FACTOR ANALYSIS OF BITTERNESS IN *Cucumis melo* var. *conomon* Mak.

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Factor analysis of bitterness in *Cucumis melo* var. *conomon* Mak."** is a bona-fide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis, entitled **"Factor analysis of bitterness in *Cucumis melo* var. *conomon* Mak."** is a record of research work done independently by **Ms. Annie Mathew** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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To the Farmers of Kerala

Introduction

INTRODUCTION

Oriental pickling melon, *Cucumis melo* var. *conomon* Mak. (2n = 24), belonging to the family Cucurbitaceae is an important vegetable crop of Kerala. It is used primarily as cooked vegetable and also as salad. It occupies a predominant place in the summer rice fallows of Kerala. It's fruit is kept as a symbol of prosperity during the festival 'Vishu'. 'Mudicode' is a well accepted variety of oriental pickling melon released from the Kerala Agricultural University. Though a non bitter variety, recently the occurrence of bitterness in fruits has caused serious set back in its production as well as marketing. Even truckloads of fruits were rejected due to the presence of a few slightly bitter fruits. Dishes made out of these bitter fruits were also unpalatable, which adds to the seriousness of this problem.

Presence of bitter principles in the family Cucurbitaceae was known as early as 1552 BC, as indicated by historical records. The bitter principles in the family Cucurbitaceae generally come under the group 'cucurbitacin'. Cucurbitacins may exist as glycosides or as free aglycones. So far 19 forms of cucurbitacins have been isolated and named as cucurbitacin A to S. Currently cucurbitacins have been isolated from members of other families viz. Cruciferae, Rosaceae and Cericidiphyllaceae (Konoshima *et al.*, 1993 and Sarkar *et al.*, 1997). The principle which gives bitterness to bitter gourd namely momordicine is also a cucurbitane triterpene, but it is structurally different from other cucurbitacins (Chandravadana, 1987 and Yasui, 2002).

Ancient Egyptians used these bitter substances as medicine due to their anticarcinogenic activity. However consumption of bitter fruits at high levels may cause stomach cramps, diarrhoea, other digestive disorders and may even result in death. Collapse of cattle and human beings due to consumption of bitter fruits was reported by Ferguson *et al.* (1983).

Different school of thoughts are there on the existence of bitterness in oriental pickling melon, which encompasses on genetic, physiologic and nutritional

aspects. However, no conclusive reports are available on bitterness in oriental pickling melon. Hence the study on inheritance of bitterness was mainly addressed in the present investigation.

There is a common thought that proximity of oriental pickling melon to other cultivated cucurbits like bitter gourd and wild bitter cucurbits may induce bitterness by way of pollen transfer. This is supported by the occurrence of the phenomenon of induction of bitterness through pollen i.e., metaxenia in cucumber and bottle gourd. In Kerala, many bitter forms of cucurbits are seen growing wild or cultivated for their medicinal value. Oriental pickling melon being highly cross pollinated, the chances of out crossing with wild is not remote. Taking into consideration the above facts, a study on the effect of foreign pollen on induction of bitterness and the possibility of gene flow from bitter forms to cultivated oriental pickling melon was conducted.

Another school of thought was that bitterness is induced by organic manures or chemical fertilizers added to the crop. Enhanced intake of nitrogen promotes nitrogen metabolism, which in turn may favour the enzymatic synthesis of cucurbitacin in cucumber (Kano *et al.*, 1999). Hence investigation was done in this aspect to find out whether the source of nutrients is having any aggregating/alleviating effect on bitterness in oriental pickling melon.

Pruning is a horticultural operation done to improve yield and quality. In cucurbits pruning at different levels may increase or decrease the yield (Kanthaswamy *et al.*, 2000; Nomura and Cardosa, 2000) and change the plant vigour. Synthesis of cucurbitacin is reported to be more in young vigorous plants (Kano *et al.*, 1997). Hence a study including pruning at different levels is important to have an idea about growth, and vigour of plant in relation to fruit bitterness. Cucurbitacin content may vary with environmental conditions, location and age of the plant. Identification of the growth stage of plant at which bitterness is minimum helps to adjust the harvesting time, thereby reducing crop loss and bitterness.

Morphological and chemical characterization of bitter and non bitter plants help in differentiating them and it gives an idea about different biochemical parameters related to bitterness.

Keeping the above facts in view, this investigation entitled 'Factor analysis of bitterness in *Cucumis melo* var. *conomon*' was undertaken with the following objectives.

1) To study the inheritance pattern of fruit bitterness.
2) To investigate the effect of foreign pollen on induction of fruit bitterness.
3) To observe whether different sources of manure influence fruit bitterness.
4) To study the effect of pruning and thinning of plant parts on bitterness of fruit.
5) To study fruit bitterness in relation to age of plant.
6) To characterize bitter and non bitter plants morphologically and biochemically in order to explore the possibility of early identification of bitter plants.

Review of Literature

2. REVIEW OF LITERATURE

The available literature on aspects pertaining to the present investigation is reviewed under the following topics. Studies on these topics in oriental pickling melon are limited. Hence reports on cucurbits in general is included here under.

2.1 Inheritance of bitterness

2.2 Source effect of manures on bitterness and yield

2.3 Effect of pruning on bitterness and yield

2.4 Bitterness in relation to age, plant part and environment

2.5 Chemical characteristics of bitter principle

2.6 Bitterness in relation to pest and disease incidence

2.1 INHERITANCE OF BITTERNESS

Literature related to gene action and inheritance of bitterness is presented in Table1. Reports on effect of metaxenia are also included.

Table 1. Studies on gene action of bitterness

Sl. No.	Crop	Plant Part Sampled	Gene Action	Reference
1	*Cucurbita* sp.	Fruit	Monogenic dominant	Contardi (1939)
2	*Lagenaria siceraria*	Fruit	Monogenic dominant	Pathak and Singh (1950)
3	Cucumber	Fruit	Monogenic dominant	Barham (1953)
4	*Cucurbita pepo*	Fruit	Monogenic dominant	Grebenscikov (1954)
5	*Cucurbita texana*	Fruit	Monogenic dominant	Grebenscikov (1955)
6	*Cucumis sativus* and *Lagenaria siceraria*	Fruit	Independent assortment of genes for bitterness and elatersase enzyme activity	Rehm (1958)
7	*Cucumis sativus*	Seedling	Monogenic dominant	Andeweg and deBruyn (1959)
8	*Cucurbita pepo*	Seedling and fruit	Suppressor gene suppressing gene for bitterness	Rehm (1960)
9	Watermelon	Fruit	Dominant bitter gene (Bi) modified by the modifier gene (Mo^{Bi}) which caused quantitative differences of bitter principles in fruits. Lack of bitterness occurs due to the presence of suppressor gene (Su^{Bi})	Chambliss *et al.* (1968)

10	*Cucurbita pepo*	Seedling and fruit	Separate gene in seedling & fruit	Rehm (1968)
11	*Cucurbita pepo*	Cotyledon	Monogenic	Sharma and Halls (1971)
12	*Cucumis* sp. *Cucurbita* sp. and *Citrullus*	Entire Plant	Monogenic dominant	Robinson *et al.* (1976)
13	*Cucumis sativus*	Entire plant	Presence of additively inherited intensifier gene	de-Ponti and Garretson (1980)
14	*Cucumis sativus* (bitter x non-bitter)	Entire plant	Monogenic dominant	Ingammer and de-Ponti (1981)
15	*Cucurbita pepo*	Fruit	Monogenic dominant	Herrington (1983)
16	*Cucurbita pepo*	Root, cotyledon and fruit	Monogenic dominant, separate gene for bitterness in root, cotyledon & fruit.	Dane *et al.* (1987)
17	*Cucurbita mixta* x *Cucurbita pepo*	Fruit	Complementary action of three dominant genes	Borchers and Taylor (1988)
18	Bitter gourd	Fruit	Additive, dominance and additive x dominance gene action	Vahab (1989)
19	Cucumber	Fruit	Independent assortment of genes for bitterness and gynoecious sex expression	Vakalounakis (1992)
20	Cucumber	Fruit	Independent segregation of the gene for bitterness from the genes for other fruit characters	Fanourakis (1993)
21	Cucumber	Fruit	Independent assortment of genes for fruit neck size and bitterness	Fanourakis and Tzifaki (1993)
22	*Cucumis melo*	Fruit	Several independent dominant genes supplementing the action of each other, if the plants are progeny of individuals from same place of origin	Dewei, *et al.* (1996)
23	Crosses between *Cucumis melo* var. *inodorus, C. melo* var. *reticulatus, C.melo* var. *cantalupensis, C. melo* var. *makuwa, C.melo* var. *conomon* and *C. melo* var. *agrestis*	Young fruit	Two independent pairs of gene, which are dominantly complementary, if the varieties are from different centers of origin	Dewei, *et al.* (1997)
24	Cucurbits	Fruit	The bitter free gene (bi) is epistastic to the bitter gene (Bt), which is responsible for increased cucurbitacin content	Robinson and Decker-Walters (1997)

25	*Cucurbita pepo* x *Cucurbita argyrosperma*	Fruit	Even though parents are non bitter, through gene interaction progeny can be bitter.	Robinson and Decker-Walters (1997)
26	Cucumber	Fruit	Bitter free gene bi_2 which is linked with the short petiole gene (sp)	Wehner *et al.* (1998)
27	*Cucumis sativus* (non bitter) x *Cucumis hardwickii* (bitter)	Fruit	Monogenic dominant	Dineshkumar (2001)

Rymal *et al.* (1984) opined that the origin of occasional bitter plants in squash producing bitter fruit may be due to its out crossing with bitter fruited wild types or due to mutations. In some cases, when bitter fruits could be traced to individual plants, the plant and mature fruit characteristics did not match with that of the cultivar indicating that genetic change has occurred.

According to Sheshadri (1986), pollen also contain bitter principle and when bitter pollen fertilizes non bitter ovule, the resulting fruit will be bitter, as seen in cucumber and bottle gourd. Metaxenic effect on nut size and time of maturation was reported by Kumar and Das (1996) in Almond (*Prunus dulcis*). Carlson (1995) reported xeniac effect on grain size in maize. In mango, metaxenia was reported by Chaudhary and Desai (1995) for fruit weight, total soluble solids and acid content. Metaxenia in *Pistacia vera* was demonstrated by Riazi *et al.* (1995) with respect to different nut and seed characteristics. Absence of xeniac effect in *Cucurbita pepo* for bitterness was reported by Robinson and Decker-Walters (1997).

2.2 SOURCE EFFECT OF MANURES ON BITTERNESS AND YIELD

Reports on source effect of manures on yield, bitterness and related characters are scarce in oriental pickling melon Hence available literature on source effect of manures on yield and other attributes of cucurbits in general are reviewed hereunder. Since the metabolism of bitter principles may be favoured by high nitrogen content, related literature on this aspect is also included.

Attia and Nassar (1958) reported that in watermelon, application of pigeon manure increased fruit size and sugar content. Khaflewsk (1984) observed that the

application of liquid manure drained from cowdung @ 50-100 m^3 ha^{-1} and FYM @ 20-60 t ha^{-1} increased the marketable yield in cucumber by 13.8 percent and 16.9 percent respectively.

In oriental pickling melon, Joseph (1985) found that the highest yield and number of fruits per plant were obtained when 1½ times the standard dose of NPK was applied, with 75 percent nitrogen as organic manures. Florescu and Chirala (1985) reported that maximum yield was obtained with pig compost 40 t ha^{-1} basally and 20 t ha^{-1} as top dressing for green house cucumber. Increase in yield with the application of cattle manure and chicken manure was reported by Omori and Sugimoto (1987) in different vegetable crops.

In a study conducted by Segovia (1988), it was observed that among the different organic manures, maximum yield was obtained for cattle manure @ 80 t ha^{-1} followed by poultry manure @ 10 t ha^{-1}. However, Ragimova (1987) observed that FYM @ 20 t ha^{-1} with N : P_2O_5 : K_2O @ 90:90:60 kg ha^{-1} along with Mn + Cu + Co produced highest yield in cucumber.

An increase of leaf nitrogen content up to 4.33 per cent of dry weight was reported in cucumber, when a nitrogen dose of 300 per cent more than that of recommended dose was applied (Al-Sahaf and Al-Khafagi, 1990)

Florescu *et al.* (1991) reported that composted urban waste @ 30 t ha^{-1} produced highest yield for cucumbers when compared to 50 t ha^{-1} of the same or FYM @ 50 t ha^{-1}.

In *Cucumis melo*, no nitrogen accumulation occurred at normal fertilizer rate (80 g N, 12 g P_2O_5, 10 g K_2O and 40 g CaO/plant) during warm season. But during the cool season, nitrogen accumulation occurred even at a fertilizer rate half of the normal amount (Kim *et al.*, 1991).

Singogo *et al.* (1991) observed that Lucerne (*Medicago sativa*) plus manure increased the fruit yield in muskmelon, and the yield was on par with that of the

highest rate of synthetic fertilizers. In *Cucumis melo*, Buzetti *et al.* (1993) reported that when different doses of nitrogen were applied, the leaf nitrogen content increased correspondingly.

Sharaifa and Haltas (1993) observed that the highest yield for both sole crop and intercrop of maize, soybean and watermelon were obtained from plots receiving highest level of poultry manure. Increase in yield with rate of applications of chicken manure and spent mushroom compost compared to that of mineral fertilizers was reported by Faria *et al.* (1994).

Bryan *et al.* (1995) observed that the yield in squash increased with the application of solid waste compost @ 60 and 120 t $acre^{-1}$. Arenfalk and Hagelskjair (1995) obtained the highest marketable yield in vegetables with mineral fertilizers or poultry manure as compared to composted FYM or composted household refuse.

Increase in tender fruit yield on addition of VAM, phosphobacterium and *Azospirillum* along with FYM was reported by Nirmala *et al.* (1999) in cucumber.

Park and Chiang (1997) reported that in aeroponic study of *Cucumis sativus* the leaf nitrogen content increased with concentration of nitrogen in the nutrient solution. Total N content in plants was higher when grown in NH_4^+ solution than grown in NO_3^- solution. Kano *et al.* (1997, 2001) observed that in cucumber, leaf nitrate content of the bitter lines was higher than that of non bitter lines. Later in 1999, they found that the total leaf nitrogen and amino acid levels were higher in bitter lines than in non bitter line, but the nitrate ion level was lower in the former. They concluded that high total N and amino acid N levels in the leaves induce bitterness in leaves and fruits by promoting N metabolism which in-turn favours the enzymatic synthesis of cucurbitacin C, the bitter factor in *Cucumis sativus.*

In cucumber, Alphuse and Saad (2000) observed an increase in vine length and yield on combined application of farmyard manure and chicken manure .In pumpkin, Bage *et al.* (2000) reported early yield with application of cowdung compared to other organic manures like mahua cake, mustard cake and surja. Abou-

Hadid, *et al.* (2001) recorded higher fruit weight in cucumber by application of chicken manure compared to other organic manures.

2.3 EFFECT OF PRUNING ON BITTERNESS AND YIELD

Literature related to effect of pruning on growth characters and bitterness of cucurbits in general are reviewed hereunder.

Aurin and Rasco (1988) reported higher markertable yield of *Luffa cylindrica* with increase in number of secondary branches owing to pruning at four leaf stage or two leaf stage, followed by pruning of primary branches.According to Arora and Malik (1989), when the plants were pruned to have two, four, six and all primary branches, the highest and earliest yield was observed for plants with six primary branches.

Gobeil and Gosselin, (1990) reported that among the four methods of pruning adopted in cucumber viz i) pruning mainstem after production of 12-14 fruits, ii) Pruning mainstem and allowing production of short secondary suckers, iii) pruning mainstem after production of 18-20 fruits and retaining one secondary branch near the top of the plant and iv) prolonged production on mainstem, the method (ii) was the most productive with 4.8 fruits $week^{-1}$ in winter and 6.2 fruits $week^{-1}$ in summer. In bittergourd, Rasco and Castillo (1990) found that pruning of lower lateral branches increased the number of flowers per plant by increasing the number of flowers on the higher lateral branches.

In cucumber, More *et al.* (1990) found that pruning of primary branches above two nodes gave the highest yield compared to the other treatments viz. i) pruning of all primary braches ii) pruning all primary branches after the first node and iii) no pruning .In musk melon, Mougou *et al.* (1991), realized that, pruning the main stem after the fourth leaf, followed by pruning primary branches after the fourth leaf and secondary branches after third leaf results in increased yield.

Jutamanee *et al.* (1993) observed that pinching the main shoot of cucumber between nodes seven and eight induced the development of bisexual and pistillate

flowers. The yield and number of fruits per plant were not significantly affected by the pruning treatments which ranged from removal of 50 per cent of leaves in early stages of growth to cutting of tip of flowering vines by 20-40 cm (moderate) as reported by Humphries and Vermillion (1994).

Pruning of tertiary shoots in *Cucumis melo* increased the marketable yield (Park, 1995). In the case of cucumber plants topped at five leaf stage, Anchiio and Lin (1995) found that, with increasing number of lateral branches, the number of fruits per plant increased from 17 (with one lateral branch) to 28.975 (with three lateral branches). Young Hah *et al.* (1995) also reported that in cucumber the highest yield was observed when the plants were pruned to have three lateral shoots in comparison to one lateral shoot or a mainshoot and one lateral shoot. You Tiao *et al.* (1996) found that in strong branching cultivars of water melon, pruning of mainstem resulted in increased fruit set and fruit weight.

Kano *et al.* (1997) reported that in *Cucumis sativus* cv. Kagafutokyuri, the fruits borne on the first lateral shoot were more bitter than on the second lateral. High occurrence of bitter fruits was observed in the first lateral shoot regardless whether the main shoot was kept as such or pruned back. In general, they observed that bitter constituent cucurbitacin C was synthesized more in young vigorous plants than in old less vigorous plants.

In bottle gourd, Damato *et al.* (1998) reported that pruning the first four basal shoots and main shoot at 180 cm above ground level, decreased yield by 46 per cent. They also observed that unpruned plants yielded fruits earlier than pruned plants. In cucumber, Paschold and Kleber (1998) observed that among the different pruning treatments given, removal of every second fruit and removal of all side shoots except the upper two resulted in the highest yield. Wang (1998) reported that in pumpkin fruit, yield was the highest when three stem per plant were retained compared to one stem and two stems per plant.

In cucumber, Kanthaswamy *et al.* (2000) reported that pruning of all primary branches after two nodes gave the highest yield compared to no pruning and pruning

of all the primary branches. However, Nomura and Cardosa (2000) observed that in cucumber yield decreased with increased defoliation. Removal of 50 per cent and 70 per cent leaves resulted in a greater number of nodes per plant, although they were smaller and less vigorous.

2.4 BITTERNESS IN RELATION TO AGE, PLANT PART AND ENVIRONMENT

Bitterness in cucurbits may change with age and environment conditions. Reports on variation in cucurbitacin content with respect to age, plant part and environment are briefly reviewed here under.

Vogel (1934) reported that the extent of bitterness in cucumber (*Cucumis sativus*) depended on genetic characters of the variety as well as growing conditions. In *Cucurbita mixta,* Grebenscikov (1954) found that seedlings from seeds of bitter fruits had non bitter roots but had small amounts of cucurbitacin B in the cotyledons.

As early as in 1957, Rehm *et al.* conducted a lot of investigations on bitterness in cucurbits. His findings about bitterness and its relation with age, plant part and environment are as follows:

- Fruits of *Acanthosicyos horrida* and *Coccinia adoensis* were bitter while they were green, but lost their bitterness rapidly during ripening. However in *Cucumis longipes* the bitter principle content increased considerably on fruit ripening.
- Very young fruit of *Cucumis pustulatus* contained approximately 0.02 per cent cucurbitacin B and traces of D, and in ripe fruits the concentration of cucurbitacin B and D increased to 0.06 per cent and 0.04 per cent respectively.
- In *Citrullus ecirrhosus*, young roots contained only cucurbitacin E, whereas thick mature roots contained cucurbitacins I, J, K and L, in addition to E. All the seedlings of cultivated *Citrullus* contained cucurbitacins in the same quantity as in the wild form.
- In *Bryonia* roots, the cucurbitacins present two months after germination were B and E, and at twelve month age, D and I were also present.

- The radicles of *Benincasa hispida* were only slightly bitter and the cotyledons were completely free from bitterness.
- In *Cucumis anguria*, no bitter principle was found in the radicle whereas, cotyledons were slightly bitter.
- Among the six cultivars of muskmelon studied only one had bitter seedlings.
- In cultivated cucumber, bitter principle present in cotyledon was initially cucurbitacin B, which changed to cucurbitacin C at fully expanded cotyledonary stage.
- In *Lagenaria siceraria*, roots were bitter in bitter fruited forms and in *Citrullus naudinianus,* roots were bitter for non bitter fruited forms also. Controversially bitter fruits and non bitter roots were present in *Coccinia hirtella, C. quinqueloba* and *Echinocystis wrightii*
- In *Luffa cylindrica*, a high concentration of cucurbitacin B in the emerging radicles was observed which decreased rapidly within a few days. The cotyledons were found to be non bitter.
- The seedlings of *Cyclanthera pedata* and *Cyclanthera explodeus* were non bitter.
- Out of the 46 cucurbitacious crops studied, only *Cucumis angolensis* and *Cucurbita foetidissima* had very bitter leaves.
- In *Cucumis humifructus*, the outer layer of the fruit was bitter, whereas the inner jelly was non bitter.
- In *Cucurbia mixta*, outer layer of fruit was non bitter, whereas inner spongy tissue around seeds was highly bitter.
- Seeds of *Luffa acutangula* and *Sicyos angulata* contained very high concentration of bitter principle localized in the perisperm. However, embryos of developing seeds were completely free from bitterness.
- In *Telfairia pedata*, the fibrous cover surrounding the seed coat was bitter.

Rehm (1960) reported that in *Cucurbita pepo*, the synthesis of bitter principle initiated with the onset of seed germination. It increased rapidly in seedlings upto six days after which it decreased.Whitaker and Davis (1962) opined that in most of the cucurbitaceous species, roots are the only bitter portion of the plant, and species with bitter fruits and non bitter roots are rare.It was observed that in *Cucurbita pepo*, the cucurbitacin B content was maximum at full cotyledon expansion stage(Sharma and Halls, 1971).

Herrington (1983) observed an association between leaf and fruit bitterness in an unidentified cultivar of *Cucurbita pepo*, while in the cultivar Custard Squash, no association was observed.

According to Swaider *et al.* (1983), high temperature of above 33°C increased the fruit bitterness in cucumber.

Jaworski *et al.* (1985) observed that the bitter principle glycoside content in the cotyledons decreased with germination, in *Cucurbita pepo* var. Blackjack. He also reported that the concentration of cucurbitacin in the placental region of the fruit was higher than in pericarp and rind. However, Dane *et al.* (1987) reported that since fruit bitterness segregated independently from seedling bitterness, selection against fruit bitterness cannot be done at seedling stage in *Cucurbita pepo.* Elawed and Jack (1992) observed that in *Cucumis melo* var. flexuosus bitter principles developed soon after seed germination, reached a maximum and then declined at first and second leaf stages.

According to Tatlioglu (1993), only those varieties with non bitter seedlings lack the gene for bitterness and depending on weather conditions at the time of fruit development, fruits of the same plant may be bitter or non bitter, (Robinson and Decker-Walters, 1997). Kano *et al.* (1997) reported that in *Cucumis sativus*, leaf bitterness increased with age of the plant.

Khanikar (1995), opined that, 80 ppm succinic acid as seed treatment, along with two foliar sprays at five-leaf stage, and first pistillate flower stage completely

eliminated fruit bitterness in *Luffa acutangula.* Among the thirty-nine varieties of bottle gourd available in Korea, two were classified as bitter by HeeDon *et al.* (2000).

Kano *et al.* (2000) observed that in *Cucumis sativus*, incidence of bitterness was higher in the plots with lower air temperature. Kano and Goto (2003) also observed that employment of vigourous root stock in *Cucumis sativus* resulted in more number of bitter fruits in the bitter lines.

The effect of air temperature on fruit quality in water melon was investigated by Kano (2004) and found that fruit taste can be increased by lowering day temperature. Monforte *et al.* (2004) reported that melon has a world wide distribution with high phenotypic variation for fruit shape, fruit weight and flesh taste.

2.5 CHEMICAL CHARACTERISTICS OF BITTER PRINCIPLE

Investigations on nature of bitter principle in cucurbits were initiated during early twentieth century. Reports on type of cucurbitacin present and their enzymatic transformations are presented below.

Berg (1912) reported that in *Ecballium elaterium* (Fa. Cucurbitaceae), the glycoside elaterinide (bitter principle) was rapidly hydrolyzed by the enzyme elaterase.

The study conducted by Enslin *et al.* (1956) in different cucurbits gave the following results:

- The genus *Acanthosicyos*, *Cucumis*, *Ecballium*, *Lagenaria* and *Sphaerosicyos* had species with high elaterase activity.
- There existed a positive association between bitter principle content and elaterase activity in *Cucumis hookeri, Cucumis sativus* and *Lagenaria siceraria*
- In species of *Citrullus, Cucurbita* and *Echinocystis,* irrespective of bitterness, the elaterase activity was very weak or absent.
- The main bitter principle in *Citrullus lanatus* and *Citrullus colocynthus* was the enol-ß glycoside of Cucurbitacin E.

Rehm (1956) observed that the fruit juices of *Cucumis melo* and *Cucurbita maxima* were capable of enzymatically transforming one cucurbitacin form to another. Occurrence of many cucurbitacins as glycoside was reported by Enslin and Rivette (1957).

In his study conducted at National Chemical Research Laboratory under South African Council for Scientific and Industrial Research, Rehm *et al.* (1957) observed the following.

- In *Cucurbita pepo* var. *ovifera*, bitter principles occurred as E and B together, E and I together, E, B, D and I together, E, B, D, G, H, I, J and K together or E alone.
- The fruits of bitter *Citrullus naudinianus* contained cucurbitacins B, D, G and H, whereas roots contained cucurbitacins E, I, J and K in addition to that in fruits.
- The most commonly occurring bitter principle was cucurbitacin B, which occurred in 91 per cent of all species investigated, followed by D (in 69%) and G and H (in 47%). Cucurbitacins C, F and L were present in *Cucumis sativus*, *Cucumis angloensis* and *Citrullus ecirrhosis* respectively.
- Only cucurbitacins B, C and E occurred alone. Cucurbitacin D was never found in the absence of B. Cucurbitacins G and H were always associated with B and D. Cucurbitacin I occurred always along with E or J. Cucurbitacin K was always associated with cucurbitacins E and I.
- The main cucurbitacin present in the radicles of bitter *Cucurbita pepo* and *Lagenaria siceraria* was cucurbitacin E and that in *Cucumis sativus* were cucurbitacin B and C.
- Bitterness in *Momordica charantia* was caused by compounds other than cucurbitacins.
- Samples of bottle gourd plant from Northern Transvaal contained cucurbitacins B, D, G and H, whereas samples from Southern Rhodesia contained cucurbitacins E and I in addition to B, D, G and H.

