by the cell, alteration of the target site of the antibiotic and synthesis of a resistant form of an essential metabolic enzyme that is normally sensitive to different antibiotics (Timoney et al., 1988).

Bacterial resistance to antibiotics

The development of bacterial strain resistant to antibiotics was recognized in the late 1950s. It later became evident that microorganisms such as Salmonella typhimurium and S. dublin, which are resistant to one or more antibiotics can be transferred to other bacteria (O' Brien et al., 1982; Gracey, 1986). Studies have shown that the use of antibiotics as feed additives results in an increase in both proportion and persistence of antibiotic resistant bacteria (Langlois et al., 1984). The feeding of low levels of antibiotics creates a selection pressure to the bacterial flora of livestock. The effect of this selection pressure has been the appearance of numerous resistant strains of Escherichia coli, Salmonella spp., Staphylococcus aureaus, Pasteurella hemolytica, P. multocida, Streptococcus agalactiae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus pleuropneumoniae, Clostridium perfringens and many other bacterial species (Timoney et al., 1988). The Swann Committee (cited by Gracey, 1986) reported that the use of antibiotics as growth promoter in livestock and poultry has led to the development of resistant strains of microorganisms. These resistant microorganisms can transmit resistance to the non-resistant bacteria. Threlfall et al. (1978) reported that the use of antibacterial drugs to control bovine salmonellosis in Britain facilitated the emergence and establishment of 204 multi-resistant types and 193 strains of Salmonella typhimurium. Lee et al. (1993) in a nationwide survey in the USA found that 57% of isolates from broiler chickens after slaughter were resistant to one or more antimicrobial agents, while 45% were resistant to two or more agents with higher resistance to tetracycline (45%) and streptomycin (41%), sulfixazole (19%), gentamycin (10%) and trimethoprim/ sulfamethoxazole (8%). In a study, in India, Mahipal et al. (1994) found 63.2 % strains of E. coli was singly or multi-resistant to different antibiotics isolated from various infections to man and animals.

Similarly, increasing drug resistance has been observed in human patients from different clinical cases. In a national survey in the USA, Riley et al. (1984) found increasing resistance to Salmonella isolated from human patients. He attributed this to increasing frequencies of anti-microbial drug-resistant Salmonella infections to animal reservoirs. Antibiotic resistance in opportunist pathogens is a major problem in human hospital practice but there has been little information about veterinary hospitals.

Multiple antibiotic resistance

R-factors were first found in members of the genus Shigella in Japan (Watanabe, 1963). Since then, they have been found in all other genera of the family Enterobacteriaceae and in the genera of Pasteurella, Vibrio, Camphylobacter, Haemophilus, Neisseria, Staphylococcus, Streptococcus, Clostridium and Pseudomonas (Temony et al., 1988). R-plasmids possess regions with the resistance genes and resistance

to a number of different antibiotics can be mediated by the same R-factor and is known as multiple antibiotic resistance (Prescott and Baggot, 1993). The prevalence of multiple drug resistance bacteria itself is a serious problem, but transfer of multiple drug resistance to other members of the family Enterobacteriaceae, particularly E. coli, Salmonella and Shigella makes it even greater concern to clinicians in curbing infections in medical and veterinary practice (Mahipal et al., 1994).

R-plasmids in human and animals

Persons who carry the largest number of R-plasmids are the individuals who are in direct contact with antibiotic such as sick people under treatment, workers in antibiotic factories and farms where antibiotics are incorporated into animal feedstuffs (Linton et al., 1972). However, individuals, who are not in contact with antibiotics also carry R-plasmids, but in minimal amounts (Pohl and Lintermans, 1986) and R-plasmids also develop and disseminate in the environment as well as in humans and animals even in the absence of any selective pressure of antibiotics (Huber, 1986).

Likewise animals raised with frequent use of antibiotics have more R-plasmids containing colibacilli flora (Pohl and Lintermans, 1986). However, R- plasmids were also observed on farms where antibiotics are not used either for treatment or for growth promotion (cited by Pohl and Lintermans, 1986). Therefore, in animals as well as in humans, R-factors are observed in the absence of any selective pressure by antibacterial agents (Pohl and Lintermans, 1986).