- In *Acanthosicyos horrida*, all species of *Cucumis* and *Lagenaria*, content of bitter principle glycosides were less due to high elaterase activity. But in *Ecballium elaterium,* inspite of high elaterase activity, bitter principle glycoside content was also high.
- In the genera *Cucurbita* and *Citrullus* (with the exception of *Citrullus naudinianus*), bitter principles mainly occurred in the form of glycosides and in *Cucumis*, it occurred as aglycone.
- Cucurbitacin B series was characteristic to the genera *Coccinia, Cucumis, Lagenaria* and *Trichomeria, w*hereas E series was a characteristic to *Citrullus*

Rehm and Wessels (1957) considered cucurbitacin B and E as the primary cucurbitacins. The metabolic pathways for cucurbitacin and other plant defense compounds share similar precursors (Balliano *et al.*, 1982). Halaweish (1993) suggested the formation of isocucurbitacin, one step ahead of normal cucurbitacin biosynthesis in *Cucurbita texana.* A total of 29 cucurbitacins and cucurbitacin glycosides were isolated from *Ibervillea sonorae*, belonging to the family Cucurbitaceae, by Achenbach and Horn (1993).

Ahmad *et al.* (1994) isolated two new forms of cucurbitacin B and S from fruits of *Luffa echinata*, in addition to already reported cucurbitacin B, isocucurbitacin B and sitosterol glucoside. Cucurbitacin F was isolated by El-Fattah (1994) from *Cucumis callosus* Occurrence of six new cucurbitacin glycosides in the seeds of *Cylanthera pedata* was reported by Tommasi *et al.* (1996).

Afifi *et al.* (1999) isolated for the first time Isocucurbitacin E and dihydroisocucurbitacin E from *Cucumis phrophetarum.* Yasui (2002) reported that the triterpenoid bitter component in bittergourd was chemically different from that in cucumber and pumpkin. Kano and Goto (2003) found that HMG-CoA reductase activity was higher in bitter fruits of *Cucumis sativus* than in non-bitter fruits.

2.6 BITTERNESS IN RELATION TO PEST AND DISEASE INCIDENCE

Bhaskaran (1971) observed that wild muskmelon (*Cucumis melo* var. *callosus*), which was resistant to fusarium wilt contained more cucurbitacin than the

susceptible variety. Sharma and Hall (1971) found that external application of cucurbitacins on non preferred seedlings of different cucurbits induced preference for Diabroticite beetles.

High cucurbitacin content was reported by Kannaiyan and Purushothaman (1973) in muskmelon (*Cucumis melo*) varieties resistant to fusarium wilt. In pumpkin, Cucurbitacins were found as feeding attractants for red pumpkin beetle by Pal *et al.* (1978). Susceptibility of non bitter cucumber to two spotted spider mites was observed by dePonti and Garretsen (1980). Action of cucurbitacins as kairomone for cucumber beetle *Diabrotica undecimpunctata* and *D. balteata* was reported by Ferguson *et al.* (1982). They also demonstrated that cotyledonary cucurbitacin content was directly related to extent of damage of seedlings by beetles in field. dePonti (1983) suggested the existence of linkage between genes for resistance against mite and bitterness in cucumber.

Metcalf *et al.* (1983) reported that the use of bitter fruit extract of *Cucurbita texana* could act as a trap for Diabroticite beetle. Nugent *et al.* (1984) observed that all the non bitter cucumber plants are resistant to cucumber beetles. Deheer and Tallamy (1991) reported that larvae of spotted cucumber beetle (*Diabrotica undecimpunctata*) consumed more of bitter roots than non bitter roots. Nonlinkage of bitterness and resistance to red spider mite in cucumber was reported by Dhillon (1992). In sponge gourd, Mehta and Sandhu (1992) found that application of crude cucurbitacin extracts from watermelon and muskmelon induced preference for feeding by *Aulacophora foveicollis*. Studies by Dhillon (1993) showed that in summer squash, varieties resistant and susceptible to cucumber beetle contained the same level of cucurbitacin. Cranshaw and Schweissing (1997) found that cucurbitacin based feeding stimulant baits are effective in controlling striped cucumber beetle (*Acalymma vittatum*) on cantaloupe.

In a study conducted by Eben *et al.* (1997), the cucumber beetles *Acalymma* sp. and *Diabrotica* sp. showed significant preference to bitter *Cucurbita pepo*. Nayar and More (1998) opined that in cucurbits cucumber beetles feed on fruit parts with the highest cucurbitacin concentration.

Materials and Methods

3. MATERIALS AND METHODS

The present investigation entitled 'Factor analysis of bitterness in *Cucumis melo* var. *conomon* Mak. was carried out at the College of Horticulture, Kerala Agricultural University, during 2000-2003. The details of the experimental site, materials and methodologies are presented hereunder.

3.1 EXPERIMENTAL SITE, SOIL AND CLIMATE

Field experiments were conducted at the Vegetable Research Farm of the Department of Olericulture, located at 10° 32' N latitude and 76°16' E longitude at an altitude of 22.5 m above mean sea level. The location has a well-drained sandy loam soil, which is acidic in reaction (pH 5.3). The area lies in tropical monsoon climatic region, with more than 80 per cent of the rainfall getting distributed through southwest and northeast monsoon showers.

3.2 EXPERIMENTAL MATERIAL

The experiment was conducted on the recommended and well accepted variety of oriental pickling melon viz., Mudicode. Though a non bitter variety, occurrence of bitter fruits has been noticed recently. The seeds from bitter fruits were collected and raised in the field during June 2000. Cultural operations and application of fertilizers and manures were carried out as per package of practices recommendations of Kerala Agricultural University (KAU, 2002). Controlled pollination was done to get selfed seeds of bitter and non bitter types.

For pollination, male and female flowers were bagged on the previous day of flower opening. Next day, at the time of anthesis, covers were removed and pollen from male flowers were transferred to the stigma of the female flowers of the same plant by gently rubbing anther on stigma. The bags on female flowers were retained for two more days to prevent further contamination by foreign pollen. The selfed fruits were evaluated for bitterness and those, which were true to type, were selected from both bitter and non bitter plants, for further advancement of generations. By

consecutive selfing and selection for four generations, homozygous bitter and non bitter lines, which were true to type for bitterness were obtained.

The seeds of these homozygous bitter and non bitter lines were multiplied and used for the different experiments.

3.3 EXPERIMENTAL METHODS

The entire study consisted of five different experiments, along with morphological and biochemical characterization.

3.3.1 Experiment 1. Studies on inheritance of bitterness in oriental pickling melon

This study was conducted to know the genetics of bitterness in *Cucumis melo* var. *conomon* and consisted of the following steps.

3.3.1.1 Generation of F_1

During September 2001, homozygous bitter (P_1) and non bitter (P_2) lines were raised in the field. The parental lines were crossed in both the directions i.e., P_1 x P_2 (F_1) and P_2 x P_1 (F_1^1) so as to evoke cytoplasmic effect(s) if any. The parental lines were also selfed to get viable seeds of bitter and non bitter types in sufficient quantities.

3.3.1.2 Generation of backcross and F_2 seeds

The hybrid seeds of the two crosses along with their parents were sown in the field during January 2002. Half the number of F_1 plants in both the combinations were then crossed back to both the parents to produce the seeds of BC_1, BC_2, BC_1^1 and BC_2^1 generations. The other half of F_1s was selfed to produce F_2 seeds. Thus sufficient seeds of the following generations viz. P_1 (bitter parent), P_2 (non bitter parent), F_1 (P_1 x P_2), F_1^1 (P_2 x P_1), F_2 (selfed F_1), F_2^1 (selfed F_1^1), BC_1 (F_1 x P_1), BC_1^1 (F_1^1 x P_2) BC_2 (F_1 x P_2) and BC_2^1 (F_1^1 x P_1) were generated.

3.3.1.3 Evaluation of parents along with F_1, F_2, BC_1, BC_2 and their reciprocals

Seeds of all these generations were raised in the field during May 2002 in Randomized Block Design with four replications. The number of plants per replication was 120, with 15 plants in each generation. Cultural operations were conducted as per the package of practices recommendations of Kerala Agricultural University (KAU, 2002).

3.3.1.4 Observations recorded

For taking observations, five plants each were selected from P_1, P_2, F_1, F_2, BC_1, BC_2, F_1^1, F_2^1, BC_1^1 and BC_2^1 and following observations were recorded.

3.3.1.4.1 Length of main vine (m)

Length of the main vine was measured from the collar region to the tip and expressed in meters.

3.3.1.4.2 Days to first male flower opening

The number of days was counted from the date of sowing to the date of opening of the first male flower.

3.3.1.4.3 Days to first female flower opening

The number of days was counted from the date of sowing to the date of opening of the first female flower.

3.3.1.4.4 Number of fruits per plant

Total number of fruits produced per plant was recorded.

3.3.1.4.5 Percentage of fruit set

Percentage of fruit set was worked out based on total number of female flowers produced and total number of fruits set.

3.3.1.4.6 Average fruit weight (kg)

Weight of fruits from each plant was taken and average was calculated and expressed in kilograms.

3.3.1.4.7 Fruit length (cm)

Fruit length was measured from the blossom end to the stalk end of the fruit and expressed in centimeters.

3.3.1.4.8 Fruit circumference (cm)

Fruit circumference was measured at the middle of the fruit and expressed in centimeters.

3.3.1.4.9 Total yield per plant (kg)

The weight of fruits harvested from each plant was recorded.

3.3.1.4.10 Evaluation of bitterness

Bitterness of immature fruits, one week after pollination, were evaluated by a panel of ten members and classified into bitter and non bitter.

3.3.1.4.11 Estimation of cucurbitacin

Estimation was conducted as per the procedure suggested by Rehm *et al.* (1957). Detailed procedure is given under 3.3.6.2.3.

3.3.1.5 Statistical analysis of data

The data collected from the present study were analyzed by using various biometrical techniques.

3.3.1.5.1 Generation mean analysis

The data from different generations were tested for the adequacy of additive – dominance model using A, B, C and D scaling test (Mather, 1949). Estimation of

various genetic components from the generation mean was done using the six-parameter model suggested by Hayman (1958).

The segregation pattern of genes governing bitterness was analyzed by χ^2 test to determine the goodness of fit of the observed ratio with the expected ratio.

3.3.1.5.2 Estimation of genetic parameters

3.3.1.5.2.1 Phenotypic and genotypic coefficient of variation

The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were estimated as follows (Burton, 1952),

$$PCV = [Vp]^{1/2} \bar{X} \times 100$$

$$GCV = [Vg]^{1/2} \bar{X} \times 100$$

Where, Vp = Phenotypic variance, Vg = Genotypic variance and $\bar{X}$ = mean.

The PCV and GCV values were classified as suggested by **Sivasubramanian and Menon (1973) as follows.**

0 to 10 per cent	- Low
11 to 20 per cent	- Medium
21 per cent and above	- High

3.3.1.5.2.2 Heritability (Broad sense)

Heritability in broad sense was estimated using the formula

$$H = (Vg/Vp) \times 100,$$

Where Vg and Vp are genotypic and phenotypic variances respectively. The range of heritability was categorized as suggested by Robinson *et al.* (1949) as

0-30 per cent	- low
31-60 per cent	- moderate
61 per cent and above	- high

3.3.1.5.2.3 Genetic advance

The genetic advance was worked out using the formula suggested by Johnson *et al.* (1955) as

Genetic advance = $K \times Vp^{½}$ (Vg/Vp)

Where K = 2.06, the constant given by Allard (1960) at five per cent selection pressure.

3.3.1.5.2.4 Estimation of inbreeding depression

Inbreeding depression was calculated using the formula,

Inbreeding depression = $[(F_1 - F_2) / F_1] \times 100$, where F_1 and F_2 are the mean values of F_1 and F_2 progeny.

3.3.1.5.2.5 Phenotypic and genotypic correlation coefficients

The phenotypic and genotypic correlation coefficients were worked out as per the formula suggested by Johnson *et al.* (1955).

3.3.1.5.2.6 Path coefficient analysis

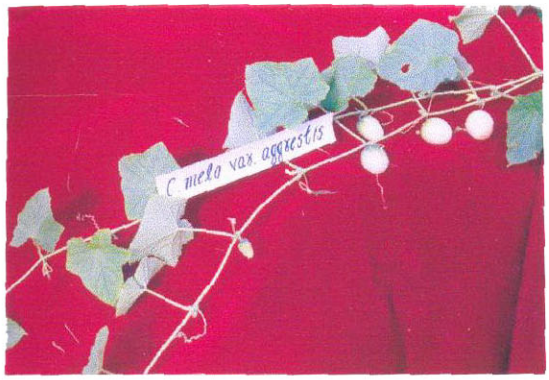
In path coefficient analysis, the correlation among cause and effect is partitioned into direct and indirect effects of casual factors on effect factor. Analysis was done as suggested by Dewey and Lu (1959).

3.3.2 Experiment 2. Effect of foreign pollen on induction of bitterness in oriental pickling melon

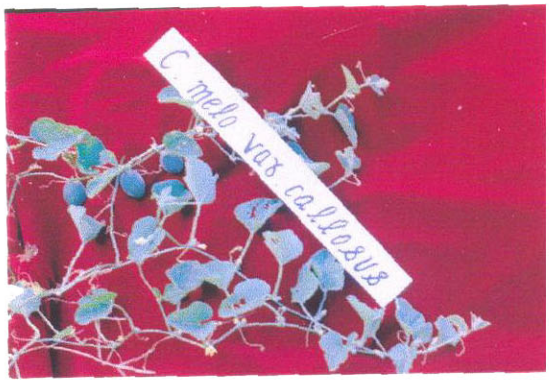
The experiment was designed to find out whether external pollen grains are inducing bitterness in cultivated oriental pickling melon and to know the possibility of gene flow from wild bitter species to cultivated non bitter forms.

3.3.2.1 Lay out and materials of the experiment

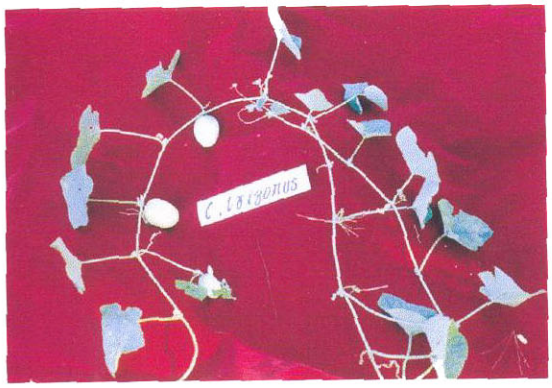
The experiment was laid out in the field during November 2001 in Randomized Block Design with three replications. Homozygous lines of bitter and



Cucumis melo var. *agrestis*

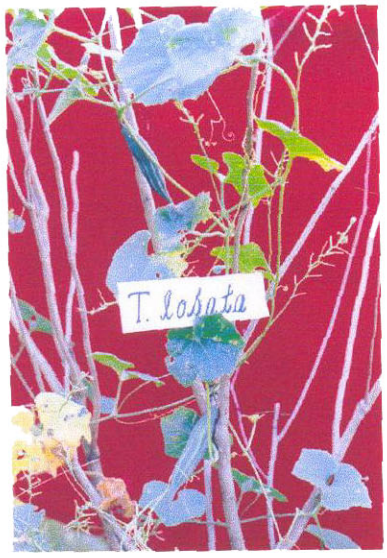


Cucumis melo var. *callosus*

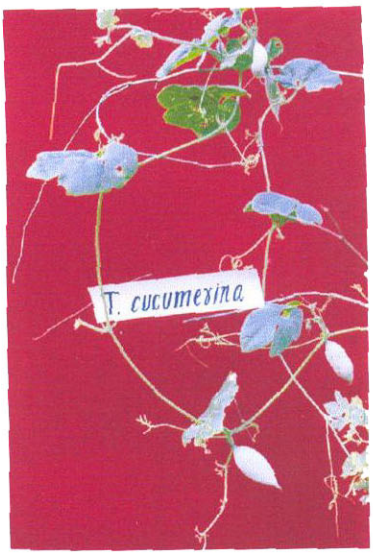


Cucumis trigonus

Luffa cylindrica (wild)



Trichosanthes lobata



Trichosanthes cucumerina

Plate 1. Different wild cucurbits used in pollination study

non bitter oriental pickling melon were raised along with other cucurbitaceous species (Plate 1) for collecting pollen for controlled pollination. Different cucurbitaceous species used in this study were

Cuumis melo var. *agrestis*
Cucumis melo var. *callosus*
Cucumis trigonus
Trichosanthes lobata
Trichosanthes cucumerina
Trichosanthes anguina
Momordica charantia and
Luffa cylindrica (wild)

The seeds of these species were obtained from NBPGR regional station, Vellanikkara.

3.3.2.2 Treatments

Pollen from the above species were applied , as described in the treatments below, for effecting controlled pollination.

T_1 - 100% pollen from non bitter oriental pickling melon (OPM)
T_2 - 100% pollen from bitter OPM
T_3 - 100% pollen from *Cucumis melo* var.*agrestis*
T_4 - 90% pollen from non bitter OPM and 10% from *Cucumis melo* var. *agrestis*
T_5 - 75% pollen from non bitter OPM and 25% from *Cucumis melo* var. *agrestis*
T_6 - 100% pollen from *Cucumis melo* var. *callosus*
T_7 - 90% pollen from non bitter OPM and 10% from *Cucumis melo* var. *callosus*
T_8 - 75% pollen from non bitter OPM and 25% from *Cucumis* melo var. *callosus*
T_9 - 100% pollen from *Cucumis trigonus*
T_{10} - 90% pollen from non bitter OPM and 10% from *Cucumis trigonus*
T_{11} - 75% pollen from non bitter OPM and 25% from *Cucumis trigonus*
T_{12} - 100% pollen from *Trichosanthes lobata*
T_{13} - 90% pollen from non bitter OPM and10% from *Trichosanthes lobata*

T_{14} - 75% pollen from non bitter OPM and 25% from *Trichosanthes lobata*

T_{15} - 100% pollen from *Trichosanthes cucumerina*

T_{16} - 90% pollen from non bitter OPM and 10% from *Trichosanthes cucumerina*

T_{17} - 75% pollen from non bitter OPM and 25% from *Trichosanthes cucumerina*

T_{18} - 100% pollen from *Trichosanthes anguina*

T_{19} - 90% pollen from non bitter OPM and 10% from *Trichosanthes anguina*

T_{20} - 75% pollen from non bitter OPM and 25% from *Trichosanthes anguina*

T_{21} - 100% pollen from *Momordica charantia*

T_{22} - 90% pollen from non bitter OPM and 10% from *Momordica charantia*

T_{23} -75% pollen from non bitter OPM and 25% from *Momordica charantia*

T_{24} - 100% pollen from *Luffa cylindrica* (wild)

T_{25} - 90% pollen from non bitter OPM and 10% from *Luffa cylindrica* (wild)

T_{26} - 75% pollen from non bitter OPM and 25% from *Luffa cylindrica* (wild)

3.3.2.3 Controlled pollination

To determine the number of flowers whose pollen is to be mixed to get different proportions as mentioned in the treatment, pollen count of different pollen sources were taken using haemocytometer.

For this, flower buds were collected just before anther dehiscence. Distilled water (0.1 ml) containing 0.05 percent teepol was taken in a glass vial. Entire anther lobe of the flower was transferred to the glass vial and crushed gently to release the pollen. The content was stirred thoroughly in order to attain an even dispersion of pollen grains in the suspension. A drop of this suspension was drawn using a micropipette and transferred to each of the two counting chambers of an Improved Neubauer haemocytometer. Each of the chambers had an area of 0.0025 mm^2 divided into square millimeter areas. The counting chambers were 0.1 mm deep so that the volume of solution that can be held in each chamber was 0.00025 ml.

The pollen grains in each of the counting chamber were counted by using low power objective of the microscope. For each accession, ten such estimates were made. The number of pollen per flower was calculated as follows:

Volume of each chamber = 0.00025 ml

If 'X' is the average number of pollen per counting chamber i.e., in 0.00025 ml., number of pollen in 0.1 ml of solution (i.e., in a single flower)

$$= \frac{X \times 0.1}{0.00025} = 400X$$

Based on pollen count per flower of each species, mixing of pollen was done as per the treatments and controlled pollination was effected. Both the male and female flowers used for crossing were bagged before flower opening. The bags were retained for two more days after pollination. The resultant fruits after pollination were evaluated for bitterness one week after pollination. The following observations were taken from the resultant fruits.

3.3.2.4 Observations recorded

Observations were recorded from three plants per treatment and average was calculated.

3.3.2.4.1 Percentage of fruit set

For each treatment, number of flowers pollinated and the number of fruits set were recorded. The percentage fruit set was worked out and recorded.

3.3.2.4.2 Fruit length (cm)

Fruit length was measured from the blossom end to the stalk end and expressed in centimeters.

3.3.2.4.3 Fruit circumference (cm)

Fruit circumference was measured at the middle of the fruit and expressed in centimeters.

3.3.2.4.4 Fruit weight (kg)

Weight of fruits from each plant was taken and average was calculated.

3.3.2.4.5 Number of seeds per fruit

Number of seeds per fruit was counted in each plant and average was taken.

3.3.2.4.6 Number of bitter and non bitter fruits in each combination

The fruits were evaluated organoleptically for bitterness and classified into bitter and non bitter.

3.3.2.4.7 Estimation of cucurbitacin

Estimation of cucurbitacin was done as per the procedure suggested by Rehm *et al.* (1957). Detailed procedure is given under 3.3.6.2.3. The placental portion of fruits at fifteen days maturity was used for analysis.

3.3.2.5 Evaluation of F_1

The seeds of treatment combinations which gave fruit set were sown in the field during February, 2002, to evaluate bitterness of fruits and observations on fruit weight, fruit length, fruit circumference and number of seeds per fruit were recorded.

3.3.2.6 Evaluation of F_2

The flowers of F_1 were selfed to get F_2. The F_2 generation was raised in the field during June, 2002, and evaluated for bitterness.

3.3.2.7 Study on pollen germination

Pollen germination studies were conducted *in vivo* to know whether the external pollen grain germinates on the stigma of oriental pickling melon and induce bitterness during pollen germination.

Fluorescence technique suggested by Kho and Baer (1968) and Kho *et al.* (1980) was used for the study. The flowers were artificially pollinated as per the treatments described. The pollinated flowers were fixed in FAA mixture (formalin 10 ml, acetic acid 10 ml and ethyl alcohol 80 ml) at 12 hours after pollination. After 24

hours of fixation, the materials were transferred into glass vials containing I N NaOH and kept for12 hours at room temperature to soften the tissues. The softened material was washed thoroughly with distilled water, then transferred to another glass vial containing 0.1 percent aniline blue in 0.1N K_2HPO_4 for 18 hours. After staining, the stigmatic portion was mounted on a microscopic slide and viewed through fluorescence microscope to observe the germinating pollen grains. In cases where the pollen grains did not germinate 12 hours after pollination, fixation study was repeated after 24 hours.

3.3.3 Experiment 3. Source effect of manures on bitterness in oriental pickling melon

Different organic sources of nitrogen along with inorganic source were applied for both bitter and non bitter plants, so as to find out whether different sources of nutrients have any alleviating/promoting effect on fruit bitterness.

3.3.3.1 Layout of the experiment

The experiment was conducted during two seasons, September 2001 to December 2001 and January 2002 to April 2002. The design adopted was Randomized Block Design with four replications. The number of plants in each replication was 30. Crop management practices were adopted as per the package of practices recommendations of the Kerala Agricultural University (KAU, 2002). Manures and fertilizers were applied as per the scheduled treatments. The average nutrient content in the different organic manures used in the study is furnished in Appendix-I.

3.3.3.2 Treatments

Different manurial treatments adopted were,

T_1 - FYM @ 25 t ha^{-1} + NPK @ 70:25:25 kg ha^{-1}

T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to70 kg N ha^{-1}

T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}

T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha^{-1}

T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}

T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}

T_7 - T_1 + lime @ 250 kg ha^{-1}

T_8 - T_5 + lime @ 250 kg ha^{-1}

T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}

T_{10} - No fertilizer/manure.

In all these treatments balance requirement of P and K were supplemented by chemical fertilizers.

Twenty five tonnes of FYM ha^{-1} and half dose of N were applied basally along with P and K. Remaining half dose of nitrogen was applied in two equal splits.

3.3.3.3 Observations recorded

Observations were recorded from three plants and average was worked out.

3.3.3.3.1 Fruit bitterness

Fruits at harvestable stage were evaluated for bitterness and classified as bitter and non bitter.

3.3.3.3.2 Total yield per plant (kg)

Total yield was recorded as described in 3.3.1.4.9.

3.3.3.3.3 Number of fruits per plant

Total number of fruits was recorded as described in 3.3.1.4.4.

3.3.3.3.4 Number of female flowers per plant

Total number of female flowers produced in a plant was counted and recorded.

3.3.3.3.5 Percentage fruit set

Percentage fruit set was recorded as described in 3.3.1.4.5.

3.3.3.3.6 Days to first female flower opening

Days to first female flower opening was recorded as described in 3.3.1.4.3.

3.3.3.3.7 Duration of the crop (days)

Number of days taken from germination to last harvest was recorded.

3.3.3.3.8 Fruit length (cm)

Fruit length was measured from stalk end to blossom end and expressed in centimeters.

3.3.3.3.9 Fruit circumference(cm)

Fruit circumference was measured at the middle of the fruit.

3.3.3.3.10 Fruit cavity length (cm)

Fruit was cut longitudinally into two and length of cavity from stalk end to blossom end was recorded.

3.3.3.3.11 Fruit cavity breadth (cm)

Fruit cavity breadth was measured at the middle of the fruit after cutting it into two halves.

3.3.3.3.12 Average fruit weight (kg)

Average fruit weight was recorded as described in 3.3.1.4.6.

3.3.3.3.13 Fruit shape

Fruit shape of bitter and non bitter fruits were observed and recorded.

3.3.3.3.14 Economics of cultivation

Economics of cultivation was worked out in terms of benefit cost ratio (BCR) as follows

$$BCR = \frac{\text{Gross return}}{\text{Cost of cultivation}}$$

3.3.3.4 Statistical analysis

Data relating to different characters were analyzed statistically using the package MSTAT C and the results were interpreted.

3.3.4 Experiment 4. Effect of pruning of leaves and branches on bitterness

Pruning of leaves and branches of bitter and non bitter plants were undertaken in order to find out whether the alteration of physiological condition of plant can promote/alleviate the production of bitter principles in oriental pickling melon.

3.3.4.1 Layout of the experiment

Bitter and non bitter oriental pickling melon lines were raised in Randomized Block Design with three replications during May, 2002 at the Vegetable Research Farm of the Department of Olericulture. The number of plants in each replication was 30. The crop was grown as per the package of practices recommendations of the Kerala Agricultural University (KAU, 2002).

3.3.4.2 Treatments

Pruning of leaves and branches were done as per the treatments detailed below:

T_1 - Control (no pruning and leaf thinning).
T_2 - Pruning of all primary branches.
T_3 - Pruning of all secondary branches.
T_4 - Pruning of all tertiary branches.
T_5 - Pruning part of primary branch, after the set of two fruits on it.
T_6 - Thinning of first two leaves of all branches.
T_7 - Thinning of every alternate leaf.
T_8 - Thinning of every fourth leaf.

T_9 - Thinning of every sixth leaf.

T_{10} - Thinning of every eighth leaf.

Leaf thinning was stopped by the 25th day of onset of flowering.

3.3.4.3 Observations

Observations on fruit bitterness, fruit yield, number of fruits per plant, number of female flowers per plant, fruit set percentage, average fruit weight, days to first female flower opening, duration of the crop, fruit length, fruit circumference, fruit cavity length and fruit cavity breadth were recorded as described in experiments 1 and 2. Other observations were recorded from three plants per replication and average was calculated.

3.3.4.3.1 Number of primary branches

Total number of primary branches borne on the plant was counted.

3.3.4.3.2 Number of secondary branches

Total number of secondary branches borne on primary branches of each plant was counted.

3.3.4.3.3 Number of tertiary branches

Total number of tertiary branches borne on secondary branches of each plant was counted.

3.3.4.3.4 Number of fruits on primary branches

Total number of fruits borne on primary branches in a plant was recorded.

3.3.4.3.5 Number of fruits on secondary branches

Total number of fruits borne on secondary branches in a plant was recorded.

3.3.4.3.6 Number of fruits on tertiary branches

Total number of fruits borne on tertiary branches in a plant was recorded.

3.3.4.3.7 Days to last flowering

Total number of days from the date of sowing to the anthesis of last flower was recorded.

3.3.4.3.8 Percentage yield loss due to pruning

Yield loss was calculated based on the yield of control plants and pruned plants and expressed as percentage.

3.3.4.4 Statistical analysis of data

Observations collected were analyzed statistically using MSTAT C package and the results were interpreted.

3.3.5 Experiment 5. Bitterness in relation to age of the plant

3.3.5.1 Layout of the experiment

Bitter and non bitter lines were raised in Randomized Block Design with three replications during January, 2002. The number of plants in each replication was 30. The crop was raised as per the package of practices recommendations of the Kerala Agricultural University (KAU, 2002). The fruits were classified into ten categories based on the number of days it took for setting from the day of sowing (DAS).

3.3.5.2 Fruit categories

The ten fruit categories were as follows.

C_1 - Fruit set between 25-30 DAS

C_2 - Fruit set between 31-35 DAS

C_3 - Fruit set between 36-40 DAS

C_4 - Fruit set between 41-45 DAS

C_5 - Fruit set between 46-50 DAS

C_6 - Fruit set between 51-55 DAS

C_7 - Fruit set between 56-60 DAS

C_8 - Fruit set between 61-65 DAS

C_9 - Fruit set between 66-70 DAS

C_{10} - Fruit set between 71-75 DAS

3.3.5.3 Observations recorded

Following observations were recorded from ten plants per replication and average was taken.

3.3.5.3.1 Fruit bitterness

The fruits formed within each category was evaluated organoleptically for bitterness and results were recorded.

3.3.5.3.2 Number of female flowers

Number of female flowers formed in a plant during each time frame was counted and recorded.

3.3.5.3.3 Number of fruits

Number of fruits formed during each time frame was counted and recorded.

3.3.6 Morphological and biochemical characterization

3.3.6.1 Morphological characters

Morphological characters of seeds, seedlings, plants and fruits were studied, so as to compare bitter and non bitter plants.

3.3.6.1.1 Seed characters

The seeds were taken at random from five different fruits of bitter and non bitter types. The following observations were recorded and average was worked out.

3.3.6.1.1.1 Seed length (cm)

Length of seed was measured using vernier calipers and was expressed in centimeters.

3.3.6.1.1.2 Seed breadth (cm)

Breadth of seed was measured at the middle of the seed using vernier calipers and was expressed in centimeters.

3.3.6.1.1.3 Seed thickness (cm)

Thickness was taken at the middle of the seed using vernier calipers and expressed in centimeters.

3.3.6.1.1.4 Hundred seed weight(g)

Hundred seeds from a fruit were counted and its weight was taken.

3.3.6.1.1.5 Seed colour

Colour of mature seeds from fully ripe fruits was visually observed and recorded.

3.3.6.1.1.6 Speed of germination

Speed of germination was calculated using the formula suggested by Maguire (1962).

$$\text{Speed of germination} = \frac{x_1}{y_1} + \frac{x_2 - x_1}{y_2} + \ldots\ldots \frac{x_n - x_{n-1}}{y_n}$$

Where x_n = number of seedlings germinated on n^{th} day

y_n = number of days from sowing to n^{th} day

3.3.6.1.1.7 Germination percentage

Seeds of bitter and non bitter fruits were sown in germination trays and the total number of seeds germinated were counted. Germination percentage was calculated as follows:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

3.3.6.1.1.8 Seed bitterness

Bitterness of the dried mature seed was evaluated organoleptically and classified into bitter and non bitter.

3.3.6.1.2 Seedling characters

Bitter and non bitter seeds were sown separately in trays containing potting mixture. Observations from the seedlings were taken at random after five days of germination and average was worked out.

3.3.6.1.2.1 Hypocotyl length (cm)

Length of the hypocotyl from the base of the cotyledon to the collar region was measured and expressed in centimeters.

3.3.6.1.2.2 Radicle length (cm)

Length of the radicle was measured from the collar region to the tip of the root and was expressed in centimeters.

3.3.6.1.2.3 Cotyledonary leaf shape

Cotyledonary leaf shape was observed visually and recorded.

3.3.6.1.2.4 Cotyledonary leaf apex shape

Shape of cotyledonary leaf apex was observed visually and recorded.

3.3.6.1.2.5 Hypocotyl hairiness

Hairiness of the hypocotyl was observed using a hand lens and classified into glabrous, sub glabrous or pubescent.

3.3.6.1.2.6 Pigmentation

The hypocotyl was observed for pigmentation and was classified into strong, moderate or weak.

3.3.6.1.2.7 Cotyledonary leaf bitterness

The cotyledonary leaf was evaluated for bitterness organoleptically and classified into bitter and non bitter.

3.3.6.1.3 Plant and fruit characters

The plant and fruit characters were recorded for bitter and non bitter plants, as described in 3.3.1.

3.3.6.2 Biochemical parameters

The following biochemical parameters were estimated separately for bitter and non bitter fruits at different stages of maturity. Estimation of total phenol, total free amino acids, cucurbitacin and polyphenol oxidase activity were conducted for fruits at 5^{th},15^{th} and 25^{th} days after fruit set. Samples were taken from rind, flesh and placenta of fruits and analyzed separately for stalk end middle and blossom end of fruit. Banding patterns of seed protein were also analyzed for bitter and non bitter seeds.

3.3.6.2.1 Total phenol

The total phenol content was estimated using Folin-Ciocalteau method suggested by Bray and Thorpe (1954). Two gram fresh sample was ground well with a mortar and pestle along with 10 ml of 80 per cent ethanol. The homogenate was shaken well and the supernatant collected and evaporated to dryness. The residue was dissolved in 10 ml of distilled water. From this, one ml was made up to three ml with distilled water. To this, 0.5 ml of Folin-Ceocalteau reagent was added, followed by two ml of 20 per cent Na_2CO_3 solution after three minutes. After thorough mixing, the solution was kept in boiling water bath for one minute for proper blue colour development. Absorbance was measured at 650 nm against a reagent blank. The total phenol content was calculated from the standard curve prepared using catechol and was expressed as mg g^{-1} of fresh weight sample.

$$\text{Total phenol (mg g}^{-1}\text{ of sample)} = \frac{\text{Factor x Absorbance x Volume to which made up(ml)}}{\text{Weight of sample (g) x Aliquot taken (ml)}}$$

3.3.6.2.2 Total free aminoacids

Total free aminoacid was estimated by following the method suggested by Bray and Thorpe (1954).

Ten grams fresh sample was ground using a mortar and pestle. To this 10 ml of 80 per cent methanol was added. Then the filtrate was collected. To 0.5 ml of this filtrate, 1 ml of ninhydrin reagent was added and the volume made up to two ml with distilled water. The reaction mixture was kept in boiling water bath for 20 minutes. Five ml of diluent solvent was added immediately and the contents were mixed well. After 15 minutes, the intensity of purple colour was read against a reagent blank in a colorimeter at 570 nm. Standard curve was prepared using different concentrations of leucine and the amino acid content was expressed as percentage equivalent of leucine.

3.3.6.2.3 Cucurbitacin

Cucurbitacin was estimated colorimetrically and chromatographic studies were conducted for the separation of different components

3.3.6.2.3.1 Colorimetric method

Cucurbitacin content was estimated as per the procedure suggested by Rehm (1957).

Twenty gram fresh sample was ground well using a mortar and pestle. To this 20 ml of saturated lead acetate and 20 ml of methanol were added and mixed thoroughly. The supernatant was collected and 25 per cent KH_2PO_4 was added drop by drop to precipitate excess lead acetate. The solution was filtered and extracted with chloroform. Extraction was repeated thrice, each with 100 ml of chloroform. The chloroform extracts were collected and evaporated to dryness at 60°C in a water bath, to get cucurbitacin. The residue was dissolved in 10 ml distilled water. To this 2.5 ml of Folin-Ciocalteau reagent was added followed by 10 ml of 20 per cent Na_2CO_3 after three minutes. This solution was kept in boiling water bath for one minute. The blue colour developed was measured at 650 nm against a reagent blank using a colorimeter. The standard curve was prepared using extract of cucurbitacin at

different concentrations. Cucurbitacin content was expressed as units of cucurbitacin using the values obtained from the graph.

3.3.6.2.3.2 Thin Layer Chromatography (TLC)

The chloroform extract of cucurbitacin was evaporated and the residue was dissolved in one ml of distilled water. This sample was applied on a precoated TLC Aluminium sheet of size 20 cm x 20 cm x 0.25 mm (silica 60 F_{254}) by means of a microlitre syringe @ 20 µl per sample per spot. The plates were developed using chloroform : acetic acid (1:1 v/v) solvent. The chromatograms were observed in a uv chamber at 366 nm and Rf value for the spots were calculated using the formula

$$Rf = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent}}$$

3.3.6.2.4 Poly phenol oxidase activity

Poly phenol oxidase activity was assayed by the method suggested by Malick and Singh (1980).

Enzyme extract was prepared by grinding two grams fresh sample in three ml of phosphate buffer (pH-6) using a mortar and pestle. The homogenate was centrifuged at 18,000 rpm for 15 minutes and the supernatant was used for the assay. Freshly prepared buffered catechol, 0.01 M (0.11 g catechol dissolved in 100 ml of phosphate buffer, pH 6.0) was used as the substrate. Three ml of phosphate buffer was taken in a cuvette and spectrophotometer reading was adjusted to zero at 495 nm. Then 0.5 ml of enzyme extract was added to it and absorbance was noted as the blank reading. To a cleaned cuvette, three ml of buffered catechol and 0.5 ml of enzyme extract was taken, mixed thoroughly and inserted immediately in to the spectrophotometer. The change in absorbance for every 30 seconds interval was recorded and plotted on a graph and the linear phase of the curve was taken for the study.

3.3.6.2.5 Seed protein

Polyacrylamide gel electrophoresis using Hoefer Mighty Small TM II gel system was used for comparing seed proteins of bitter and non bitter seeds. Acrylamide monomers were polymerized with N–N methylene bis acrylamide to obtain the gel. Freshly prepared ammonium persulphate acted as catalyst and N,N,N'N' – tetramethyl ethylene diamine (TEMED) as chain initiator.

3.3.6.2.5.1 Preparation of stock solution

For preparation of the gel, the following stock solutions were prepared.

1) Monomer stock solution (30%)

Acrylamide 29.2 ml

Bis acrylamide 0.8 ml

Volume made up to 100 ml with distilled water

2) 4 x Resolving gel buffer (1.5 M tris HCl, pH 8.8)

Trisbase - 36.8 g

pH adjusted to 8.8 with 0.1 N HCl and made up to 200 ml with distilled water.

3) 4 x stacking gel buffer (0.5 M tris – HCl, pH 6.8)

Trisbase 6 g

The base was dissolved in 50 ml water and pH adjusted to 6.8 with O.1N HCl and then volume made up to 100 ml.

4) Initiator - 10% Ammonium persulphate (APS)

Ammonium Persulphate – 0.1g

APS was dissolved in distilled water and volume made up to 1 ml with distilled water.

5) Treatment buffer

4 x stacking gel buffer	– 5 ml
Glycerol	- 2g
Bromophenol blue 1 % solution	– 1 ml

Volume was made upto 10 ml with distilled water.

6) TEMED (N, N, N^1, N^1 - tetramethyl ethylene diamine)

Buffers and monomers were stored in amber coloured bottles at 4°C. Ammonium persulphate solution was prepared fresh each time.

3.3.6.2.5.2 Preparation of gel

Resolving gel of 7.5% strength was prepared by mixing the following.

Monomer	- 2.7 ml
Resolving buffer	- 2.5 ml
APS	- 100 µl
TEMED	- 10 µl
Distilled water	- 4.69 ml.

Gel solution was poured gently into the chamber between the glass plate and alumina sheet mounted on the stand, using a micropipette. Three fourth of the slab was filled with resolving gel and remaining part with distilled water and allowed to polymerise.

After 60 minutes, the distilled water was poured out and stacking gel was poured. Combs were placed on the top to make wells, and the glass plates were mounted in the polymerization stand. The stacking gel was prepared by mixing the following chemicals.

Monomer	- 0.7 ml
Stacking buffer	- 1.25 ml
APS	- 50 µl
TEMED	- 10 µl
Distilled water	- 3.0 ml

3.3.6.2.5.3 Electrophoretic run

After polymerization, the comb was removed and gel installed in electrophoresis apparatus. It was then filled with electrode buffer and connected to

electrical circuit. The apparatus was kept in a cooled condition. Electrode buffer was prepared by mixing the following.

Trisbase	- 1.52 g
Glycine	- 7.2 g
Distilled water	- 500 ml

The prepared sample was mixed with treatment buffer in the ratio 1:1. Fifteen µl each of the sample was loaded into each well using a micropipette.

A constant current of 7 mA was maintained till the end of running.

3.3.6.2.5.4 Preparation of sample

Half a gram of sample was weighed after the removal of seed coat. The sample was ground well using a mortar and pestle along with extraction buffer (0.1 M Tris – HCl, pH 7.6). The sample and extraction buffer were mixed in the ratio 1 : 3. The homogenized sample was centrifuged at 10,000 rpm at 5°C for 20 minutes. The supernatant was taken in vials and stored at subzero temperature.

3.3.6.2.5.5 Staining of gel

After running, the gels were immersed in the staining solution and placed on the shaker and kept overnight. Staining solution was prepared by mixing the following chemicals.

Methanol	- 50 ml
Acetic acid	- 40 ml
Distilled water	- 100 ml
Coomasie brilliant blue	- 0.20 g

3.3.6.2.5.6 Destaining of gel

After proper staining, the gel was distained by placing it in distaining solution until the protein bands became viable. Destaining solution was prepared by mixing the following chemicals.

Methanol - 5 ml

Acetic acid - 4 ml

Water - 100 ml

The gel was then photographed and documented.

3.3.7 Pest and disease incidence

Occurrence of major pests and diseases in relation to bitterness were observed and recorded.

3.3.8 Weather parameters

The incidence of bitterness in relation to weather conditions was observed and recorded. Weather parameters during the period of study were collected from the meteorological observatory of the College of Horticulture, Vellanikkara and is furnished in Appendix-II.

Results

4. RESULTS

Oriental pickling melon, one of the most popular cucurbitaceous vegetable of Kerala often suffers difficulty in marketing because of bitter fruits getting mixed in the lot of non bitter types. This being a serious problem, investigations were taken up to identify the factors responsible for bitterness and the results obtained are discussed hereunder.

4.1 STUDIES ON INHERITANCE OF BITTERNESS

In order to find out gene action governing bitterness, bitter (P_1) and non bitter (P_2) parental lines along with F_1, F_2, BC_1, BC_2 (Plate 2) and their reciprocals were evaluated and the results obtained are presented below.

4.1.1 Gene action

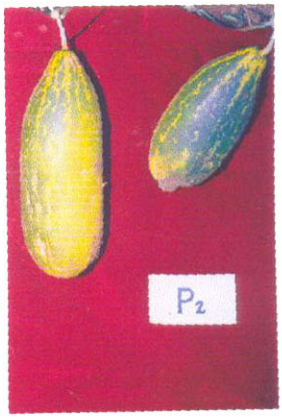
The individual plants of P_1, P_2, F_1, F_2, BC_1, BC_2 and their reciprocals were evaluated for fruit bitterness and classified into bitter and non bitter (Tables 2 to 6). All the F_1 hybrids out of the crosses between bitter and non bitter plants were fully bitter. Using χ^2 test, the goodness of fit of observed values of F_2 and back crosses for the monohybrid ratio 3:1 was tested (Tables 2 and 3). The probability of occurrence of values in the ratio 3:1 was 30-50 per cent and 20-30 per cent for F_2 of P_1xP_2 and P_2xP_1 respectively. Thus it was concluded that the population does not fit well in the 3:1 ratio.

As the next step, fitness of values for the dihybrid ratio for inhibitory gene action, 13:3, was tested and probability values of 80 per cent and 90-95 per cent were obtained for F_2 and its reciprocal (Tables 4 and 5). Hence it can be seen that the observed values of F_2 fit more appropriately in the ratio 13:3. The segregation pattern of genes as per the ratio 13:3 due to inhibitory gene action is given in Table 7.

χ^2 values for F_1, F_2, BC_1, BC_2 and their reciprocals is given in Table 6. The back cross progeny of F_1 with bitter parent gave rise to plants with only bitter fruits and that with non bitter parents gave rise to plants with both bitter and non bitter fruits in the ratio 1:1 with 80 per cent probability.

Results of scaling test for cucurbitacin and different yield attributes (Table 8) revealed that epistatic effect was nonsignificant for the characters cucurbitacin content, days to first female flower opening, fruit weight and fruit length. For the

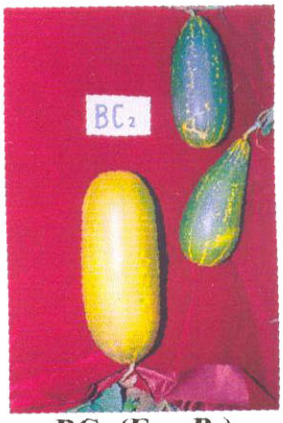
P_1 (Bitter)



P_2 (Non bitter)

F_1 (P_1 x P_2)

BC_1 (F_1 x P_1)



BC_2 (F_1 x P_2)

F_2

Plate 2. Fruits of different generations used in the inheritance study

Table 2. χ^2 test for goodness of fit of F_2 (bitter x non bitter) for the ratio 3:1

Class	Observed No. (O)	Expected No.(E)	d=O-E	$\chi^2 = \frac{d^2}{E}$	Probability range
Bitter	48	45	+3	0.2	30-50%
Non Bitter	12	15	-3	0.6	
Total	60	60		0.8	

Table 3. χ^2 test for goodness of fit of F_2-reciprocal (non bitter x bitter) for the ratio 3:1

Class	Observed No. (O)	Expected No.(E)	d=O-E	$\chi^2 = \frac{d^2}{E}$	Probability range
Bitter	49	45	+4	0.36	20-30%
Non Bitter	11	15	-4	1.06	
Total	60	60		1.42	

Table 4. χ^2 test for goodness of fit of F_2 (bitter x non bitter) for the ratio 13:3

Class	Observed No. (O)	Expected No.(E)	d=O-E	$\chi^2 = \frac{d^2}{E}$	Probability range
Bitter	48	48.75	-0.75	0.01	80%
Non Bitter	12	11.25	+0.75	0.05	
Total	60	60		0.06	

Table 5. χ^2 test for goodness of fit of F_2 - reciprocal (non bitter x bitter) for the ratio 13:3

Class	Observed No. (O)	Expected No.(E)	D=O-E	$\chi^2 = \frac{d^2}{E}$	Probability range
Bitter	49	48.75	+0.25	0.001	90-95%
Non Bitter	11	11.25	-0.25	0.006	
Total	60	60		0.007	

Table 6. Ratio of bitter and non bitter forms of oriental pickling melon in F_1, F_2, BC_1, BC_2 and their reciprocal generations

Generation	Sample size	Bitter frequency	Non bitter frequency	Expected ratio	χ^2 value	Probability (%)
F_1 (bitter x non bitter)	60	60	-			
Reciprocal of F_1 (F_1')	60	60	-			
F_2 (bitter x non bitter)	60	48	12	13:3	0.06	80
Reciprocal of F_2 (F_2')	60	49	11	13:3	0.007	90-95
BC_1 (F_1 x bitter)	60	60	-			
BC_2 (F_1 x non bitter)	60	29	31	1:1	0.06	80
BC_1 (F_1' x non bitter)	60	29	31	1:1	0.06	80
BC_2' (F_1' x bitter)	60	60	-			

Table 7. Segregation pattern of genes controlling bitterness

nn II (Bitter parent) x NNii (Non bitter parent)

F_1 : NnIi (Bitter)

F2:

Gametes	NI	Ni	nI	ni
NI	NNII (Bitter)	NNIi (Bitter)	NnII (Bitter)	NnIi (Bitter)
Ni	NNIi (Bitter)	NNii (Non bitter)	NnIi (Bitter)	Nnii (Non bitter)
nI	NnII (Bitter)	NnIi (Bitter)	nnII (Bitter)	nnIi (Bitter)
Ni	NnIi (Bitter)	Nnii (Non bitter)	nnIi (Bitter)	nnii (Bitter)

Table 8. Scaling test for epistatic effect

Sl. No.	Characters	Scales			
		A	B	C	D
1	Length of vine (m)	-0.0125	-1.125*	-2.763*	-0.8125*
2	Days to first male flower opening	3.170*	1.338	0.838	-1.835
3	Days to first female flower opening	0.835	-3.830	7.160	5.078
4	Fruit weight (kg)	-0.175	0.075	0.350	0.225
5	Number of fruits	-0.575	-0.365	-5.84*	-2.45*
6	Percentage fruit set	-4.43*	-9.90	-12.08	1.13
7	Fruit length (cm)	1.27	1.13	4.50	1.05
8	Fruit circumference (cm)	2.30*	0.25	1.75	-0.40
9	Yield (kg)	-1.49	-0.25	-2.54*	-0.40
10	Cucurbitacin content (Units g^{-1})	1.10	0.000	3.30	1.10

* Significant at 5% level

character length of vine, scales B, C and D were significant, indicating the presence of all the three types of non allelic gene interactions. The scale A alone was significant for days to first male flower opening, percentage fruitset and fruit circumference. For the character number of fruits per plant, scales C and D were significant and for fruit yield, scale C was significant. The significance of scale C suggests dominance x dominance, type of non allelic gene interaction and that of scale D reveals additive x additive type of gene interaction.

Gene action for different characters obtained through six-parameter model is given in Table 9. Significant positive additive (0.88), dominance (2.09), additive x additive (1.63) and additive x dominance (0.56) effect was noticed for vine length. High negative dominance x dominance (-8.17) interaction was observed for days to first male flower opening. Significant additive effect was noted for the characters fruit weight (-0.28), fruit set percentage (7.13), fruit circumference (1.60) and cucurbitacin content (33.0). In all these cases, interaction effects were nonsignificant. Number of fruits registered significantly high dominance (6.28) and additive x additive effect (4.90).

4.1.2 Correlation between cucurbitacin content and different characters

Result of genotypic and phenotypic correlation among different characters under study is presented in Table 10.

Cucurbitacin content showed significant positive correlation, both genotypic and phenotypic, with the character vine length (r_g = 0.76 and r_p = 0.63), number of fruits per plant (r_g = 0.89 and r_p = 0.64) and fruit set percentage (r_g = 0.93 and r_p = 0.66). Genotypic correlation was found to be significant and positive for fruit circumference with cucurbitacin content (r_g = 0.90). Significant and negative correlation was observed for the characters days to first female flower (r_g = -0.84), fruit weight (r_g = -0.99, r_p = -0.54) and fruit length (r_g = -1.09, r_p = -0.63) with cucurbitacin content.