Public health significance of antibiotic resistance in animals

Many antibiotics that are used in animal feed are also used to treat diseases in man. Such use of antibiotics in feed raised the concern among public health authorities and consumers because such level of the drug use may occur bacterial resistant in the gastrointestinal tract of these animals (WHO, 1985). Such resistance can be transferred to bacterial inhabitants of the gastrointestinal tract (McCapes et al., 1991) through food chain. The feeding of low levels of antibiotic such as tetracycline and penicillin in poultry, swine and calves to promote growth has resulted in a great increase in the reservoir of resistant bacteria (Timoney et al., 1988). These resistant bacteria from animals may reach the human population. This is well established with Salmonella infections (Prescott and Baggot, 1993). Likewise there is considerable evidence that some antibiotic resistant E. coli can colonize the intestines of humans long enough for transfer of antibiotic resistance to occur (Wells and James, 1973). However, the colonization of the digestive tract by a foreign strain is generally very difficult as the conditions in the intestinal environment are unfavorable for foreign strain.

The danger of passing antibiotic resistance from animals to man or vice-versa through their donor bacteria, as some of their serotypes are of zoonotic nature, has got the importance in present day chemotherapy (Linton, 1986). A potential health hazard to consumers can be expected from resistant bacteria. If the organism is resistant to antibiotics, then initial treatment may be ineffective both in man and animals and an alternative treatment need to be applied (McCapes et al., 1991).

Control of antibiotic resistance

A number of approaches can be taken to limit the development and spread of antibiotic resistance. All our efforts should be directed towards reducing the selection pressure as much as possible. Antibiotics should be administered at therapeutic doses only for short periods, prolonged use may select resistant strains (Prescott and Baggot, 1993). Price and Sleight (1970) mentioned that decreased use or withdrawal of certain drugs followed by dramatic reduction in resistance to these and other antibiotics. It is commonly assumed that misuse and inappropriate use of antibiotics is the main cause of antibiotic resistance. Thus, the control of antibiotic resistance depends on the careful and appropriate use of antibiotics.

Conclusion

Resistant and multiresistant organisms may develop even without the use of antibiotics, however, their indiscriminate and prolonged use in human and animal practice may enhance the emergence and development of resistant and multiresistant bacteria. These resistance can be transferred between animal and man and vice versa. Thus, prudent use of antibiotics both in human and animals is needed.

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GENETIC RESOURCES POLICY INITIATIVE PROJECT

IPGRI (International Plant Genetic Resources Institute) and IDRC (Canadian Development Cooperation) are presently implementing Genetic Resources Policy Initiative (GRPI) project in Nepal from 2002. Initially, the project will focus its work in six countries (Nepal,Vietnam, Egypt, Ethiopia, Zambia and Peru) and three regions (Andean Community, West and Central Africa and East Africa).

In Nepal, the leading organization is NARC, and within NARC, the Agriculture Botany Division is coordinating the GRPI project activities. A Multi stakeholder Task Force has been formed involving key stakeholders from both public and private sectors. It comprises representatives of many stakeholders involved in the genetic resources-related issues: Government organizations (Ministry, Departments, Research Institute), I/NGOs, Private sector (agro entrepreneurs), farmers, and CBOs etc. Over its four year cycle, the project will engage (i) collecting and synthesizing data regarding demand from national stakeholders in developing countries for capacity strengthening and research service linked to genetic resources; (ii) identifying gaps between those demands and existing information and training possibilities; (iii) on-the-ground training, capacity building and research with national stake-holders within selected countries.

The specific objectives are:

- to assess the demands of developing countries for different research and capacity building services in the field of genetic resources
- to act as a 'knowledge broker' linking demands with existing information resources
- to identify critical gaps between information and resources that are currently available and the demands for research and capacity building services
- to support participatory action research in different countries or regions where needs cannot be met through the direct use of existing materials
- to initiate and support capacity strengthening activities involving national policy makers
- to strengthen and facilitate regional networks active in the field and
- to create an independent and neutral institution, with an international governance structure, that will be able to coordinate and encourage the realization of these objectives

NARC is an autonomous National Institute responsible for agricultural research and outreach in Nepal, including the conservation of agricultural biodiversity and use of that diversity to improve farmers well-being.

IPGRI is an International Agricultural Research Institute with a mandate to advance the conservation and use of plant genetic resources for present and future generations.

नेपाल कृषि अनुसन्धान परिषद

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