Table 9. Gene action for different characters in OPM

Sl. No.	Characters	m mean	d additive	h dominance	i additive x additive	j additive x dominance	l dominance x dominance
1	Length of vine (m)	2.21	0.88*	2.09*	1.63*	0.56*	-0.49
2	Days to first male flower opening	30.25	0.50	4.41	3.66	0.91	-8.17*
3	Days to first female flower opening	38.33	0.25	-10.74	-10.14	2.33	13.15
4	Fruit weight (kg)	1.04	-0.28*	-0.53	-0.45	-0.13	0.55
5	Number of fruits plant^{-1}	5.83	0.85	6.28*	4.90*	-0.10	-3.96
6	Percentage fruit set	53.65	7.13*	1.36	-2.25	2.74	16.57
7	Fruit length (cm)	29.97	-1.85	-3.10	-2.10	0.07	-0.30
8	Fruit circumference (cm)	28.87	1.60*	2.42	0.79	1.03	-3.35
9	Yield (kg plant^{-1})	5.97	-0.55	1.13	0.80	-0.62	0.94
10	Cucurbitacin (Units g^{-1})	81.4	33.00*	28.04	-2.20	0.55	1.10

* Significant at 5% level

Table 10. Phenotypic and genotypic correlations for different characters

Characters	Length of vine (m)	Days to first male flower opening	Days to first female flower opening	Fruit weight (kg)	Number of fruits plant^{-1}	Percentage fruit set	Fruit length (cm)	Fruit circumference (cm)	Yield (kg plant^{-1})	Cucurbitacin (units g^{-1})
Length of vine (m)	**1.00**	-0.02	0.68*	-0.65*	0.71*	0.63*	-0.70*	-0.47	0.03	0.63*
Days to first male flower opening	-0.12	**1.00**	-0.27	0.12	0.16	0.40	0.24	-0.19	0.15	0.16
Days to first female flower opening	-0.72*	-0.08	**1.00**	-0.53*	0.35	0.35	-0.51	-0.16	0.11	0.23
Fruit weight (kg)	-1.01**	-0.29	0.83**	**1.00**	-0.50*	-0.55*	0.66*	0.33	0.27	-0.54*
No. of fruits plant^{-1}	1.02**	0.08	-0.97**	-0.91**	**1.00**	0.50	-0.47	-0.18	-0.04	0.64*
Percentage fruit set	1.04**	-0.47	-0.82*	-1.09**	0.99**	**1.00**	-0.74*	-0.65*	0.07	0.66*
Fruit length (cm)	-1.09**	0.18	1.20**	1.13**	-1.13**	-1.28**	**1.00**	0.60*	0.14	-0.63*
Fruit circumference (cm)	0.71*	0.91*	-0.59	-0.86**	0.77*	0.67	-0.61	**1.00**	0.07	-0.39
Yield (kg plant^{-1})	0.44	-0.65	-0.58	-0.10	0.50	0.27	-0.81	-0.51	**1.00**	0.09
Cucurbitacin (units g^{-1})	0.76**	0.05	-0.84**	-0.99**	0.89**	0.93**	-1.09**	0.90**	-0.08	**1.00**

Figures in upper diagonal indicate phenotypic correlation values and lower diagonal indicate genotypic correlation values

* Significant at 5% level

** Significant at 1% level

Among different yield and yield contributing factors, significant positive genotypic correlation was observed between fruit weight and days to first female flower anthesis (r_g = 0.83); fruit weight and fruit length (rg = 0.66); number of fruits and vine length (r_g = 1.02); percentage fruit set and vine length (r_g = 1.04); percentage fruit set and number of fruits (r_g = 0.99); fruit length and days to first female flower (r_g = 1.20); fruit length and fruit weight (r_g = 1.13); fruit circumference and vine length (r_g = 0.71); fruit circumference and days to first male flower opening (r_g = 0.91) and fruit circumference and number of fruits (r_g = 0.77). Significant negative genotypic correlation was observed for vine length and days to first female flower (r_g = -0.72). vine length and fruit weight (r_g = -1.01), percentage fruit set and days to first female flower (rg = -0.82). Percentage fruit set and fruit weight (r_g = -1.09), fruit length and vine length (r_g = -1.09), fruit length and number of fruits (r_g = -1.13), fruit length and percentage fruit set (r_g = -1.28) fruit circumference and fruit weight (r_g = -0.86).

On analyzing the phenotypic correlation value, it can be seen that vine length is significantly and positively correlated with days to first female flower (r_p = 0.68), number of fruits (r_p = 0.71), and percentage fruit set (r_p = 0.63). Significant negative correlation between vine length and the characters, fruit weight (r_p = -0.65) and fruit length (r_p = -0.70) were observed. Positive and significant phenotypic correlation was recorded for fruit weight and fruit length (r_p = 0.66); and fruit length and fruit circumference (r_p = 0.60). However the character fruit weight was significantly and negatively correlated with days to first female flower (r_p = -0.53), number of fruits (r_p = -0.50) and fruit set percentage (r_p = -0.55). Significant negative correlation was also observed for the character percentage fruit set with fruit length (r_p = -0.74) and fruit circumference (r_p = -0.65).

4.1.3 Direct and indirect effect of different characters on bitterness

High positive direct effect was noticed for the characters (Table 11 and Fig.1) length of vine (0.72) and days to first male flower (0.64) on cucurbitacin content. However the characters fruit weight (-1.42), number of fruits (-0.70), fruit set percentage (-0.49), fruit length (-0.43) and fruit circumference (- 0.94) exerted high direct effect, which was negative.

Table 11. Direct and indirect effect of different characters on fruit bitterness

Characters	Length of Vine (m)	Days to first male flower opening	Days to first Female flower opening	Fruit weight (kg)	Number of fruits plant^{-1}	Percentage fruit set	Fruit length (cm)	Fruit circumference (cm)	Yield (kg plant^{-1})
Length of vine (m)	**0.72**	-0.07	0.15	1.43	-0.72	-0.52	0.45	-0.70	-0.03
Days to first male flower opening	-0.09	**0.64**	0.01	0.39	-0.05	0.22	-0.06	-0.88	-0.12
Days to first female flower opening	-0.50	-0.03	**-0.22**	-1.15	0.68	0.38	-0.50	0.55	-0.04
Fruit weight (kg)	-0.72	-0.17	-0.18	**-1.42**	0.64	0.53	-0.49	-0.83	0.00
Number of fruits plant^{-1}	0.73	0.04	0.22	1.30	**-0.70**	-0.48	0.49	-0.76	0.03
Percentage fruit set	0.76	-0.29	0.17	1.55	-0.68	**-0.49**	0.53	-0.64	-0.02
Fruit length (cm)	-0.75	0.09	-0.26	-1.62	0.80	0.60	**-0.43**	0.57	-0.05
Fruit circumference (cm)	0.53	0.60	0.13	1.25	-0.56	-0.33	0.26	**-0.94**	0.04
Yield (kg plant^{-1})	0.37	-1.20	0.13	0.09	-0.32	-0.16	0.35	0.59	**0.06**

0.0 to 0.09 - negligible 0.1 to 0.19 - low 0.2 to 0.29 - moderate 0.3 to 0.98 - high

Residual effect : 0.306

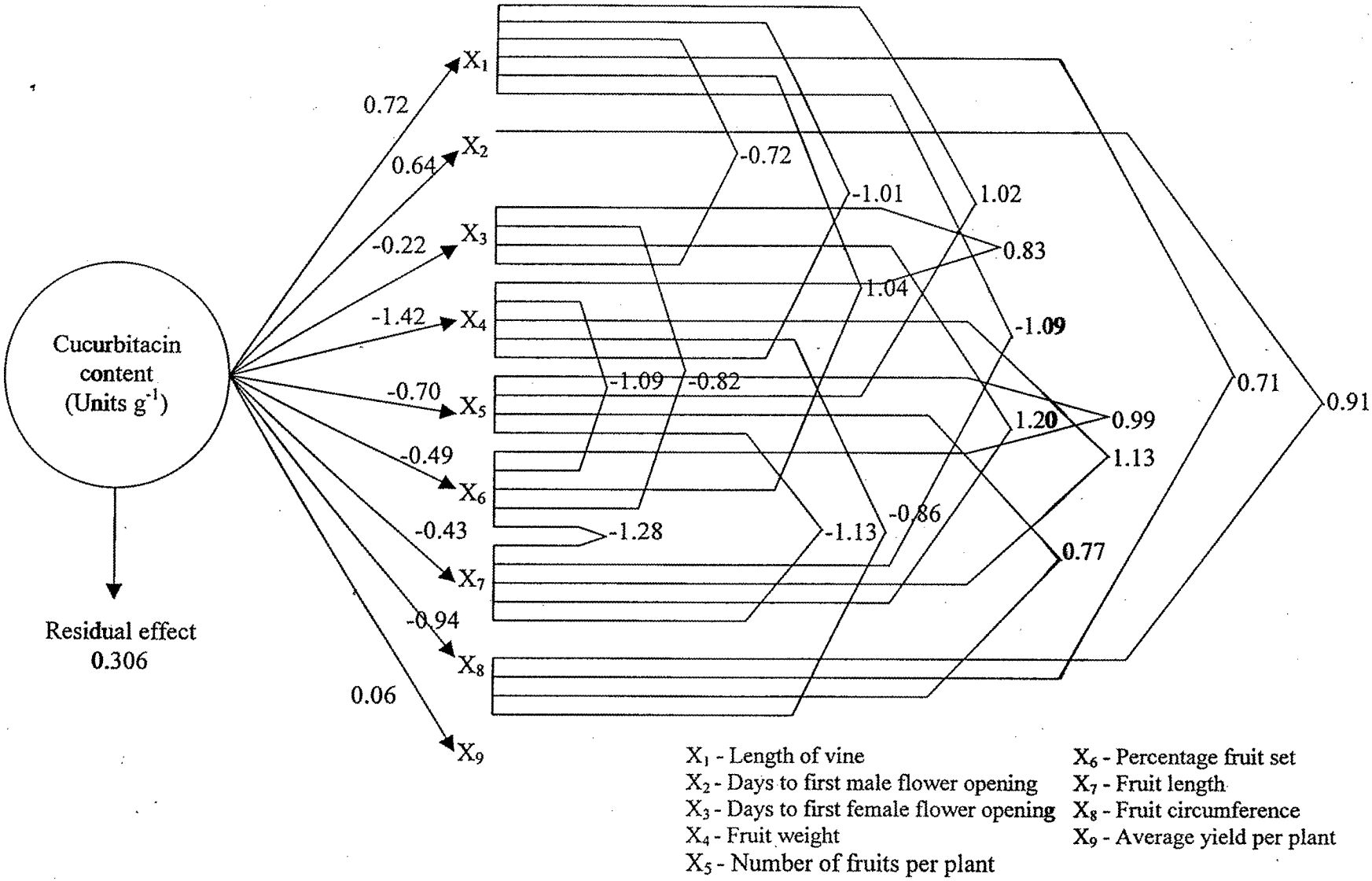


Fig. 1. Path diagram showing effect of different characters on cucurbitacin content

With respect to indirect effect, most of the characters influenced cucurbitacin content through length of vine, fruit weight, number of fruits, percentage fruit set, fruit length and fruit circumference.

4.1.4 Variability among different characters

The phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability, expected genetic advance as percentage of mean and inbreeding depression are presented in Table 12 and Figs.2 to 4.

High PCV and GCV values were recorded for cucurbitacin content (32.89 and 32.83 respectively). PCV value was moderate for length of vine (19.72), fruit weight (17.98) and number of fruits (17.94). The characters days to first male flower, days to first female flower, percentage fruit set, fruit length, fruit circumference and yield registered low PCV and GCV values. GCV values were moderate for length of vine (16.31), fruit weight (12.87) and number of fruits (12.67).

The cucurbitacin content recorded the highest heritability (99), followed by length of vine (68). Moderate heritability was noted for percentage fruit set (55), fruit circumference (52), fruit weight (51), number of fruits (49), fruit length (43), days to first male flower (37) and days to first female flower (33). Very low heritability value was observed for yield per plant (5). Genetic advance was low for all the characters studied except for cucurbitacin content (52.86).Inbreeding depression values ranged from 0.12 (fruit weight) to 14.30 (cucurbitacin content).

4.2 EFFECT OF FOREIGN POLLEN ON INDUCTION OF BITTERNESS

Pollen from nine different cucurbits was mixed as per the treatments and controlled pollination was done to know its effect on bitterness of fruits in oriental pickling melon. The possibility of gene flow from wild to cultivated species was also studied.

4.2.1 Pollen count

Pollen count of the nine cucurbit species used in the study is given in Table 13, based on which mixing of pollen was done. Highest pollen count was observed for

Table 12. Heritability, genetic advance, coefficients of variation and inbreeding depression for cucurbitacin content and yield attributes

Sl. No.	Characters	Heritability (%)	Genetic advance	Genotypic Coefficient of Variation	Phenotypic Coefficient of Variation	Inbreeding depression (%)
1	Length of vine (m)	68	0.74	16.31	19.72	0.92
2	Days to first male flower opening	37	0.78	2.05	3.35	0.17
3	Days to first female flower opening	33	1.58	3.62	6.29	2.08
4	Fruit weight (kg)	51	0.18	12.87	17.98	0.12
5	Number of fruits plant^{-1}	49	1.26	12.67	17.94	2.15
6	Percentage fruit set	55	5.75	6.86	9.22	4.82
7	Fruit length (cm)	43	1.70	4.29	6.54	1.63
8	Fruit circumference (cm)	52	1.33	3.10	4.26	0.38
9	Yield (kg plant^{-1})	05	0.07	2.36	9.74	0.80
10	Cucurbitacin (Units g^{-1})	99	52.86	32.83	32.89	14.30

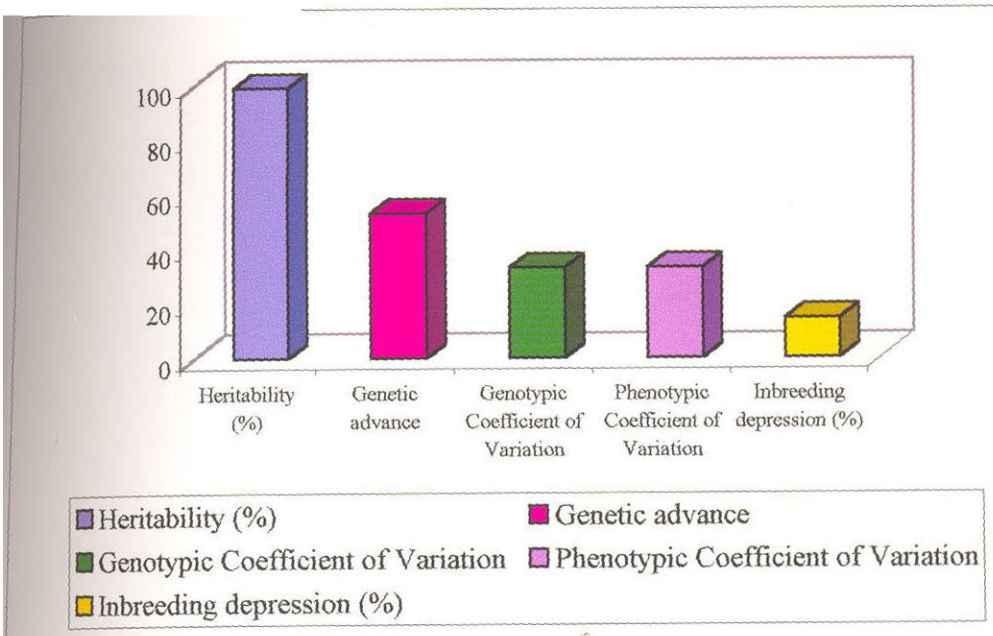


Fig. 2. Variability, heritability and inbreeding depression for cucurbitacin

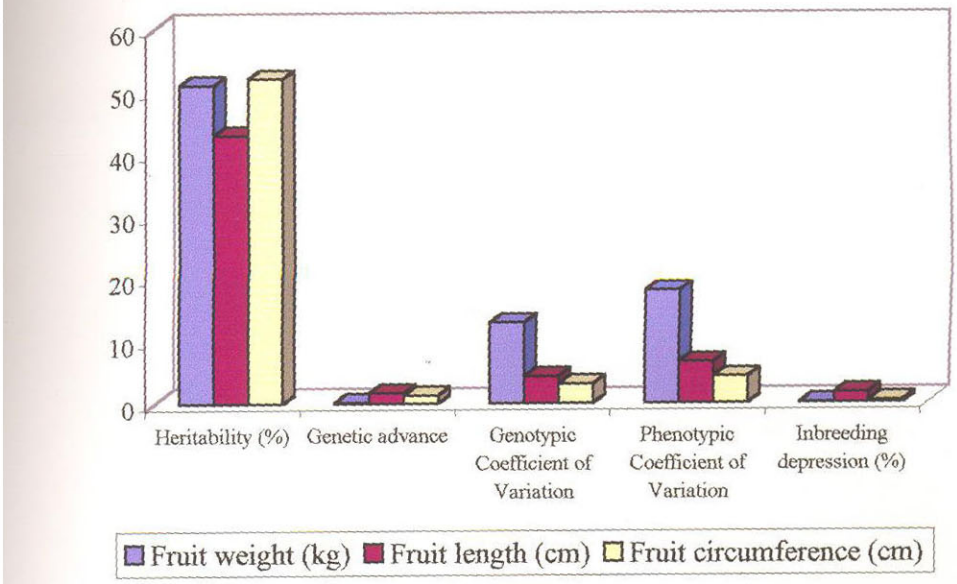


Fig. 3. Variability, heritability and inbreeding depression for fruit characters

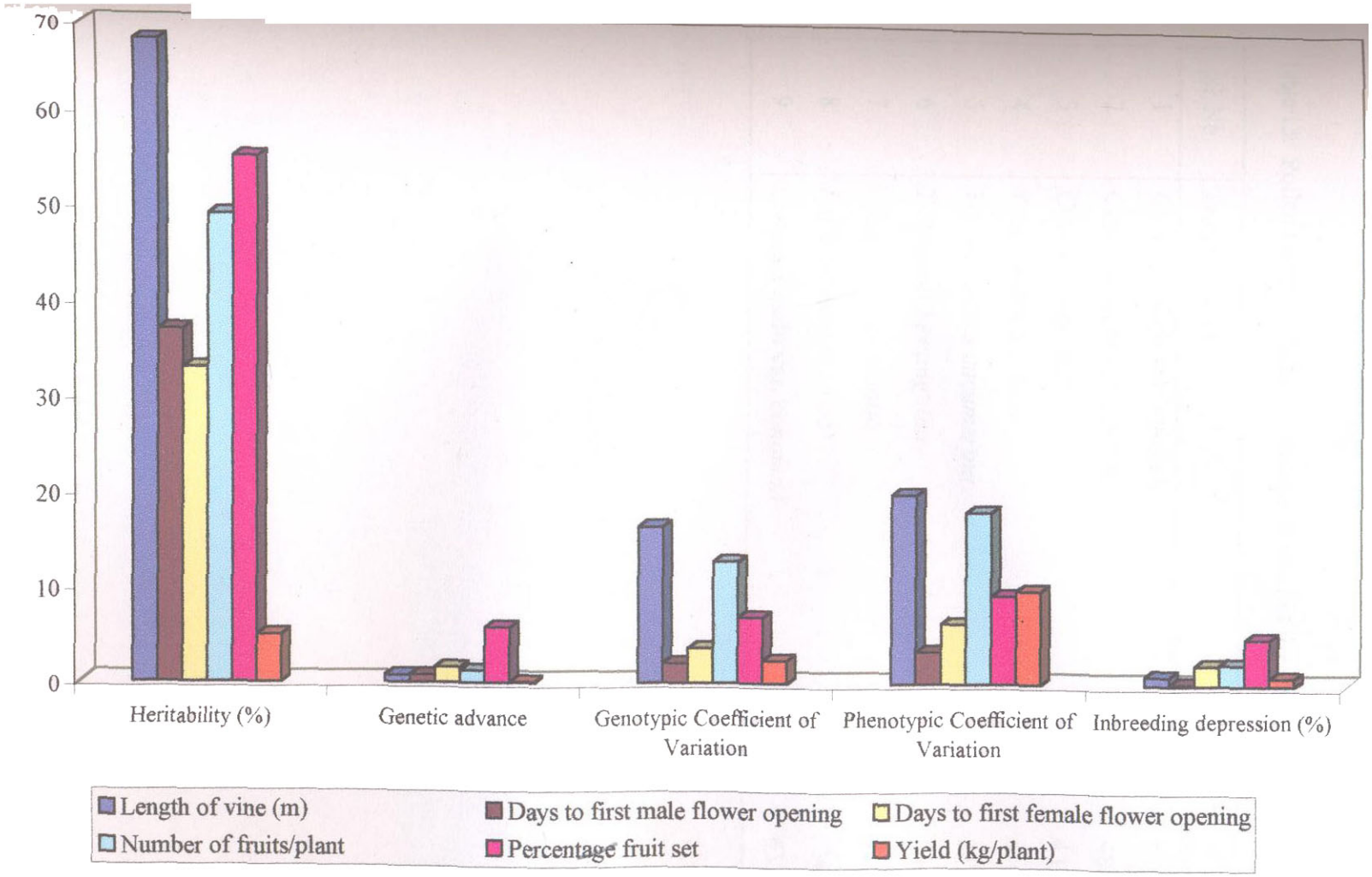


Fig. 4. Variability, heritability and inbreeding depression for plant characters

Table 13. Pollen count of different cucurbits used in the study

Sl. No.	Pollen source	No. of pollen/ flower
1	*Cucumis melo* var. *agrestis*	5200
2	*Cucumis melo* var. *callosus*	4800
3	*Cucumis trigonus*	4320
4	*Trichosanthes lobata*	4100
5	*Trichosanthes cucumerina*	3020
6	*Trichosanthes anguina*	2080
7	*Momordica charantia*	2400
8	*Luffa cylindrica* (wild)	12400
9	*Cucumis melo* var. *conomon*	6320

Luffa cylindrica (12,400 flower^{-1}) and lowest for *Trichosanthes anguina* (2080 flowers^{-1}).

4.2.2 Effect of mixed pollination on fruit set

Effect of pollination, as per the different treatment combinations, on fruit set is summarized in Table 14. The highest fruit set was recorded for T_2 (84.46%). Among the different species,when used alone, only *C. melo* var. *callosus* gave fruit set, with an average of 40 percentage (Table 15). The treatments T_3, T_9, T_{12}, T_{15}, T_{18}, T_{21}, and T_{24} did not set fruits at all.

4.2.3 Effect of mixed pollination on fruit bitterness of F_0

All the treatments, which gave fruit set, had non bitter fruits and the cucurbitacin content ranged from 0.145 units g^{-1} (T_8) to 0.160 units g^{-1} (T_{22}) (Table 14).

4.2.4 Effect of mixed pollination on other fruit characters of F_0

The various fruit characters under the different treatments are presented in Table 16.

Significant differences among the treatments were observed for the fruit characters viz. fruit length, fruit circumference, fruit weight and number of seeds per fruit.The fruit length ranged from 30.5 cm (T_{11}, T_{22} and T_{25}) to 23.40 cm (T_6). The treatment T_{25} recorded the highest fruit circumference (27.5 cm) and treatment T_{19} recorded the lowest (20.60 cm). Fruit weight was maximum for T_{20} and T_5 (1.33 kg) and minimum for T_6 (0.80 kg). Number of seeds per fruit ranged from 433.4 (T_6) to 628.4 (T_2).

4.2.5 Evaluation of F_1 of the cross involving foreign pollen

The results of evaluation of fruit bitterness in F_1 generation is presented in Table 17. All the treatments in which fruits were set, resulted in F_1 plants with non bitter fruits, except for T_6, T_7 and T_8. F_1 of T_6 produced bitter fruited plants, while that

Table 14. Fruit set and fruit quality of F_0 generation in different pollen combinations

Treatments	No. of flowers pollinated	No. of fruits set	Fruit set %	Fruit quality (B/NB)	Cucurbitacin (units g^{-1})
T_1	15	12.33	82.20	NB	0.156
T_2	15	12.67	84.46	NB	0.150
T_3	15	00.00	0.00	-	-
T_4	15	12.33	82.20	NB	0.155
T_5	15	10.00	66.60	NB	0.148
T_6	15	6.00	40.00	NB	0.156
T_7	15	12.33	82.20	NB	0.149
T_8	15	11.67	77.80	NB	0.145
T_9	15	00.00	0.00	-	-
T_{10}	15	10.67	71.33	NB	0.148
T_{11}	15	9.33	62.20	NB	0.151
T_{12}	15	00.00	0.00	-	-
T_{13}	15	9.67	64.47	NB	0.150
T_{14}	15	9.00	60.00	NB	0.151
T_{15}	15	00.00	0.00	-	-
T_{16}	15	10.00	66.60	NB	0.155
T_{17}	15	9.00	60.00	NB	0.149
T_{18}	15	00.00	0.00	-	-
T_{19}	15	11.33	75.53	NB	0.151
T_{20}	15	10.00	66.60	NB	0.154
T_{21}	15	00.00	0.00	-	
T_{22}	15	11.33	75.53	NB	0.160
T_{23}	15	9.67	64.46	NB	0.155
T_{24}	15	00.00	0.00	-	
T_{25}	15	11.33	75.53	NB	0.158
T_{26}	15	10.00	80.00	NB	0.154

T_1 - 100% pollen from non bitter oriental pickling melon (OPM); T_2 - 100% pollen from bitter OPM; T_3 - 100% pollen from *Cucumis melo* var.*agrestis;* T_4 - 90% pollen from non bitter OPM and 10% from *Cucumis melo* var. *agrestis;* T_5 - 75% pollen from non bitter OPM and 25% from *Cucumis melo* var. *agrestis;* T_6 - 100% pollen from *Cucumis melo* var. *callosus;* T_7 - 90% pollen from non bitter OPM and 10% from *Cucumis melo* var. *callosus;* T_8 - 75% pollen from non bitter OPM and 25% from *Cucumis* melo var. *callosus;* T_9 - 100% pollen from *Cucumis trigonus;* T_{10} - 90% pollen from non bitter OPM and 10% from *Cucumis trigonus;* T_{11} - 75% pollen from non bitter OPM and 25% from *Cucumis trigonus;* T_{12} - 100% pollen from *Trichosanthes lobata;* T_{13} - 90% pollen from non bitter OPM and10% from *Trichosanthes lobata;* T_{14} - 75% pollen from non bitter OPM and 25% from *Trichosanthes lobata;* T_{15} - 100% pollen from *Trichosanthes cucumerina;* T_{16} - 90% pollen from non bitter OPM and 10% from *Trichosanthes cucumerina;* T_{17} - 75% pollen from non bitter OPM and 25% from *Trichosanthes cucumerina;* T_{18} - 100% pollen from *Trichosanthes anguina;* T_{19} - 90% pollen from non bitter OPM and 10% from *Trichosanthes anguina;* T_{20} - 75% pollen from non bitter OPM and 25% from *Trichosanthes anguina;* T_{21} - 100% pollen from *Momordica charantia;* T_{22} - 90% pollen from non bitter OPM and 10% from *Momordica charantia;* T_{23} -75% pollen from non bitter OPM and 25% from *Momordica charantia;* T_{24} - 100% pollen from *Luffa cylindrica* (wild); T_{25} - 90% pollen from non bitter OPM and 10% from *Luffa cylindrica* (wild); T_{26} - 75% pollen from non bitter OPM and 25% from *Luffa cylindrica* (wild)

Table 15. Crossability between oriental pickling melon and other cucurbits.

Sl. No.	Pollen source	Fruit set Percentage	Fruit quality of F_0 (B/NB)	Fruit quality of F_1 (B/NB)
1	***Cucumis melo* var. *agrestis*** (bitter)	**0.00**	-	-
2	*Cucumis melo* var. *callosus* (bitter)	40.00	NB	B
3	*Cucumis trigonus* (bitter)	0.00	-	-
4	*Trichosanthes lobata* (bitter)	0.00	-	-
5	*Trichosanthes cucumerina* (bitter)	0.00	-	-
6	*Trichosanthes anguina* (non bitter)	0.00	-	-
7	*Momordica charantia* (bitter)	0.00	-	-
8	*Luffa cylindrica* (wild smoothgourd) (bitter)	0.00	-	-

Table 16. Fruit characters of F_0 in different pollen combinations

Treatments	Fruit length (cm)	Fruit circumference (cm)	Fruit weight (kg)	No. of seeds per fruit
T_1	30.43	25.33	1.23	598.6
T_2	29.30	25.43	1.25	628.4
T_3	+	+	+	+
T_4	27.36	23.50	1.20	612.0
T_5	28.50	24.43	1.33	588.2
T_6	23.40	25.83	0.800	433.4
T_7	30.43	25.50	1.000	584.5
T_8	27.00	25.30	0.85	498.2
T_9	+	+	+	+
T_{10}	29.36	24.40	1.05	619.6
T_{11}	30.50	24.36	1.00	599.2
T_{12}	+	+	+	+
T_{13}	30.36	26.46	1.10	612.4
T_{14}	28.50	25.43	1.00	606.3
T_{15}	+	+	+	+
T_{16}	30.46	24.50	1.15	614.0
T_{17}	29.40	26.50	1.05	590.6
T_{18}	+	+	+	+
T_{19}	24.40	20.60	0.933	511.2
T_{20}	28.36	24.46	1.33	498.5
T_{21}	+	+	+	+
T_{22}	30.50	25.30	1.00	598.6
T_{23}	30.00	25.50	0.90	581.4
T_{24}	+	+	+	+
T_{25}	30.50	27.50	1.15	599.2
T_{26}	26.50	23.46	0.95	538.6
CD	1.33	2.09	0.22	8.22

+ - not considered since no fruit set was obtained; T_1 - 100% pollen from non bitter oriental pickling melon (OPM); T_2 - 100% pollen from bitter OPM; T_3 - 100% pollen from *Cucumis melo* var.*agrestis;* T_4 - 90% pollen from non bitter OPM and 10% from *Cucumis melo* var. *agrestis;* T_5 - 75% pollen from non bitter OPM and 25% from *Cucumis melo* var. *agrestis;* T_6 - 100% pollen from *Cucumis melo* var. *callosus;* T_7 - 90% pollen from non bitter OPM and 10% from *Cucumis melo* var. *callosus;* T_8 - 75% pollen from non bitter OPM and 25% from *Cucumis* melo var. *callosus;* T_9 - 100% pollen from *Cucumis trigonus;* T_{10} - 90% pollen from non bitter OPM and 10% from *Cucumis trigonus;* T_{11} - 75% pollen from non bitter OPM and 25% from *Cucumis trigonus;* T_{12} - 100% pollen from *Trichosanthes lobata;* T_{13} - 90% pollen from non bitter OPM and10% from *Trichosanthes lobata;* T_{14} - 75% pollen from non bitter OPM and 25% from *Trichosanthes lobata;* T_{15} - 100% pollen from *Trichosanthes cucumerina;* T_{16} - 90% pollen from non bitter OPM and 10% from *Trichosanthes cucumerina;* T_{17} - 75% pollen from non bitter OPM and 25% from *Trichosanthes cucumerina;* T_{18} - 100% pollen from *Trichosanthes anguina;* T_{19} - 90% pollen from non bitter OPM and 10% from *Trichosanthes anguina;* T_{20} - 75% pollen from non bitter OPM and 25% from *Trichosanthes anguina;* T_{21} - 100% pollen from *Momordica charantia;* T_{22} - 90% pollen from non bitter OPM and 10% from *Momordica charantia;* T_{23} -75% pollen from non bitter OPM and 25% from *Momordica charantia;* T_{24} - 100% pollen from *Luffa cylindrica* (wild); T_{25} - 90% pollen from non bitter OPM and 10% from *Luffa cylindrica* (wild); T_{26} - 75% pollen from non bitter OPM and 25% from *Luffa cylindrica* (wild)

Table 17. Fruit quality of F_1s under different pollen treatment combinations

Treatment	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}
Fruit quality	NB	NB	-	NB	NB	B	B & NB	B & NB	-	NB	NB	_	NB

Treatment	T_{14}	T_{15}	T_{16}	T_{17}	T_{18}	T_{19}	T_{20}	T_{21}	T_{22}	T_{23}	T_{24}	T_{25}	T_{26}
Fruit quality	NB	-	NB	NB	-	NB	NB	-	NB	NB	-	NB	NB

B – Bitter
NB – Non bitter

Table 18. Fruit characteristics of parents and F_1 of the cross *C.melo* var. *conomon* x *C. melo* var.*callosus*

Generation	Fruit Weight (g)	Fruit Length (cm)	Fruit Circumference (cm)	No. of seeds per fruit
P_1 (*C.melo* var. *conomon*)	1200	28.5	25.24	605.33
P_2 (*C.melo* var. *callosus*)	50	12.33	10.22	312.24
F_1	200	19.67	14.46	468.44

of T_7 and T_8 produced both bitter and non bitter fruited plants. The fruits of F_1 plants of the cross *C. melo* var. *conomon* x *C. melo* var. *callosus* showed characters of both the species (Plate 4). Fruit characters of this cross is presented in Table 18. Average fruit weight for F_1 was 200 g and contained 468.44 seeds. The average length and circumference of these fruits were 19.67 cm and 14.46 cm respectively.

4.2.6 Evaluation of F_2 plants of the cross *C. melo* var. *conomon* x *C. melo* var. *callosus*

The bitter F_1 plants were selfed to produce F_2 (Plate 4), which gave rise to bitter and non bitter fruited plants, segregating in the ratio 13:3 with 50-70 per cent probability (Table 19).

Table 19. χ^2 test for the cross *C. melo* var. *conomon* x *C. melo* var. *callosus* for the ratio 13:3

Class	Observed No. (O)	Expected No.(E)	d=O-E	$\chi^2 = \frac{d^2}{E}$	Probability range
Bitter	51	52.8	-1.8	0.06	50-70%
Non Bitter	14	12.2	+1.8	0.27	
Total	65	65		0.33	

4.2.7 *In vivo* pollen germination studies

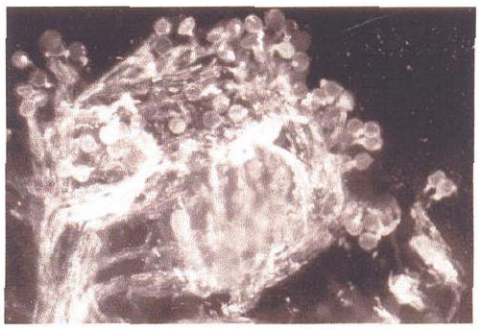
Photographs of pollen germination of nine pollen sources used in the study, on the stigma of oriental pickling melon are given in Plate 3.

Except for *Trichosanthes lobata* and *Trichosanthes cucmerina*, all other species showed pollen germination on the stigma of oriental pickling melon, but did not result in fruit set and bitterness.

4.3 SOURCE EFFECT OF MANURES ON BITTERNESS

The study was undertaken to know whether different sources of nutrients have any role in altering the expression of bitterness in the fruits of oriental pickling melon. The results obtained on the occurrence of bitterness and yield attributes during the two seasons (September to December 2001 and January to April 2002) of study are presented hereunder.

Cucumis melo var. *agrestis*

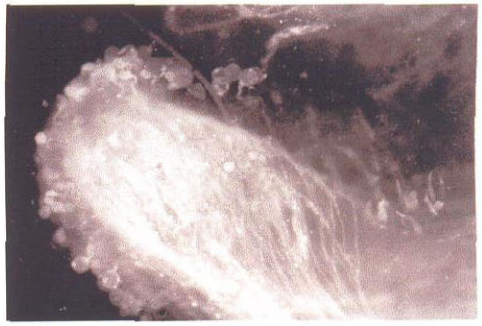


Cucumis melo var. *callosus*

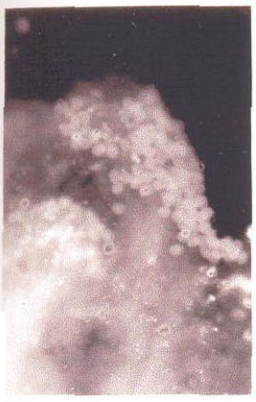
Cucumis trigonus

Trichosanthes lobata

Trichosanthes anguina



Luffa cylindrica (wild)

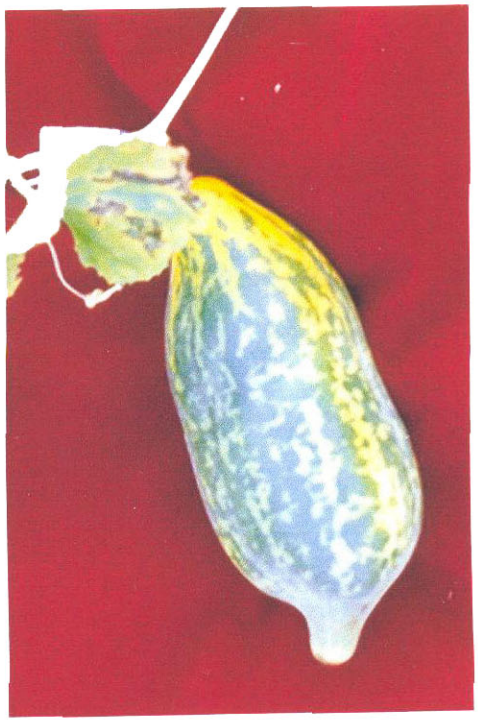


Trichosanthes cucumerina

Momordica charantia

Cucumis melo var. conomon

Plate 3. *In vivo* pollen germination of different cucurbits on stigma of oriental pickling melon



F_0

F_1

Plate 4. F_0 and F_1 fruits of the cross *Cucumis melo* var. *conomon* x *Cucumis melo* var. *callosus*

Analysis of data showed that there existed significant difference among treatments for all the characters studied (Tables 21-26). The effect of season was significant for the characters yield per plant, number of fruits per plant, number of female flowers per plant, duration of the crop and breadth of fruit cavity. However the interaction between seasons and treatments were non-significant for the characters fruit weight, breadth of fruit cavity and fruit length.

4.3.1 Effect of source of nutrients on bitterness

The fruits were evaluated for bitterness and the results are presented in Table 20. The source of nutrients did not change the expression of bitterness in both bitter and non bitter plants. All the fruits produced on bitter plants under the different sources of nutrients were bitter and that on non bitter plants were non bitter.

4.3.2 Effect of source of nutrients on yield and yield attributes

4.3.2.1 Fruit yield (kg per plant)

The data on fruit yield of bitter and non bitter plants during both the seasons are given in Table 21. Bitter and non bitter plants did not show significant difference among themselves for fruit yield (Fig.5). No significant difference was observed for this character with respect to fruit type. The highest yield during both the seasons was recorded for the treatment T_2 (7.93 and 7.79 kg for non bitter and bitter plants during first season and 5.90 and 5.85 kg for non bitter and bitter plants during second season). For bitter plants, during first season, T_1 (6.96 kg) was the second best treatment, which was on par with T_7 and T_8. During second season, the treatments T_1, T_4, T_5, T_6 and T_7 were on par. In the case of non bitter plants, the second highest yield was recorded for the treatment T_1 during the first season and for T_4 during the second season. In all the cases, lowest yield was recorded for the treatment T_{10}.

4.3.2.2 Number of fruits per plant

In the case of bitter plants, the highest number of fruits was observed during first season for the treatment T_2 (8.75), which was on par with T_1 (8.17) (Table 21). During second season, the highest number of fruits were obtained for the treatment T_2 (7.08) and was significantly superior to all other treatments.

Table 20. Effect of source of nutrients on bitterness of fruit in bitter and non bitter plants.

Treatment	Quality of fruit - S_1		Quality of fruit - S_2	
	Bitter plant	Non bitter plant	Bitter plant	Non bitter plant
T_1	B	NB	B	NB
T_2	B	NB	B	NB
T_3	B	NB	B	NB
T_4	B	NB	B	NB
T_5	B	NB	B	NB
T_6	B	NB	B	NB
T_7	B	NB	B	NB
T_8	B	NB	B	NB
T_9	B	NB	B	NB
T_{10}	B	NB	B	NB

T_1 - FYM @ 25 t ha^{-1} + urea equivalent to 70 kg N ha^{-1}; T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to 70 kg N ha^{-1}; T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}; T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha'1; T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}; T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}; T_7 - T_1 + lime @ 250 kg ha^{-1}; T_8 - T_5 + lime @ 250 kg ha^{-1}; T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}; T_{10} - No fertilizer/manure ; T- treatments; F- fruit type; S-season.

Table 21. Effect of sources of nutrients on yield and number of fruits per plant

Treatments	Yield per plant (kg)						No. of fruits per plant					
	S_1		S_2		Mean		S_1		S_2		Mean	
	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB
T_1	6.96	6.85	4.50	4.60	5.73	5.72	8.17	6.33	5.33	4.17	6.75	5.25
T_2	7.93	7.79	5.90	5.85	6.91	6.82	8.75	6.91	7.08	5.17	7.91	6.04
T_3	5.44	5.51	4.23	4.37	4.88	4.94	6.86	5.78	5.58	4.25	6.22	5.01
T_4	5.78	5.98	4.73	4.75	5.23	5.36	6.25	5.75	5.83	4.50	6.04	5.12
T_5	5.96	6.02	4.42	4.54	5.19	5.28	5.88	5.30	5.33	4.58	5.60	4.94
T_6	3.77	4.00	4.57	4.57	4.17	4.28	4.78	4.19	6.08	4.92	5.43	4.55
T_7	6.73	6.96	4.71	4.58	5.72	5.57	7.17	6.42	6.08	4.92	6.62	5.67
T_8	6.69	6.80	4.08	4.27	5.38	5.57	7.25	6.33	4.83	4.67	6.04	5.50
T_9	6.44	6.52	4.19	4.31	5.32	5.41	6.08	5.28	5.00	4.58	5.54	4.93
T_{10}	1.92	1.71	1.10	0.90	1.51	1.30	3.58	3.17	2.08	1.75	2.83	2.46
Mean	5.76	5.82	3.77	4.27	4.76	5.04	6.47	5.55	4.71	4.35	5.59	4.95
CD	T - 0.367 F - NS TxF - NS		T - 0.40 F - NS TxF - NS		S - 2.70 TxS - 2.00		T - 0.60 F - 0.55 TxF - 0.84		T - 0.55 F - 0.45 TxF - 0.78		S - 2.98 TxS - 2.20	

T_1 - FYM @ 25 t ha^{-1} + urea equivalent to 70 kg N ha^{-1}; T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to 70 kg N ha^{-1}; T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}; T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha^{-1}; T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}; T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}; T_7 - T_1 + lime @ 250 kg ha^{-1}; T_8 - T_5 + lime @ 250 kg ha^{-1}; T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}; T_{10} - No fertilizer/manure ; T-treatments; F- fruit type; S-season.

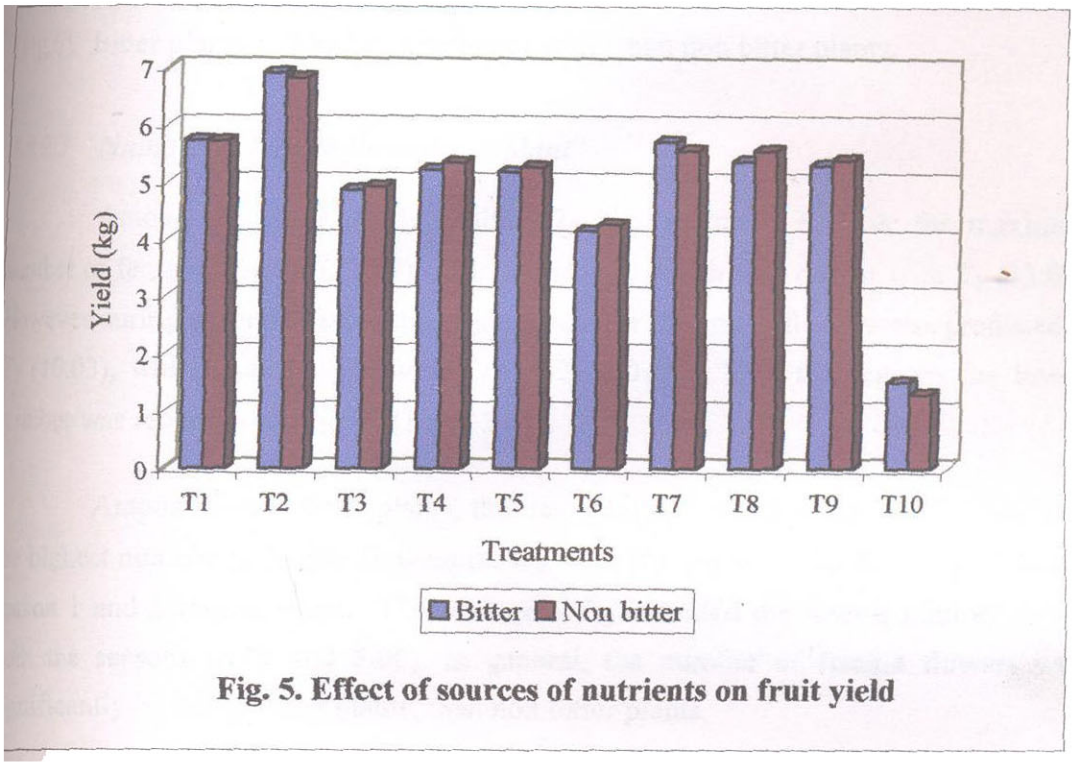


Fig. 5. Effect of sources of nutrients on fruit yield

T_1 - FYM @ 25 t ha^{-1} + urea equivalent to 70 kg N ha^{-1}; T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to 70 kg N ha^{-1}; T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}; T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha^{-1}; T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}; T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}; T_7 - T_1 + lime @ 250 kg ha^{-1}; T_8 - T_5 + lime @ 250 kg ha^{-1}; T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}; T_{10} - No fertilizer/manure

In the case of non bitter plants, during both the seasons, the treatment T_2 recorded the highest number of fruits (6.91 and 5.17), which was on par with T_1, T_7 and T_8 during first season and with T_6, T_7 and T_8 during second season. In all the cases, lowest number of fruits was recorded for T_{10}. Significant difference between bitter and non bitter plants was observed during both the seasons for number of fruits (Fig.6). Bitter plants had higher number of fruits than non bitter plants.

4.3.2.3 Number of female flowers per plant

Among the bitter plants (Table 22), the treatment T_1 gave the maximum number of female flowers (13.17) during season I, which was on par with T_2 (13.00). However during second season, the highest number of female flowers was produced in T_2 (10.03), which was on par with T_1 (9.42). During both the seasons the lowest number was recorded for T_{10} (6.83 and 5.83).

Among the non bitter plants, the treatment with poultry manure (T_2) recorded the highest number of female flowers during both the seasons. (10.92 and 8.67 during season 1 and 2 respectively). The treatment T_{10} recorded the lowest number during both the seasons (6.75 and 5.00). In general, the number of female flowers were significantly higher in bitter plants, than non bitter plants.

4.3.2.4 Fruit set (%)

The percentage fruit set in bitter plants were greater than that of non bitter plants during both the seasons (Table 22).

In the case of bitter plants, during first season, percentage fruit set was highest for the treatment T_8 (70.98), which was not significantly superior to T_2 (67.48) and T_7 (68.22). During second season, T_7 recorded the highest value (70.43), which was on par with T_2, T_4, T_5, T_6 and T_9. Lowest fruit set percentage was recorded for T_{10} during both the seasons (53.50 and 35.83).

Among the non bitter plants, percentage of fruit set was the highest for T_8 (70.97) during first season and for T_6 during second season (63.13). During first season, T_8 was on par with T_7 (67.34) and during second season T_6 was on par with T_2, T_5, T_7 and T_8. Lowest value was recorded for T_{10} during both the seasons (46.67 and 33.37).

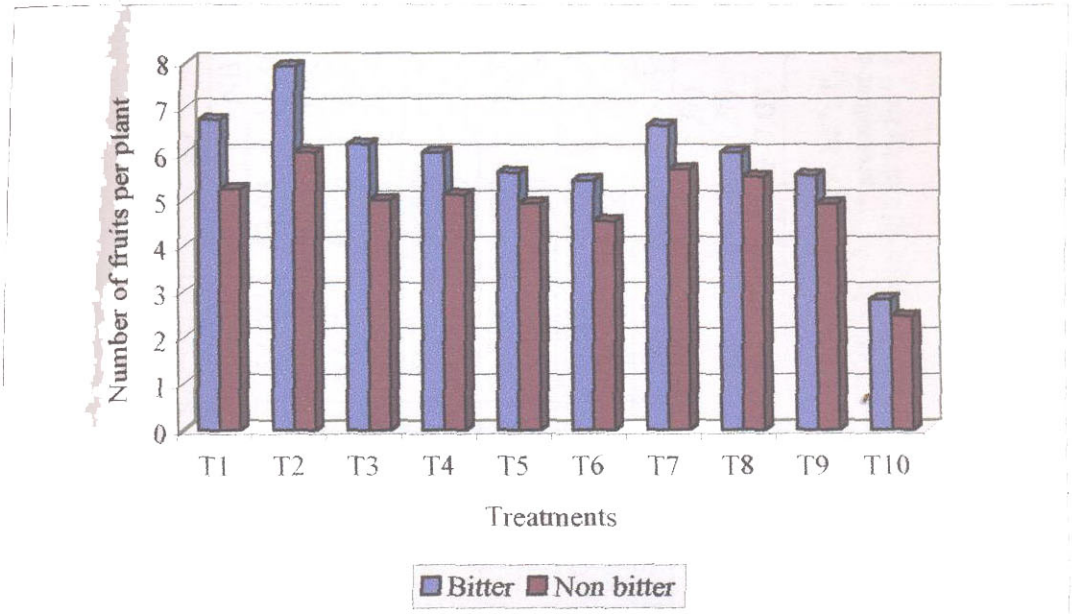


Fig. 6. Effect of sources of nutrients on number of fruits per plant

T_1 - FYM @ 25 t ha^{-1} + urea equivalent to 70 kg N ha^{-1}; T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to 70 kg N ha^{-1}; T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}; T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha^{-1}; T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}; T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}; T_7 - T_1 + lime @ 250 kg ha^{-1}; T_8 - T_5 + lime @ 250 kg ha^{-1}; T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}; T_{10} - No fertilizer/manure

Table 22. Effect of sources of nutrients on number of female flowers per plant and fruit set percentage

Treatments	No. of female flower per plant						Fruit set (%)					
	S_1		S_2		Mean		S_1		S_2		Mean	
	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB
T_1	13.17	10.67	9.42	7.67	10.79	9.17	61.98	58.86	63.03	55.48	62.50	57.17
T_2	13.00	10.92	10.03	8.67	11.51	9.79	67.48	62.25	68.72	60.29	68.10	61.27
T_3	12.08	10.50	9.42	8.42	10.75	9.46	56.83	55.12	59.09	50.49	57.96	52.80
T_4	9.92	9.92	8.83	8.00	9.37	8.96	58.74	57.61	65.54	56.35	62.14	56.98
T_5	10.50	9.42	7.92	7.42	9.21	8.42	59.92	56.34	66.88	61.59	63.40	58.96
T_6	9.00	8.00	9.17	7.75	9.08	7.87	53.13	52.43	65.89	63.13	59.51	57.78
T_7	10.58	9.42	8.58	8.03	9.50	8.72	68.22	67.34	70.43	60.71	69.32	64.02
T_8	10.25	9.00	7.67	7.42	8.96	8.21	70.98	70.97	63.00	62.90	66.99	66.93
T_9	9.58	8.67	7.58	8.25	8.58	8.46	62.83	61.10	65.52	55.29	64.17	58.19
T_{10}	6.83	6.75	5.83	5.00	6.33	5.87	53.50	46.67	35.83	33.37	44.66	40.02
Mean	10.49	9.33	8.37	7.66	9.43	8.49	61.36	58.86	62.39	55.96	61.87	57.41
CD	T-0.92 F-0.42 TxF-NS		T-0.65 F-0.45 TxF-NS		S - 1.55 TxS -1.14		T- 5.56 F- 1.45 TxF-NS		T- 5.00 F- 1.59 TxF-7.07		S- NS TxS-18.74	

T_1 - FYM @ 25 t ha^{-1} + urea equivalent to 70 kg N ha^{-1}; T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to 70 kg N ha^{-1}; T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}; T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha^{-1}1; T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}; T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}; T_7 - T_1 + lime @ 250 kg ha^{-1}; T_8 - T_5 + lime @ 250 kg ha^{-1}; T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}; T_{10} - No fertilizer/manure; T- treatments; F- fruit type; S-season.

4.3.2.5 Days to first female flower opening

Among the bitter plants, during first season flowering occurred in 37.83 days for T_7 (Table 23), which was on par with T_1 (38.00 days), T_4 (38.58 days) and T_8 (38.33 days). During second season, earliest flowering was recorded for T_1 (39.00 days). For both the seasons, the treatment, which took maximum number of days for flowering, was T_{10} (42.5 and 41.58 days respectively).

In the case of non bitter plants, early flowering was observed for T_2 during both the seasons (39.83 and 40.50 days). The treatment T_5 took maximum number of days for flowering during the first season (43.67 days) and the treatment T_{10} during second season (43.08 days).

Significant difference was observed for days to first female flower anthesis, among bitter and non bitter plants.

4.3.2.6 Duration of the crop (days)

In general, duration of the crop was significantly higher for non bitter plants than bitter plants (Table 23).

Among bitter plants, maximum crop duration was observed during both the seasons for the treatment T_5 (75.67 days and 63.83 days) which was on par with T_3, T_4, T_7 and T_9 during season I and T_3 and T_4 during season II. Lowest duration (71.0 days and 59.42 days) was recorded for T_{10}, during both the seasons.

Among non bitter plants, T_3 and T_8 recorded the maximum duration (77.25 days) and T_{10} recorded the minimum duration (71.83 days) during the first season. Analysis of data on second season revealed that maximum duration of 66.17 days was recorded for T_4 and T_9. Minimum duration of 60.58 days was observed for T_{10}.

4.3.2.7 Fruit length (cm)

Data on fruit length for bitter and non bitter plants under different treatments is given in Table 24. It was found that the length of fruits on non bitter plants were significantly higher than that of bitter plants during both the seasons.

Table 23. Effect of sources of nutrients on days to first female flower opening and duration of crop

Treatments	Days to first female flower opening						Duration of crop (days)					
	S_1		S_2		Mean		S_1		S_2		Mean	
	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB
T_1	38.00	41.42	39.00	41.50	38.50	41.46	74.08	77.00	62.17	64.17	68.12	70.58
T_2	39.67	39.83	40.33	40.50	40.00	40.16	74.33	76.92	61.67	65.67	68.00	71.29
T_3	40.58	43.42	40.42	42.00	40.50	42.71	75.42	77.25	63.50	65.67	69.46	71.46
T_4	38.58	41.50	40.06	42.42	39.32	40.78	75.58	75.92	63.17	66.17	69.37	71.04
T_5	41.00	43.67	41.58	42.42	41.29	43.04	75.67	76.75	63.83	64.58	69.75	70.66
T_6	41.42	42.42	41.00	42.25	41.21	42.33	72.25	73.67	61.67	62.83	66.96	67.67
T_7	37.83	40.75	40.17	41.92	39.00	41.33	75.33	77.08	62.00	65.92	68.66	71.50
T_8	38.33	41.08	40.00	41.92	39.16	41.50	74.33	77.25	62.50	65.00	68.41	71.12
T_9	40.67	41.50	40.42	42.50	40.54	42.00	75.00	76.67	62.50	66.17	68.75	71.42
T_{10}	42.50	42.83	41.58	43.08	42.04	42.95	71.00	71.83	59.42	60.58	65.21	66.20
Mean	39.85	41.84	40.45	37.85	40.15	39.84	74.29	76.03	62.24	64.67	68.26	70.35
CD	T-1.03 F-0.49 TxF-1.46		T-0.80 F-0.52 TxF-1.14		S - NS TxS - 2.36		T- 1.01 F- 1.50 TxF-NS		T- 0.93 F- 0.51 TxF-1.31		S- 2.84 TxS-2.21	

T_1 - FYM @ 25 t ha^{-1} + urea equivalent to 70 kg N ha^{-1}; T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to 70 kg N ha^{-1}; T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}; T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha^{-1}; T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}; T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}; T_7 - T_1 + lime @ 250 kg ha^{-1}; T_8 - T_5 + lime @ 250 kg ha^{-1}; T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}; T_{10} - No fertilizer/manure; T- treatments; F- fruit type; S-season.

Table 24. Effect of source of nutrients on fruit length and fruit circumference

Treatments	Fruit length (cm)						Fruit circumference (cm)					
	S_1		S_2		Mean		S_1		S_2		Mean	
	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB
T_1	25.18	33.34	25.04	28.70	25.11	31.02	27.28	28.76	26.83	24.06	27.05	26.42
T_2	25.90	34.02	24.61	32.96	25.25	33.49	26.98	28.93	26.46	28.55	26.72	28.74
T_3	25.78	29.79	24.94	32.01	25.36	30.90	26.56	25.70	25.50	27.14	26.03	26.42
T_4	26.62	31.11	24.95	34.37	25.78	32.74	28.39	27.38	25.58	28.20	26.99	27.79
T_5	27.55	32.45	25.73	33.46	26.64	32.95	29.22	27.67	27.42	29.92	28.32	28.83
T_6	25.52	31.48	26.20	31.96	25.86	31.72	26.35	27.40	27.93	27.08	27.14	27.24
T_7	27.17	33.13	25.28	32.70	26.22	32.91	28.63	28.57	27.00	27.91	27.81	28.24
T_8	26.40	31.90	24.99	30.21	25.70	31.05	28.24	27.80	26.62	26.46	27.43	27.13
T_9	28.58	34.22	25.14	30.53	26.86	32.37	30.03	29.13	26.88	26.38	28.45	27.75
T_{10}	20.23	23.00	21.15	25.18	20.87	24.09	21.59	21.64	22.68	23.85	22.13	22.75
Mean	25.89	31.44	24.83	31.20	25.36	31.32	27.32	27.29	26.28	26.96	26.80	27.12
CD	T-2.08 F-0.78 TxF-NS		T-2.04 F-1.36 TxF-NS		S - NS TxS-NS		T- 1.67 F- NS TxF-NS		T- 1.80 F- NS TxF-NS		S- NS TxS-3.93	

T_1 - FYM @ 25 t ha^{-1} + urea equivalent to 70 kg N ha^{-1}; T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to 70 kg N ha^{-1}; T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}; T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha1; T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}; T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}; T_7 - T_1 + lime @ 250 kg ha^{-1}; T_8 - T_5 + lime @ 250 kg ha^{-1}; T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}; T_{10} - No fertilizer/manure; T- treatments; F- fruit type; S-season.

In the case of bitter plants, fruit length was highest for the treatment T_9 (28.58 cm) during first season, which was on par with T_4 (26.62 cm), T_5 (27.55 cm) and T_7 (27.17 cm). During second season, fruit length was the highest for T_6 (26.20 cm), which was on par with all other treatments except T_{10}. The treatment T_{10} recorded lowest length of fruits, during both the seasons (20.23 cm and 21.15 cm).

In the case of non bitter plants, the highest fruit length was recorded for the treatment T_9 (34.22 cm) during first season and for T_4 (34.37 cm) during the second season. Lowest value during both the seasons was registered for T_{10} (23.00 cm and 25.18 cm).

4.3.2.8 Fruit circumference (cm)

The data on fruit circumference of bitter and non bitter plants is presented in Table 24.

In the case of bitter plants, fruit circumference, during first season, was the highest for T_9 (30.03 cm) which was on par with T_4, T_5 and T_7. During second season, the treatments T_1 (26.83 cm), T_2 (26.46 cm), T_5 (27.42 cm), T_7 (27.00 cm) and T_8 (26.62 cm) and T_9 (26.88 cm) were on par with T_6, the highest one (27.93 cm). Lowest values during both seasons were recorded for T_{10} (21.59 cm and 22.68 cm).

Among the non bitter plants, during first season, the treatment T_9 (29.13 cm) recorded the highest fruit circumference and T_{10} the lowest (21.64 cm). During second season, the highest and lowest values were recorded for the treatments T_5 (29.92 cm) and T_{10} (23.85 cm) respectively.

4.3.2.9 Fruit cavity length (cm)

The length of fruit cavity of bitter and non bitter plants under various treatments is given in Table 25. Among the bitter plants length of fruit cavity, during first season, was highest for T_9 (25.75 cm), which was significantly different from other treatments. T_{10} recorded the lowest cavity length (14.39 cm). During second season, the value ranged from 16.37 cm (T_{10}) to 22.11 cm (T_5).

Table 25. Effect of source of nutrients on fruit cavity length and fruit cavity breadth

Treatments	Fruit cavity length (cm)						Fruit cavity breadth (cm)					
	S_1		S_2		Mean		S_1		S_2		Mean	
	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB
T_1	18.36	23.76	20.95	20.08	19.65	22.30	4.14	4.66	4.34	3.82	4.24	4.24
T_2	22.56	23.53	19.84	23.45	21.20	23.49	4.45	4.99	4.26	4.74	4.35	4.87
T_3	21.09	21.51	19.46	22.99	20.28	22.25	4.36	3.91	4.14	4.46	4.25	4.18
T_4	22.03	23.58	20.31	24.03	21.17	23.80	4.61	4.78	4.23	4.83	4.43	4.80
T_5	23.83	23.17	22.11	23.84	22.97	23.50	4.91	4.55	4.55	4.73	4.73	4.64
T_6	20.45	22.48	22.08	22.27	21.27	22.38	4.31	4.47	4.53	4.58	4.42	4.53
T_7	23.26	23.78	20.83	22.33	22.04	23.05	4.68	4.73	4.33	4.50	4.51	4.62
T_8	22.48	23.31	20.23	22.33	21.35	22.82	4.61	4.60	4.23	4.32	4.42	4.46
T_9	25.75	24.13	20.73	21.59	23.24	22.86	5.01	4.77	4.46	4.36	4.73	4.56
T_{10}	14.39	16.51	16.37	17.93	15.38	17.22	3.33	3.78	3.77	3.96	3.55	3.87
Mean	21.42	22.57	20.29	22.16	20.85	22.36	4.44	4.52	4.28	4.42	4.36	4.47
CD	T-1.47 F-NS TxF-2.08		T-1.42 F-1.52 TxF-NS		S-NS TxS-4.18		T-0.41 F-NS TxF-NS		T-0.37 F-NS TxF-NS		S-0.13 TxS-NS	

T_1 - FYM @ 25 t ha^{-1} + urea equivalent to 70 kg N ha^{-1}; T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to 70 kg N ha^{-1}; T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}; T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha^{-1}; T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}; T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}; T_7 - T_1 + lime @ 250 kg ha^{-1}; T_8 - T_5 + lime @ 250 kg ha^{-1}; T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}; T_{10} - No fertilizer/manure ; T-treatments; F- fruit type; S-season.

Among the non bitter plants, fruit cavity length varied from 16.51 cm (T_{10}) to 24.13 cm (T_9) during season I and from 17.93 cm (T_{10}) to 24.03 cm (T_4) during season 2. In general, length of fruit cavity showed significant difference between the bitter and non bitter plants.

4.3.2.10 Fruit cavity breadth (cm)

Significant difference among the treatments was observed for bitter and non bitter plants during both the seasons (Table 25).

In the case of bitter plants, during first season, T_9 recorded the highest fruit cavity breadth (5.01 cm), which was on par with T_4, T_5, T_7 and T_8. During second season, highest fruit cavity breadth was observed for T_5 (4.55 cm). During both the seasons, lowest value was observed for T_{10} (3.33 cm and 3.77 cm respectively).

In the case of non bitter plants, during first season, T_2 recorded the highest fruit cavity breadth (4.99 cm), which was not significantly different from T_1, T_4, T_7, T_8 and T_9. Highest value during second season was recorded for T_4 (4.83 cm). Lowest fruit cavity breadth during first and second seasons were recorded for T_{10} and T_1 respectively (3.78 and 3.82 cm).

4.3.2.11 Average fruit weight (kg)

Average fruit weight was significantly higher for non bitter fruits than bitter fruits during both the seasons (Table 26 and Fig.7).

In the case of bitter plants, highest average fruit weight was recorded for T_9 (1.04 kg) during first season, which was on par with T_4 (0.94 kg), T_5 (0.99 kg) and T_7 (0.95 kg). During second season T_5 recorded the highest value (0.90 kg), which was on par with T_1, T_2, T_3, T_4, T_6, T_7, T_8 and T_9. Lowest fruit weight during both the seasons was recorded for T_{10} (0.49 and 0.53 kg).

Among the non bitter plants, maximum fruit weight was recorded for T_2 during both the seasons, (1.16 kg and 1.13 kg) which was on par with T_1, T_4, T_5, T_7, T_8 and T_9 during first season and with T_1, T_3, T_4, T_5, T_6 and T_7 during second season.

Table 26. Effect of sources of nutrients on average fruit weight in bitter and non bitter plants

Treatments	Average fruit weight(kg)					
	S_1		S_2		Mean	
	B	NB	B	NB	B	NB
T_1	0.84	1.13	0.83	1.06	0.84	1.10
T_2	0.90	1.16	0.83	1.13	0.86	1.15
T_3	0.86	0.93	0.80	1.08	0.83	1.00
T_4	0.94	1.10	0.85	1.14	0.89	1.07
T_5	0.99	1.08	0.90	1.10	0.94	1.09
T_6	0.87	1.03	0.86	1.09	0.87	1.06
T_7	0.95	1.13	0.87	1.11	0.91	1.12
T_8	0.90	1.07	0.88	1.00	0.89	1.04
T_9	1.04	1.15	0.86	1.00	0.95	1.09
T_{10}	0.49	0.55	0.53	0.57	0.51	0.56
Mean	0.87	1.02	0.82	1.03	0.85	1.03
CD	T-0.10 F-0.07 TxF-NS		T-0.09 F-0.05 TxF-NS		S-NS TxS-NS	

T_1 - FYM @ 25 t ha^{-1} + urea equivalent to 70 kg N ha^{-1}; T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to 70 kg N ha^{-1}; T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}; T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha1; T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}; T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}; T_7 - T_1 + lime @ 250 kg ha^{-1}; T_8 - T_5 + lime @ 250 kg ha^{-1}; T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}; T_{10} - No fertilizer/manure ; T- treatments; F- fruit type; S-season.

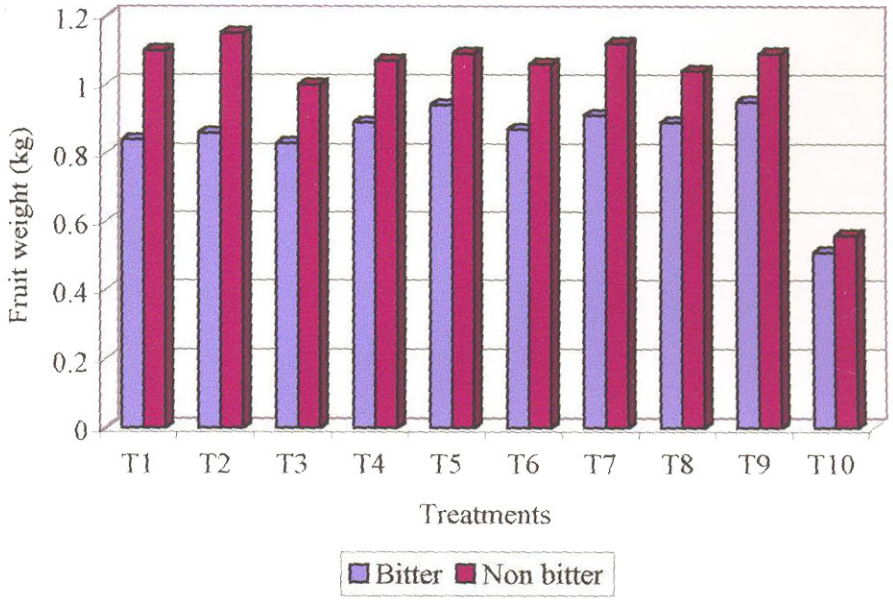


Fig. 7. Effect of sources of nutrients on average fruit weight

T_1 - FYM @ 25 t ha^{-1} + urea equivalent to 70 kg N ha^{-1}; T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to 70 kg N ha^{-1}; T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}; T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha^{-1}; T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}; T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}; T_7 - T_1 + lime @ 250 kg ha^{-1}; T_8 - T_5 + lime @ 250 kg ha^{-1}; T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}; T_{10} - No fertilizer/manure

During both the seasons, lowest fruit weight was recorded for T_{10} (0.55 kg and 0.57 kg).

4.3.2.12 Fruit shape

The fruit shape was oblong with stalk end slightly pointed in the case of non bitter fruits and slightly flattened in the case of bitter fruits.

4.3.2.13 Economics of cultivation

On analyzing the benefit:cost ratio (B:C) of different treatments adopted (Table 27), it was observed that use of poultry manure resulted in the highest B:C ratio (1.85). It was closely followed by T_7 (1.69) and T_1 (1.68).The lowest B:C ratio was obtained for the treatment T_6 (0.87).

4.4 EFFECT OF PRUNING OF LEAVES AND BRANCHES ON FRUIT BITTERNESS

Pruning is an operation generally carried out for maintaining better source sink relationship and thereby higher yield. According to the intensity of pruning adopted, the plant vigour and in turn various metabolic processes may change. In the present study, pruning and leaf thinning operations at various intensities were adopted to find out whether the production of bitter principles is inhibited / initiated by change in vigour of the plant. The results obtained regarding bitterness and other yield contributing factors are presented below.

4.4.1 Fruit bitterness

Evaluation of fruit bitterness revealed that irrespective of the pruning and leaf thinning operations adopted, all the fruits borne on bitter plants were bitter and that on non bitter plants were non bitter (Table 28).

4.4.2 Fruit yield (kg per plant)

Yield from bitter and non bitter lines are presented in Table 29. Significant difference was observed among various treatments for bitter and non bitter plants (Fig.8). However, interaction effect was nonsignificant.

Table 27. Effect of different sources of nutrients on gross and net returns in nonbitter oriental pickling melon and their benefit cost ratios

Sl.No.	Treatments	Cost of cultivation/cent (Rs.)	Gross return/cent (Rs.)	Net return/cent (Rs.)	Benefit cost ratio
1	T_1	245.30	411.00	165.70	1.68
2	T_2	253.00	467.40	214.40	1.85
3	T_3	282.85	330.60	47.75	1.17
4	T_4	266.00	358.80	92.80	1.35
5	T_5	282.80	361.20	78.40	1.28
6	T_6	276.30	240.00	-36.30	0.87
7	T_7	247.50	417.60	170.10	1.69
8	T_8	284.85	408.00	123.15	1.43
9	T_9	245.60	391.20	145.60	1.59

T_1 - FYM @ 25 t ha^{-1} + urea equivalent to 70 kg N ha^{-1}; T_2 - FYM @ 25 t ha^{-1} + poultry manure equivalent to 70 kg N ha^{-1}; T_3 - FYM @ 25 t ha^{-1} + goat manure equivalent to 70 kg N ha^{-1}; T_4 - FYM @ 25 t ha^{-1} + FYM equivalent to 70 kg N ha^{-1}; T_5 - FYM @ 25 t ha^{-1} + groundnut cake equivalent to 70 kg N ha^{-1}; T_6 - FYM @ 25 t ha^{-1} + neem cake equivalent to 70 kg N ha^{-1}; T_7 - T_1 + lime @ 250 kg ha^{-1}; T_8 - T_5 + lime @ 250 kg ha^{-1}; T_9 - T_1 + furadan @ 0.5 kg a.i. ha^{-1}; T- treatments.

Table 28. Effect of different levels of pruning on fruit bitterness

Treatments	Bitter	Non bitter
T_1	B	NB
T_2	B	NB
T_3	B	NB
T_4	B	NB
T_5	B	NB
T_6	B	NB
T_7	B	NB
T_8	B	NB
T_9	B	NB
T_{10}	B	NB

T_1 - Control; T_2 - Pruning of all primary branches; T_3 - Pruning of all secondary branches; T_4 - Pruning of all tertiary branches; T_5 - Pruning part of primary branch, after the set of two fruits on it; T_6 - Thinning of first two leaves of all branches; T_7 - thinning of every alternate leaf; T_8 - Thinning of every fourth leaf; T_9 - Thinning of every sixth leaf; T_{10} - Thinning of every eighth leaf.

Table 29. Effect of different levels of pruning on fruit yield, number of fruits, number of female flowers and fruit set percentage

Treatments	Fruit yield per plant (kg)			No. of fruits per plant			No. of female flowers per plant			Fruit set (%)		
	B	NB	Mean	B	NB	Mean	B	NB	Mean	B	NB	Mean
T_1	6.51	6.36	6.44	7.66	5.66	6.16	9.99	8.88	9.44	76.61	62.38	69.49
T_2	0.65	0.90	0.78	1.33	1.00	1.17	2.33	2.00	2.17	57.08	49.99	53.54
T_3	4.86	4.63	4.75	5.11	3.88	4.50	6.99	6.55	6.77	73.11	59.66	66.38
T_4	5.84	5.67	5.76	6.88	5.22	6.05	10.22	9.00	9.61	67.33	64.33	65.83
T_5	4.65	4.66	4.66	6.55	4.10	5.33	8.99	6.49	7.74	72.33	63.88	68.11
T_6	5.47	5.86	5.67	6.44	5.44	6.05	9.99	9.10	9.55	64.46	62.66	63.56
T_7	5.26	5.76	5.51	7.10	5.55	5.94	9.99	8.22	9.11	71.33	67.33	69.33
T_8	5.30	5.93	5.62	6.88	5.00	5.94	10.44	8.33	9.39	65.99	60.00	62.99
T_9	5.30	6.02	5.66	5.77	5.21	5.49	10.22	8.66	9.44	56.66	60.33	58.49
T_{10}	5.40	5.91	5.65	7.33	5.33	6.33	10.00	8.44	9.22	73.33	63.33	68.33
Mean	4.92	5.17	5.04	6.11	4.62	5.41	8.92	7.57	8.24	67.82	61.46	64.61
CD	F-0.21 T-0.46 FxT-NS			F-0.20 T-0.45 FxT-0.64			F-0.59 T-0.61 FxT-0.87			F-2.62 T-5.86 FxT-NS		

T_1 - Control; T_2 - Pruning of all primary branches; T_3 - Pruning of all secondary branches; T_4 - Pruning of all tertiary branches; T_5 - Pruning part of primary branch, after the set of two fruits on it; T_6 - Thinning of first two leaves of all branches; T_7 - thinning of every alternate leaf; T_8 - Thinning of every fourth leaf; T_9 - Thinning of every sixth leaf; T_{10} - Thinning of every eighth leaf; F - fruit type; T - treatment.

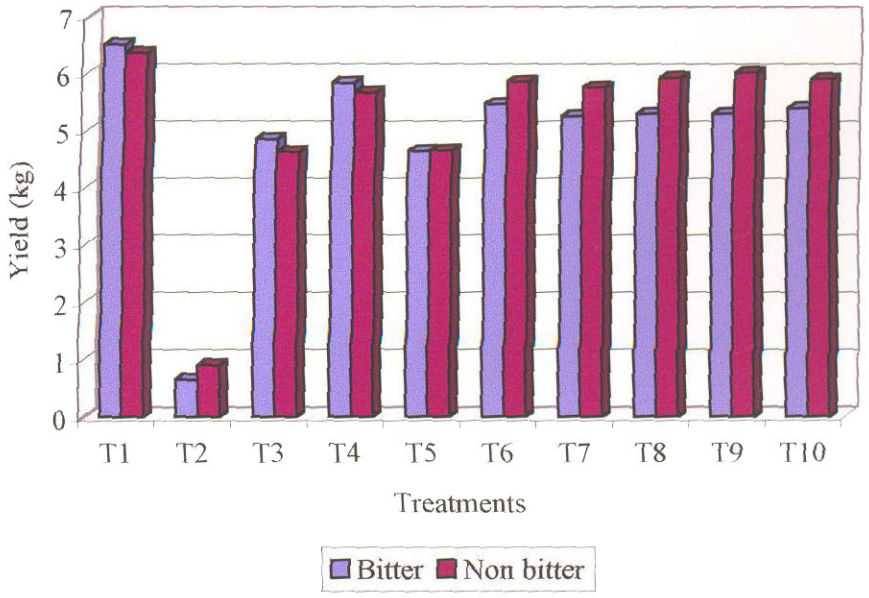


Fig. 8. Effect of pruning on fruit yield

T_1 - Control; T_2 - Pruning of all primary branches; T_3 - Pruning of all secondary branches; T_4 - Pruning of all tertiary branches; T_5 - Pruning part of primary branch, after the set of two fruits on it; T_6 - Thinning of first two leaves of all branches; T_7 - thinning of every alternate leaf; T_8 - Thinning of every fourth leaf; T_9 - Thinning of every sixth leaf; T_{10} - Thinning of every eighth leaf

The highest fruit yield was observed for the treatment T_1, both in bitter and non bitter types (6.51 and 6.36 kg respectively). Among non bitter plants, T_1 was on par with T_8 (5.93 kg), T_9 (6.02 kg) and T_{10} (5.91 kg). Lowest yield among bitter and non bitter plants was recorded for T_2 (0.65 kg and 0.90 kg respectively).

4.4.3 Number of fruits per plant

Data on number of fruits per plant is given in Table 29 and Fig.9. Significant effect was noticed on number of fruits due to the different treatments, fruit type and their interaction. Fruit number ranged from 1.33 (T_2) to 7.66 (T_1) among bitter plants and the treatment T_1 was found to be on par with T_{10} (7.33). Among non-bitter plants, T_1 had the maximum number of fruits (5.66), which was on par with T_4 (5.22), T_6 (5.44), T_7 (5.55), T_9 (5.21) and T_{10} (5.33). Minimum number of fruits was recorded for T_2 (1.00).

4.4.4 Number of female flowers per plant

Data on effect of pruning on production of female flowers is given in Table 29. Number of female flowers were higher in bitter plants, than in non bitter plants. Significant difference was noticed in the number of flowers among bitter and non bitter plants, for the various treatments adopted. Among the bitter plants, the treatments T_1 (9.99), T_4 (10.22), T_6 (9.99), T_7 (9.99), T_8 (10.44), T_9 (10.22) and T_{10} (10.00) were on par and the treatment T_2 (2.33) recorded the lowest number. In the case of non bitter plants,the treatments T_1 (8.88), T_4 (9.00), T_6 (9.10) and T_9 (8.66) were on par.The treatment T_2 (2.00) recorded the least number of female flowers per plant.

4.4.5 Fruit set percentage

Data on effect of pruning on fruit set percentage is furnished in Table 29. There was significant difference among the treatments and fruit types for fruit set percentage, but their interaction effect was nonsignificant. Among bitter plants, fruit set percentage was the highest for T_1 (76.61), which was not significantly different from T_3 (73.10), T_5 (72.33), T_7 (71.33) and T_{10} (73.33). Among non bitter plants, highest fruit set percentage was observed for T_7 (67.33), which was on par with T_1

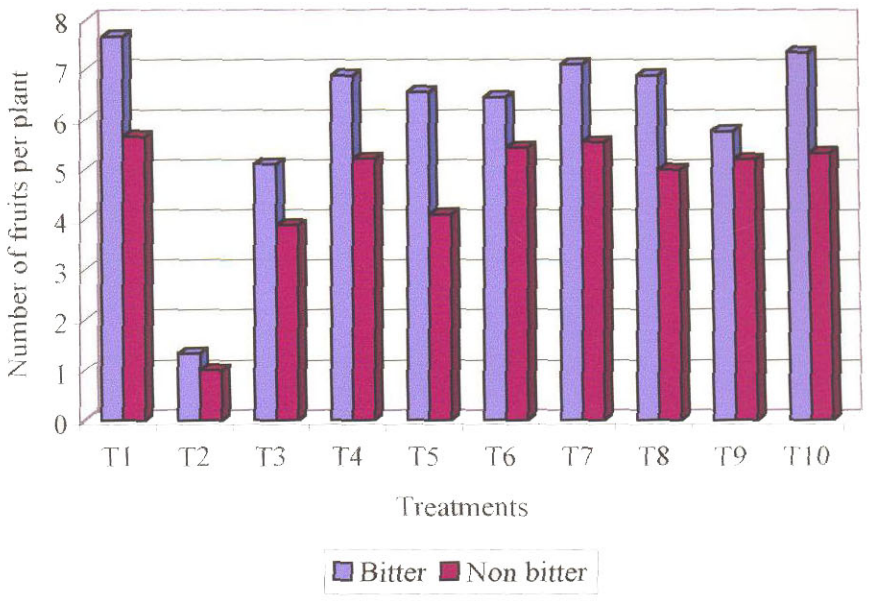


Fig. 9. Effect of pruning on number of fruits per plant

T_1 - Control; T_2 - Pruning of all primary branches; T_3 - Pruning of all secondary branches; T_4 - Pruning of all tertiary branches; T_5 - Pruning part of primary branch, after the set of two fruits on it; T_6 - Thinning of first two leaves of all branches; T_7 - thinning of every alternate leaf; T_8 - Thinning of every fourth leaf; T_9 - Thinning of every sixth leaf; T_{10} - Thinning of every eighth leaf

(62.38), T_4 (64.33), T_5 (63.88), T_6 (62.66) and T_{10} (63.33). Lowest fruit set percentage in both the cases were recorded for the treatment T_2 (57.08 and 49.99).

4.4.6 Average fruit weight (kg)

Data on average fruit weight in bitter and non bitter plants is presented in Table 30 and Fig.10. Significant difference in weight of fruit was noticed among bitter and non bitter plants for the various treatments adopted. In the case of bitter plants, T_1 recorded the highest fruit weight (0.90 kg) and was on par with T_3 (0.85 kg), T_4 (0.85 kg), T_6 (0.85 kg), T_8 (0.80 kg), T_9 (0.81 kg) and T_{10} (0.80 kg). T_2 recorded an average fruit weight of 0.65 kg. Among non bitter plants, the treatments T_1 (1.20 kg), T_3 (1.18 kg), T_5 (1.15 kg), T_6 (1.25 kg) and T_9 (1.22 kg) were on par. Average fruit weight was the lowest for T_2 (0.90 kg).

4.4.7 Days to first female flower opening

Data on days to first female flower opening is presented in Table 30. Significant difference was observed among the treatments for bitter and non-bitter plants. Bitter fruited types were earlier than non bitter types. Among bitter plants, T_1 and T_4 (37.22 days) recorded the earliest flowering, which were not significantly different from T_3 (37.99 days), T_5 (37.77 days), T_7 (37.66 days), T_8 (37.77 days) T_9 (37.55 days) and T_{10} (37.33 days). For the treatment T_2, it took 40.33 days for flowering in bitter type and 44.44 days in non bitter type. In the case of non bitter plants, T_1 (40.77 days), T_3 (40.66 days), T_4 (40.22 days), T_5 (40.44 days), T_6 (40.88 days), T_7 (40.66 days), T_8 (40.99 days), T_9 (40.77 days) and T_{10} (40.22 days) were on par.

4.4.8 Duration of the crop (days)

Results of the effect of pruning on duration of the crop is presented in Table 30. The effect of treatments, fruit types and their interaction were significant for duration of the crop. Duration of the crop was significantly higher for non bitter plants than bitter plants.

Table 30. Effect of pruning on average fruit weight, days to first female flower opening, duration of the crop and fruit length

Treatments	Average fruit weight(kg)			Days to first female flower opening			Duration of the crop (days)			Fruit length (cm)		
	B	NB	Mean	B	NB	Mean	B	NB	Mean	B	NB	Mean
T_1	0.90	1.20	1.05	37.22	40.77	38.99	74.29	76.25	75.27	26.66	32.99	29.83
T_2	0.65	0.90	0.78	40.33	44.44	42.39	65.01	69.22	67.12	22.88	29.77	26.33
T_3	0.85	1.18	1.02	37.99	40.66	39.33	73.21	75.66	74.44	26.22	35.66	30.94
T_4	0.85	1.10	0.98	37.22	40.22	38.72	71.55	77.44	74.49	26.33	36.33	31.33
T_5	0.77	1.15	0.96	37.77	40.44	39.11	70.88	76.44	73.66	24.33	30.44	27.39
T_6	0.85	1.25	1.05	38.33	40.88	39.60	72.66	77.11	74.88	25.99	36.22	31.10
T_7	0.68	1.11	0.90	37.66	40.66	39.16	71.55	77.33	74.44	24.80	31.33	28.07
T_8	0.80	1.10	0.95	37.77	40.99	39.38	73.10	77.33	75.21	27.11	36.33	31.72
T_9	0.81	1.22	1.02	37.55	40.77	39.16	72.11	76.44	74.27	26.44	35.88	31.16
T_{10}	0.80	1.11	0.96	37.33	40.22	38.77	72.66	77.99	75.33	25.99	35.33	30.66
Mean	0.79	1.13	0.96	37.92	41.00	39.46	71.70	76.12	73.91	25.68	34.03	29.86
CD	F - 0.17 T - 0.10 FxT - NS			F - 0.98 T - 0.94 FxT - NS			F - 0.64 T - 1.14 FxT - 1.61			F - 3.09 T - 1.68 FxT - NS		

T_1 - Control; T_2 - Pruning of all primary branches; T_3 - Pruning of all secondary branches; T_4 - Pruning of all tertiary branches; T_5 - Pruning part of primary branch, after the set of two fruits on it; T_6 - Thinning of first two leaves of all branches; T_7 - thinning of every alternate leaf; T_8 - Thinning of every fourth leaf; T_9 - Thinning of every sixth leaf; T_{10} - Thinning of every eighth leaf; F - fruit type; T - treatment.

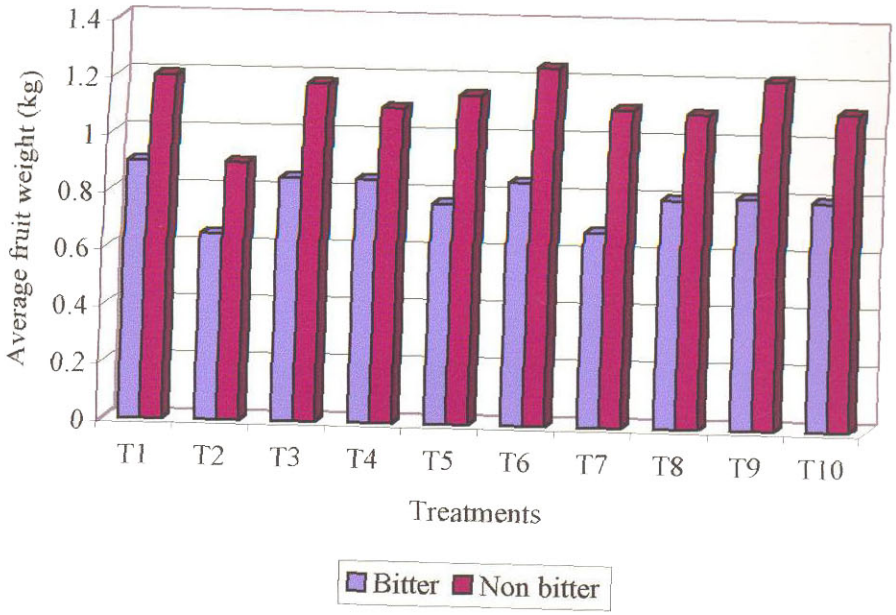


Fig. 10. Effect of pruning on average fruit weight

T_1 - Control; T_2 - Pruning of all primary branches; T_3 - Pruning of all secondary branches; T_4 - Pruning of all tertiary branches; T_5 - Pruning part of primary branch, after the set of two fruits on it; T_6 - Thinning of first two leaves of all branches; T_7 - thinning of every alternate leaf; T_8 - Thinning of every fourth leaf; T_9 - Thinning of every sixth leaf; T_{10} - Thinning of every eighth leaf

Considering the bitter plants, T_1 recorded the maximum duration (74.29 days), which was not significantly higher than T_3 (73.21 days).The treatment T_2 recorded the lowest duration of 65.01 days. In the case of non bitter plants, the treatments T_4 (77.44 days), T_6 (77.11 days), T_7 (77.33 days), T_8 (77.33 days) and T_{10} (77.99 days) were on par and the lowest duration was observed for T_2 (69.22 days).

4.4.9 Fruit length (cm)

Effect of different pruning treatments on fruit length is given in Table 30. Significant difference was observed among treatments and fruit types for length of fruit. It was significantly higher for non bitter fruits the bitter fruits. Among bitter plants, highest fruit length was observed for the treatment T_8 (27.11 cm), which was on par with that of all other treatments except T_2 (22.88 cm), T_5 (24.33 cm) and T_7 (24.80 cm). Among non bitter plants, length of fruit was the highest for T_4 and T_8 (36.33 cm) and lowest for T_2 (29.77 cm).

4.4.10 Fruit circumference (cm)

Data on effect of pruning on circumference of fruit is presented in Table 31.

Bitter and non bitter plants did not show significant difference among themselves for circumference of fruit. However, circumference of the fruit varied significantly with treatments. Among bitter plants, the highest circumference of fruit was recorded by the treatment T_1 (28.66 cm), which was on par with T_3 (28.46 cm), T_4 (28.33 cm), T_6 (28.44 cm), T_8 (28.44 cm), T_9 (28.55 cm) and T_{10} (28.55 cm). Among non bitter plants, the treatments T_3 (30.22 cm), T_4 (30.33 cm), T_6 (30.22 cm), T_8 (30.88 cm), T_9 (30.55 cm) and T_{10} (29.77 cm) were on par. The lowest measurements for both bitter and non bitter plants were recorded by T_2 (21.88 cm and 24.33 cm).

4.4.11 Fruit cavity length (cm)

Data on effect of pruning on length of fruit cavity is given in Table 31. Significant variation in length of fruit cavity was observed with respect to treatments adopted and type of fruit. The treatment T_9 recorded the highest length (22.88 cm) among bitter plants, which was not significantly different from T_1 (21.77 cm), T_3

Table 31. Effect of different levels of pruning on fruit circumference, fruit cavity length, fruit cavity breadth and number of primary branches

Treatments	Fruit circumference(cm)			Fruit cavity length(cm)			Fruit cavity breadth (cm)			Number of primary branches per plant		
	B	NB	Mean	B	NB	Mean	B	NB	Mean	B	NB	Mean
T_1	28.66	28.55	28.60	21.77	22.18	21.97	4.18	4.62	4.40	4.10	3.99	4.05
T_2	21.88	24.33	23.11	16.11	20.11	18.11	4.00	4.45	4.22	--	--	--
T_3	28.46	30.22	29.44	21.88	24.22	23.05	4.22	4.64	4.43	3.22	3.11	3.17
T_4	28.33	30.33	29.49	21.55	24.44	22.99	4.48	4.58	4.53	3.66	3.33	3.50
T_5	22.99	24.66	23.83	16.44	21.10	18.77	4.37	4.53	4.45	3.55	3.55	3.55
T_6	28.44	30.22	29.33	21.88	22.99	22.44	4.46	4.32	4.39	3.77	3.33	3.66
T_7	23.11	24.66	23.88	17.88	20.88	19.38	4.27	4.59	4.43	3.66	3.55	3.60
T_8	28.44	30.88	29.66	22.66	26.44	24.55	4.49	4.40	4.44	3.77	3.77	3.77
T_9	28.55	30.55	29.55	22.88	27.10	24.99	4.40	4.41	4.41	3.77	3.77	3.77
T_{10}	28.55	29.77	29.16	21.44	25.66	23.55	4.11	4.40	4.25	3.66	3.44	3.55
Mean	26.79	28.41	27.60	20.45	23.51	21.98	4.33	4.51	4.42	3.68	3.54	3.61
CD	F - NS T - 1.67 FxT - NS			F - 0.65 T - 1.46 FxT - NS			F - NS T - NS FxT - NS			F - NS T - NS FxT - NS		

T_1 - Control; T_2 - Pruning of all primary branches; T_3 - Pruning of all secondary branches; T_4 - Pruning of all tertiary branches; T_5 - Pruning part of primary branch, after the set of two fruits on it; T_6 - Thinning of first two leaves of all branches; T_7 - thinning of every alternate leaf; T_8 - Thinning of every fourth leaf; T_9 - Thinning of every sixth leaf; T_{10} - Thinning of every eighth leaf; F - fruit type; T - treatment.

(21.88 cm), T_4 (21.55 cm), T_6 (21.88 cm), T_8 (22.66 cm) and T_{10} (21.44 cm). In the case of non bitter plants, the highest fruit cavity length was recorded by T_9 (27.10 cm), followed by T_8 (26.44 cm) and T_{10} (25.66 cm). For both the fruit types, the lowest length of fruit cavity was observed for T_2 (16.11 cm and 20 .11 cm respectively).

4.4.12 Fruit cavity breadth (cm)

Results obtained on effect of pruning on breadth of fruit cavity are furnished in Table 31. No significant difference was observed between the treatments or fruit type for breadth of fruit cavity. Among bitter plants, the values ranged from 4.10 cm (T_2) to 4.49 cm (T_8) and among non bitter plants, from 4.32 cm (T_6) to 4.64 cm (T_3).

4.4.13 Number of primary branches per plant

The difference in number of primary branches with respect to fruit type and various treatments adopted were not significant (Table 31). Number of primary branches ranged from 3.22 (T_3) to 4.10 (T_1) in the case of bitter plants and from 3.11 (T_3) to 3.99 (T_1) in the case of non bitter plants.

4.4.14 Number of secondary branches per plant

Number of secondary branches did not vary significantly either between fruit type or between treatments (Table 32). Among bitter plants, maximum number of primary branches was observed for T_9 (5.88) and minimum for T_1 and T_8 (5.55). In the case of non bitter types, the number of secondary branches ranged from 5.33 (T_7) to 5.88 (T_5, T_6 and T_8).

4.4.15 Number of fruits on primary branches

Data relating to number of fruits on primary branches is presented in Table 32. No significant difference was observed for number of fruits formed on primary branches between treatments or between fruit types. It ranged from 3.77 (T_1, T_4 and T_9) to 5.11 (T_3) in the case of bitter plants and from 2.44 (T_8) to 3.88 (T_3) in the case of non bitter plants.

Table 32. Effect of pruning on number of secondary branches per plant, number of fruits on primary branches, number of fruits on secondary branches and loss in yield (%).

Treatment	Number of secondary branches per plant			Number of fruits on primary branches			Number of fruits on secondary branches			Loss in yield due to pruning (%)		
	B	NB	Mean	B	NB	Mean	B	NB	Mean	B	NB	Mean
T_1	5.55	5.77	5.66	3.77	3.55	3.66	3.88	1.99	2.94	--	--	
T_2	--	--	--	--	--	--	--	--	--	90.00	85.88	87.94
T_3	--	--	--	5.11	3.88	4.49	--	--	--	25.34	27.20	26.27
T_4	5.77	5.66	5.72	3.77	3.66	3.72	3.11	2.11	2.61	10.29	10.85	10.57
T_5	5.77	5.88	5.83	3.88	2.55	3.22	2.66	1.55	2.10	28.50	26.73	27.62
T_6	5.66	5.88	5.77	4.99	3.55	4.23	1.66	2.11	1.88	15.98	7.86	11.92
T_7	5.66	5.33	5.49	3.88	2.88	3.38	3.22	2.66	2.94	19.20	9.43	14.32
T_8	5.55	5.88	5.71	4.43	2.44	3.44	2.44	2.33	2.39	18.50	6.76	12.63
T_9	5.88	5.44	5.66	3.77	3.55	3.66	1.99	1.66	1.83	18.58	5.35	11.97
T_{10}	5.66	5.66	5.66	4.22	2.88	3.55	3.11	2.44	2.77	16.74	7.07	11.91
Mean	5.69	5.68	5.69	4.20	3.22	3.71	2.77	2.10	2.45	27.01	20.79	23.90
CD	F - NS T - NS FxT - NS			F - NS T - NS FxT - NS			F - NS T - NS FxT - NS			F - 2.5 T - 5.4 FxT - NS		

T_1 - Control; T_2 - Pruning of all primary branches; T_3 - Pruning of all secondary branches; T_4 - Pruning of all tertiary branches; T_5 - Pruning part of primary branch, after the set of two fruits on it; T_6 - Thinning of first two leaves of all branches; T_7 - thinning of every alternate leaf; T_8 - Thinning of every fourth leaf; T_9 - Thinning of every sixth leaf; T_{10} - Thinning of every eighth leaf; F - fruit type; T - treatment.

4.4.16 Number of fruits on secondary branches

Data on effect of pruning on number of fruits developed on secondary branches is given in Table 32. All the treatments in bitter and non bitter plants were on par. With respect to fruit types also, no significant difference was observed. Among bitter plants, the number of fruits on secondary branches ranged from 1.66 (T_6) to 3.88 (T_1). Among non bitter plants, the highest number of fruits on secondary branches was recorded for T_7 (2.66) and the lowest for T_5 (1.55).

4.4.17 Percentage loss in yield due to pruning

Data on percentage loss in yield due to pruning is given in Table 32. Among bitter plants, maximum loss was observed for T_2 (90%) and minimum loss was recorded for T_4 (10.29%). Among non bitter plants, 85.88 % loss was recorded for T_2, followed by T_3 (27.20%). The percentage loss was minimum for T_9 (5.35%).

4.5 BITTERNESS IN RELATION TO AGE OF THE PLANT

The results relating to fruit bitterness, number of female flowers and number of fruits during different stages of plant growth are presented in Table 33.

4.5.1 Fruit bitterness

Expression of bitterness was not affected by the age of the plant at which fruits were formed as evident from the Table 33. The fruits formed in bitter plants under different age categories were always bitter and that on non bitter plants were free from bitterness.

4.5.2 Number of female flowers

Production of flowers started during C_3 (36-40 DAS) and ended during C_{10} (71-75 DAS) in bitter plants. Average number of female flowers produced per plant was 12.16, and maximum flower production was observed during C_5.

On non bitter plants, production of flowers started from C_4 (41-45 DAS), reached maximum during C_6 (51-55 DAS) and continued up to C_9 (66-70 DAS). Average number of flowers produced per plant was 8.88.

Table 33. Number of female flowers, number of fruits and fruit bitterness during each time frame

Age of plants	No. of female flowers		No. of fruits		Fruit Bitterness	
	Bitter (B)	Non bitter (NB)	Bitter (B)	Non bitter (NB)	Bitter (B)	Non bitter (NB)
C_1 (25-30 DAS)	-	-	-	-	-	-
C_2 (31-35 DAS)	-	-	-	-	-	-
C_3 (36-40 DAS)	1.88	-	1.50	-	B	-
C_4 (41-45 DAS)	2.36	1.10	2.00	1.10	B	NB
C_5 (46-50 DAS)	2.89	1.46	1.00	1.22	B	NB
C_6 (51-55 DAS)	1.67	2.22	1.73	1.56	B	NB
C_7 (56-60 DAS)	1.11	2.00	1.09	1.33	B	NB
C_8 (61-65 DAS)	1.0	1.00	1.00	0.66	B	NB
C_9 (66-70 DAS)	0.25	1.10	-	1.00	-	NB
C_{10} (71-75 DAS)	1.00	-	1.00	-	B	-
Total	12.16	8.88	8.82	6.65		

DAS - Days after sowing

4.5.3 Number of fruits

On bitter plants maximum number of fruits were produced during C_4 (41-45 DAS) and on non bitter plants, during C_6 (51-55 DAS). Total number of fruits produced on bitter plants were 8.82 and on non bitter plants were 6.65.

4.6 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION

Various morphological and biochemical characters of bitter and non bitter plants were studied to characterize a bitter plant and to investigate into the various factors imparting bitterness with a view to probe into the possibility of identification of bitter plant types during early stages.

4.6.1 Morphological characters

The different qualitative and quantitative characters of bitter and non bitter plants are presented in Table 34 and Plate 5. Comparison between the two types is also given in Figures 11 and 12.

4.6.1.1 Seed and seedling characters

4.6.1.1.1 Seed length (cm)

The average length of seeds from bitter plants was 0.85 cm and was significantly lower than that of seeds from non bitter plants (1.05 cm).

4.6.1.1.2 Seed breadth (cm)

Average breadth of seeds from bitter plants (0.31cm) was significantly lower than that from non bitter plants (0.35 cm).

4.6.1.1.3 Seed thickness (cm)

Average thickness of seeds from bitter and non bitter plants were 0.14 cm and 0.16 cm respectively and the difference was significant.

4.6.1.1.4 Hundred seed weight (g)

Weight of hundred seeds was significantly higher for non bitter plants (2.38 g) compared to seeds from bitter plants (1.78 g).

Table 34. Morphological characters of bitter and non bitter seeds, seedlings, plants and fruits.

Sl. No.	Characters	Bitter plant	Non bitter plant	t-value
1	**Seed characters**			
1-1	Seed length (cm)	0.85	1.05	13.83**
1-2	Seed breadth (cm)	0.31	0.35	9.49**
1-3	Seed thickness (cm)	0.14	0.16	2.81*
1-4	Hundred seed weight (g)	1.78	2.38	53.01**
1-5	Speed of germination	9.75	7.08	12.81*
1-6	Germination percentage	88.00	71.00	26.16**
1-7	Seed colour	tan	tan	
1-8	Seed bitterness	non bitter	non bitter	
2	**Seedling characters (5 DAG)**			
2-1	Hypocotyl length (cm)	10.37	7.79	15.87**
2-2	Redicle length (cm)	3.49	2.59	8.72**
2-3	Cotyledonary leaf shape	obtuse	obtuse	
2-4	Cotyledonary leaf apex shape	round tipped	flat tipped	
2-5	Hypocotyl hairiness	subglabrous	subglabrous	
2-6	Pigmentation	weak	weak	
2-7	Cotyledonary leaf bitterness	bitter	non bitter	
3	**Plant characters**			
3-1	Vine length (m)	2.94	2.29	7.98**
3-2	Days to first female flower	34.30	36.64	9.05**
3-3	Number of female flowers	12.28	10.86	5.96**
3-4	Fruit set percentage	61.51	50.71	4.93**
3-5	Number of fruits per plant	7.55	5.49	8.72**
3-6	Yield per plant (kg)	6.16	6.01	NS
4	**Fruit characters**			
4-1	Average fruit weight (kg)	0.83	1.10	6.59**
4-2	Fruit length (cm)	26.88	31.48	11.52**
4-3	Fruit circumference (cm)	28.37	27.57	NS
4-4	Fruit cavity length (cm)	20.86	28.65	13.97**
4-5	Fruit cavity breadth (cm)	5.40	5.74	NS

** Significant at 5% level

* Significant at 1% level

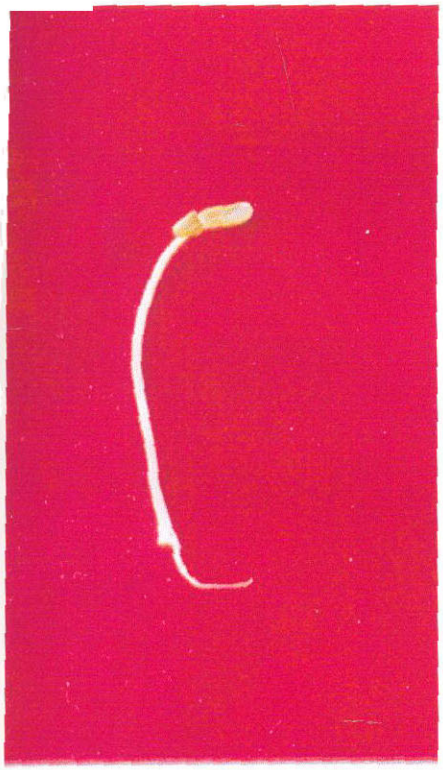
DAG - Days after germination

SEEDS

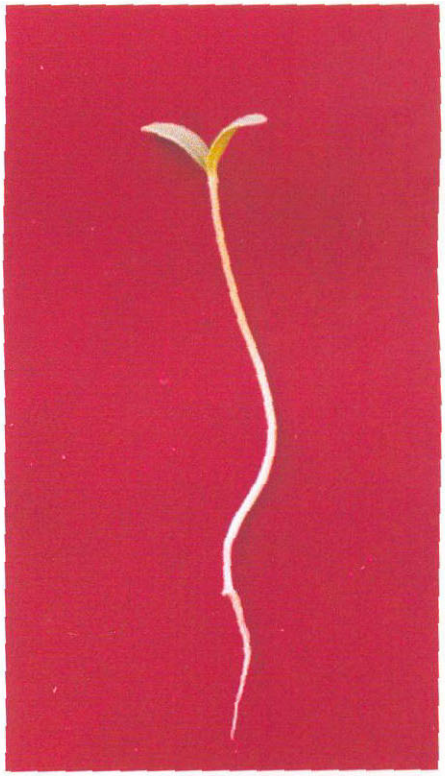
Bitter

Non bitter

SEEDLINGS (5 Days after germination)



Bitter



Non bitter

Plate 5. Seeds and seedlings from bitter and non bitter fruits

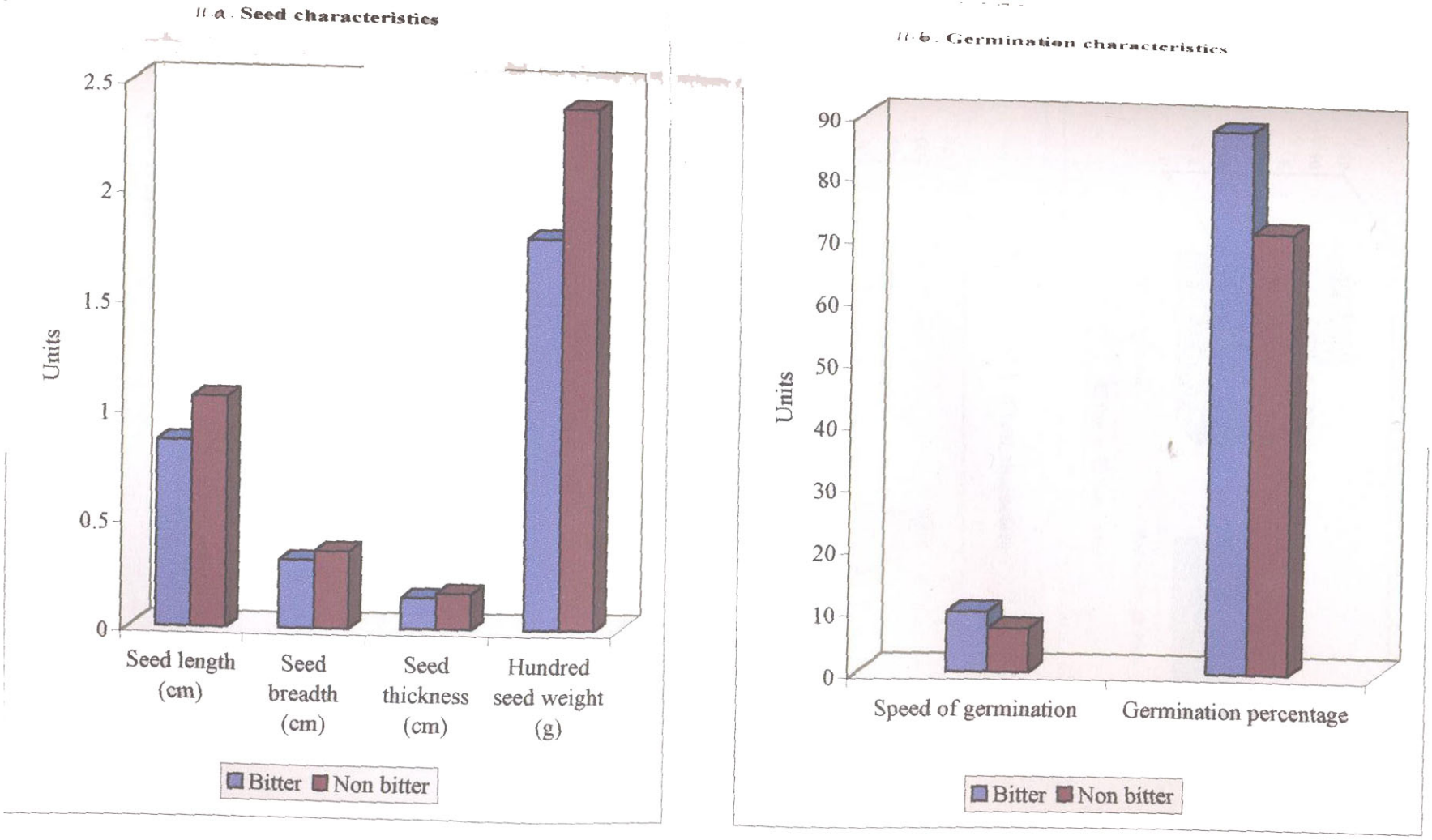


Fig. 11. Morphological characters of bitter and non bitter seeds

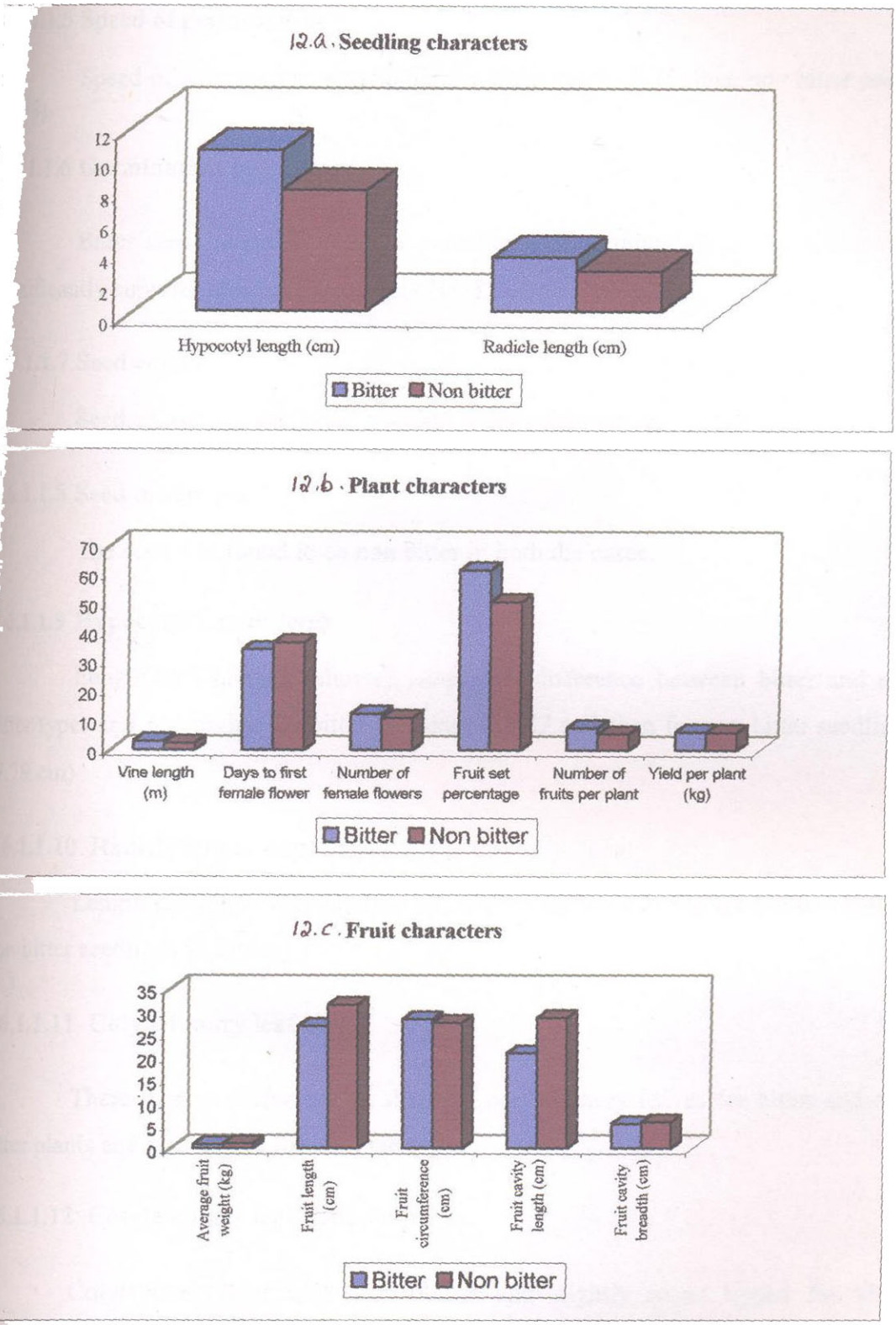


Fig. 12. Morphological characters of bitter and non bitter seedlings, plants and fruits

4.6.1.1.5 Speed of germination

Speed of germination was higher for bitter seeds (9.75) than non bitter seeds (7.08).

4.6.1.1.6 Germination percentage

Bitter seeds recorded a higher percentage of germination (88%), which was significantly superior to non bitter seeds (71%).

4.6.1.1.7 Seed colour

Seed colour for both bitter and non bitter seeds was tan.

4.6.1.1.8 Seed bitterness

The seed was found to be non bitter in both the cases.

4.6.1.1.9 Hypocotyl length (cm)

Length of hypocotyl showed significant difference between bitter and non bitter types and was higher for bitter seedlings (10.37 cm) than for non bitter seedlings (7.79 cm)

4.6.1.1.10 Radicle length (cm)

Length of radicle was significantly higher for bitter seedlings (3.49 cm) than non bitter seedlings (2.59 cm).

4.6.1.1.11 Cotyledonary leaf shape

There was no difference in shape of cotyledonary leaves for bitter and non bitter plants and was obtuse for both the types.

4.6.1.1.12 Cotyledonary leaf apex shape

Cotyledonary leaf apex was obtuse and slightly round tipped for bitter seedlings. For non bitter seedlings, it was obtuse and slightly flat tipped.

4.6.1.1.13 Hypocotyl hairiness

There was no difference in hairiness for the seedlings of both bitter and non bitter plants. Both the seedlings belonged to the group subglabrous.

4.6.1.1.14 Pigmentation

Pigmentation was observed for hypocotyl of both bitter and non bitter seedlings and was classified under the group weak.

4.6.1.1.15 Cotyledonary leaf bitterness

Cotyledonary leaves from bitter fruits tasted bitter and that from non bitter fruits were non bitter.

4.6.1.2 Plant and fruit characters

4.6.1.2.1 Vine length (m)

Average length of vine for bitter plants (2.94 m) was significantly higher than that of non bitter plants (2.29 m).

4.6.1.2.2 Days to first female flower

Significant difference was observed for number of days required for anthesis of first female flower, in bitter (34.30 days) and non bitter (36.64 days) plants.

4.6.1.2.3 Number of female flowers per plant

Number of female flowers produced was significantly higher for bitter plants (12.28) than non bitter plants (10.86).

4.6.1.2.4 Fruit set percentage

Percentage of fruit set was significantly lower in non bitter plants (50.71%) than in bitter plants (61.51%).

4.6.1.2.5 Number of fruits per plant

Number of fruits per plant was significantly higher for bitter plants (7.55) than for non bitter plants (5.49).

4.6.1.2.6 Yield per plant (kg)

No significant difference was observed for yield in bitter and non bitter plants (6.16 kg and 6.01 kg).

4.6.1.2.7 Average fruit weight (kg)

Average fruit weight was significantly higher for non bitter plants (1.10 kg) than that for bitter plants (0.83 kg).

4.6.1.2.8 Fruit length (cm)

Bitter fruits recorded an average length of 26.88 cm and was significantly different from that of non bitter fruits (31.48 cm).

4.6.1.2.9 Fruit circumference (cm)

No significant difference was observed for circumference of fruit in bitter and non bitter plants (28.37 cm and 27.57 cm)

4.6.1.2.10 Fruit cavity length (cm)

Length of fruit cavity was significantly higher for non bitter plants (28.65 cm) than bitter plants (20.86 cm).

4.6.1.2.11 Fruit cavity breadth (cm)

Breadth of fruit cavity was 5.40 cm for bitter plants and 5.74 cm for non bitter plants. No significant difference was observed between the two.

4.6.2 Biochemical characterization

Bitterness is the expression of various biochemical activities inside the plant. Bitter and non bitter fruits were analysed for the various biochemical constituents like cucurbitacin, total phenols, aminoacids and polyphenol oxidase at different stages of fruit maturity. Electrophoretic studies on seed proteins were also conducted. The results obtained are presented below.

4.6.2.1 Cucurbitacin (units g^{-1})

Content of cucurbitacin, in different parts of bitter and non bitter fruits at 5, 15 and 25 days after flower opening is given in Table 35. It's content in bitter fruits was always higher than that in non bitter fruits of the corresponding age.

4.6.2.1.1 Cucurbitacin in bitter fruit

There was significant reduction in cucurbitacin content of the bitter fruits with fruit maturity. At five days maturity, cucurbitacin content in the rind was 0.095 units g^{-1}, which decreased to 0.077 units g^{-1} by fifteen days and then to 0.069 units g^{-1} by twenty five days. In the case of flesh and placenta also, cucurbitacin content was highest at five days maturity (0.238 units g^{-1} and 0.289 units g^{-1}), which got reduced to 0.210 units g^{-1} and 0.226 units g^{-1} by twenty five days.

Significant difference was observed for cucurbitacin content in rind, flesh and placenta of a single bitter fruit.

On analyzing the cucurbitacin content of different parts viz. rind, flesh and placenta, it was found maximum in the placental portion (0.289 units g^{-1}), followed by flesh and rind (0.238 units g^{-1} and 0.095 units g^{-1}) at five days maturity. On fifteenth day, the cucurbitacin content in placenta was 0.249 units g^{-1} and that in flesh and rind were 0.221 and 0.077 units g^{-1}. The cucurbitacin content in placenta, flesh and rind were 0.226, 0.210 and 0.069 units g^{-1} respectively on 25th day.

Significant variation in cucurbitacin content was observed between stalk end, blossom end and middle portion of a fruit. On fifth day, cucurbitacin content was significantly higher at stalk end (0.214 units g^{-1}), followed by middle portion (0.205 units g^{-1}) and blossom end (0.202 units g^{-1}), which were on par. Though the content was reduced, a similar gradation was recorded on 15th day, with stalk end containing 0.187 units g^{-1}, middle portion 0.180 units g^{-1} and blossom end 0.178 units g^{-1}. In the case of twenty five days mature fruit, cucurbitacin content at blossom end and middle portion were on par (0.167 units g^{-1}) which was significantly lesser than that at stalk end (0.170 units g^{-1}). On considering the entire fruit, cucurbitacin content was highest for the placental portion at stalk end during all the stages of fruit maturity.

Table 35. Cucurbitacin content (units g^{-1}) of bitter and non bitter fruits on 5, 15 and 25 days after flowering

DAF	5 days after flowering (DAF)								15 days after flowering (DAF)								25 days after flowering(DAF)							
Fruit part	Blossom end (BE)		Middle (MID)		Stalk end (SE)		Mean		Blossom end (BE)		Middle (MID)		Stalk end (SE)		Mean		Blossom end (BE)		Middle (MID)		Stalk end (SE)		Mean	
Fruit type / Fruit part	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB
Rind	0.099	0.061	0.093	0.068	0.093	0.073	0.095	0.067	0.076	0.049	0.077	0.052	0.077	0.056	0.077	0.052	0.067	0.044	0.068	0.034	0.071	0.052	0.069	0.043
Flesh	0.232	0.146	0.229	0.149	0.252	0.152	0.238	0.149	0.217	0.127	0.216	0.141	0.230	0.144	0.221	0.137	0.207	0.115	0.211	0.121	0.210	0.132	0.210	0.123
Placenta	0.274	0.191	0.294	0.198	0.298	0.198	0.289	0.195	0.242	0.183	0.249	0.170	0.255	0.175	0.249	0.176	0.228	0.155	0.223	0.148	0.228	0.174	0.226	0.159
Mean	0.202	0.133	0.205	0.138	0.214	0.141	0.207	0.138	0.178	0.120	0.180	0.121	0.187	0.125	0.182	0.122	0.167	0.091	0.167	0.101	0.170	0.119	0.168	0.108

CD for fruit type (B/NB) : 0.002
CD for rind, flesh & placenta : 0.003
CD for BE, MID, SE : 0.003
CD for age (5, 15, 25 & DAF) : 0.002

4.6.2.1.2 Cucurbitacin in non bitter fruit

Significant reduction in cucurbitacin content was observed in non bitter fruits, with maturity. The cucurbitacin content in the rind at five days maturity stage was 0.067 units g^{-1}, which reduced to 0.052 units g^{-1} after ten days and then to 0.043 units g^{-1} after another ten days. Similar trend was observed for flesh and placenta also. The cucurbitacin content of flesh and placenta at five days maturity stage was 0.149 and 0.195 units g^{-1}. At fifteen days maturity stage it was 0.137 and 0.176 units g^{-1} and it decreased to 0.123 and 0.159 units g^{-1} by 25th day.

Significant difference in cucurbitacin content was observed for rind, flesh and placenta. Its content in placenta, flesh and rind were 0.195, 0.149 and 0.067 units g^{-1} at five days maturity; 0.176, 0.137 and 0.052 units g^{-1} at fifteen days maturity and 0.159, 0.123 and 0.043 units g^{-1} at twenty five days maturity.

Within the fruit, cucurbitacin content was highest at the stalk end (0.141 units g^{-1}), followed by middle portion (0.138 units g^{-1}) and blossom end (0.133 units g^{-1}) at five days maturity. In the case of fifteen days old fruit also, cucurbitacin content was highest at the stalk end (0.125 g^{-1}). It's content in blossom end and middle portion were on par (0.120 and 0.121 units g^{-1}). At twenty five days old stage, cucurbitacin content observed for stalk end, middle portion and blossom end were 0.119, 0.101 and 0.091 units g^{-1} respectively.

4.6.2.1.3 TLC profile of cucurbitacin

The TLC profile showed that there existed only a single spot for all the portions sampled. The results obtained are presented in Table 36. Among bitter and non bitter plants, Rf value was highest for placenta (Plate 6) during all the stages of fruit maturity, followed by flesh and rind. At five days maturity stage, Rf value for cucurbitacin in bitter and non bitter fruits were the highest for placenta (0.260 and 0.276), followed by flesh (0.252 and 0.224) and rind (0.240 and 0.196). At 15 days maturity, in the case of bitter fruits, Rf value in rind, flesh and placenta were 0.273, 0.345 and 0.354 respectively. At similar maturity sage, in non bitter fruits, Rf values were 0.136, 0.362 and 0.381 respectively. On twenty fifth day Rf values were 0.238

(rind), 0.252 (flesh) and 0.286 (placenta) for bitter fruits and 0.176 (rind) 0.214 (flesh) and 0.257 (placenta) for non bitter fruits.

Table 36. Rf values for cucurbitacin of bitter and non bitter fruits

Fruit part	5 DAF		15 DAF		25 DAF	
	Bitter	Non bitter	Bitter	Non bitter	Bitter	Non bitter
Rind	0.240	0.196	0.273	0.136	0.238	0.176
Flesh	0.252	0.224	0.345	0.362	0.252	0.214
Placenta	0.260	0.276	0.354	0.381	0.286	0.257

DAF - Days after flowering

4.6.2.2 Total phenol (mg g^{-1})

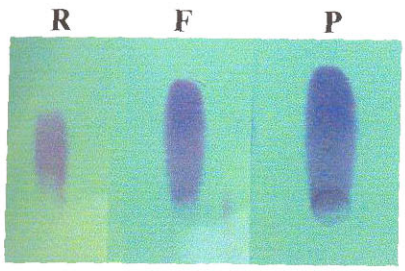
Phenol content (mg g^{-1}) in different parts of bitter and non bitter fruits at 5, 15 and 25 days after flowering is presented in Table 37.

Phenol content in bitter fruit was always higher than non bitter fruit. It was found to decrease with age of the fruit in both bitter and non bitter fruits. Significant difference in phenol content was observed with respect to type of fruit, maturity of fruit and part of the fruit.

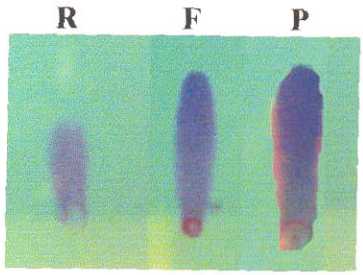
4.6.2.2.1 Phenol content in bitter fruit

The phenol content in rind decreased from 0.392 mg g^{-1} (at five days maturity) to 0.286 mg g^{-1} (at twenty five days maturity). Phenol content in flesh and placenta of five days old fruit were 0.162 and 0.120 mg g^{-1}, which decreased to 0.113 and 0.096 mg g^{-1} at fifteen days maturity and finally to 0.056 and 0.039 mg g^{-1} at twenty five days maturity.

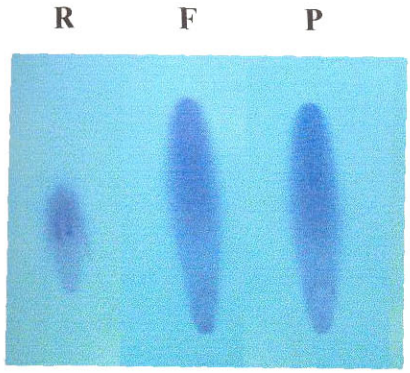
Considering rind, flesh and placenta of a fruit, phenol content was highest in the rind. Rind of five days old fruit contained 0.392 mg g^{-1} phenol, while its flesh and placenta had 0.162 and 0.120 mg g^{-1} respectively. Similarly at 15 days old stage, the phenol content in rind, flesh and placenta were 0.333, 0.113 and 0.096 mg g^{-1} respectively. In the case of 25 days old fruit, phenol content in rind was 0.286 mg g^{-1} followed by flesh (0.056 mg g^{-1}) and placenta (0.039 mg g^{-1}).



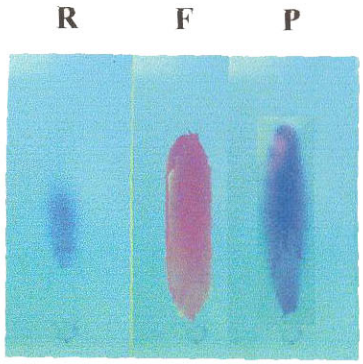
5 DAF (B)



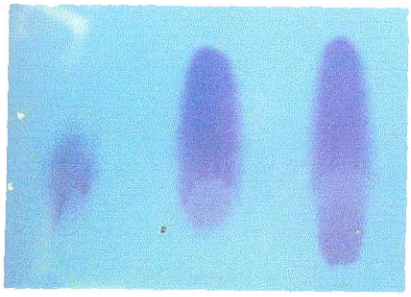
5 DAF (NB)



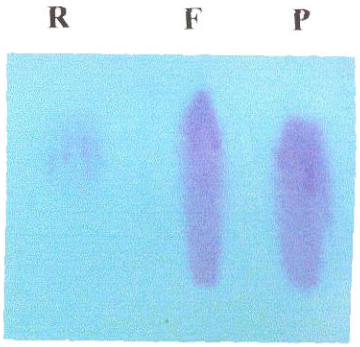
15 DAF (B)



15 DAF (NB)



25 DAF (B)



25 DAF (NB)

DAF - Days after flowering; R - Rind; F - Flesh; P - Placenta; B - Bitter; NB - Non bitter

Plate 6. TLC profile of cucurbitacin in bitter and non bitter fruits

Table 37. Phenol content (mg g^{-1}) of bitter and non bitter fruits on 5, 15 and 25 days after flowering

DAF	5 days after flowering(DAF)								15 days after flowering (DAF)								25 days after flowering (DAF)							
Fruit part	Blossom end (BE)		Middle (MID)		Stalk end (SE)		Mean		Blossom end (BE)		Middle (MID)		Stalk end (SE)		Mean		Blossom end (BE)		Middle (MID)		Stalk end (SE)		Mean	
Fruit type / Fruit part	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB
Rind	0.372	0.317	0.401	0.313	0.402	0.354	0.392	0.328	0.339	0.295	0.332	0.299	0.328	0.268	0.333	0.287	0.275	0.217	0.301	0.247	0.281	0.258	0.286	0.241
Flesh	0.155	0.132	0.165	0.136	0.167	0.143	0.162	0.137	0.114	0.088	0.123	0.082	0.101	0.094	0.113	0.088	0.045	0.035	0.056	0.037	0.069	0.049	0.056	0.040
Placenta	0.121	0.104	0.113	0.092	0.125	0.116	0.120	0.104	0.099	0.065	0.100	0.058	0.089	0.060	0.096	0.061	0.037	0.022	0.039	0.023	0.040	0.027	0.039	0.024
Mean	0.216	0.184	0.226	0.180	0.231	0.204	0.225	0.190	0.184	0.149	0.185	0.146	0.173	0.141	0.181	0.145	0.119	0.091	0.132	0.102	0.13	0.111	0.127	0.102

CD for fruit type (B/NB) : 0.005
CD for rind, flesh & placenta : 0.005
CD for BE, MID, SE : 0.005
CD for age (5, 15, 25 & DAF) : 0.005

Within the fruit, average phenol content was the highest at the stalk end of the fruit (0.231 mg g^{-1}), followed by middle portion (0.226 mg g^{-1}) and blossom end (0.216 mg g^{-1}) at five days old stage. In the case of 15 days old fruit, phenol content was highest at middle portion (0.185 mg g^{-1}), which was on par with blossom end (0.184 mg g^{-1}). The phenol content at stalk end was 0.173 mg g^{-1}. The phenol content at stalk end and middle portion were on par (0.130 and 0.132 mg g^{-1}) at 25 days old stage. At this stage, average phenol content at blossom end was 0.119 mg g^{-1}.

4.6.2.2.2 Phenol content in non bitter fruit

At five days maturity stage, phenol content in rind, flesh and placenta of non bitter fruits were 0.328, 0.137 and 0.104 mg g^{-1} respectively, which reduced to 0.287, 0.088 and 0.061 mg g^{-1} at 15 days maturity stage and to 0.241, 0.040 and 0.024 mg g^{-1} at twenty five days maturity stage.

The highest phenol content was recorded for rind of the fruit at 5, 15 and 25 days old stage (0.328, 0.287 and 0.241 mg g^{-1}). The average phenol content in the flesh was 0.137 mg g^{-1} at five days maturity, 0.088 mg g^{-1} at 15 days maturity and 0.04 mg g^{-1} at 25 days maturity. Lowest phenol content was recorded for placenta, which ranged from 0.104 mg g^{-1} (5 days old fruit) to 0.024 mg g^{-1} (25 days old fruit).

Within the fruit, phenol content at the stalk end was significantly higher on 5^{th} day (0.204 mg g^{-1}), 15^{th} day (0.141 mg g^{-1}) and 25^{th} day (0.111 mg g^{-1}) of flowering. In the blossom end and middle portion, phenol content were 0.184 and 0.180 on 5^{th} day; 0.149 and 0.146 mg g^{-1} on 15^{th} day and 0.091 and 0.102 mg g^{-1} on 25^{th} day.

4.6.2.3 Total free aminoacids (mg g^{-1})

Significant difference in amino acid content was observed with respect to age of the fruit, part of the fruit and fruit type (Table 38).

4.6.2.3.1 Amino acid content in bitter fruit

The amino acid content was the highest at five days maturity stage of fruit (rind = 0.056; flesh = 0.042 and placenta = 0.040 mg g^{-1}), followed by 15 days

Table 38. Amino acid content (mg g^{-1}) at different parts of bitter and non bitter fruits on 5, 15 and 25 days after flowering

DAF	5 days after flowering(DAF)								15 days after flowering(DAF)								25 days after flowering(DAF)							
Fruit part	Blossom end (BE)		Middle (MID)		Stalk end (SE)		Mean		Blossom end (BE)		Middle (MID)		Stalk end (SE)		Mean		Blossom end (BE)		Middle (MID)		Stalk end (SE)		Mean	
Fruit type / Fruit part	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB
Rind	0.056	0.028	0.054	0.031	0.058	0.027	0.056	0.029	0.071	0.037	0.074	0.038	0.073	0.039	0.073	0.039	0.087	0.047	0.086	0.052	0.085	0.052	0.086	0.050
Flesh	0.039	0.025	0.039	0.022	0.047	0.023	0.042	0.023	0.056	0.031	0.059	0.034	0.058	0.032	0.058	0.032	0.072	0.040	0.069	0.044	0.069	0.046	0.070	0.043
Placenta	0.039	0.021	0.041	0.021	0.041	0.020	0.040	0.021	0.048	0.028	0.051	0.028	0.054	0.025	0.051	0.027	0.067	0.038	0.061	0.039	0.059	0.030	0.062	0.036
Mean	0.045	0.025	0.045	0.025	0.049	0.023	0.046	0.024	0.058	0.032	0.061	0.033	0.062	0.032	0.061	0.033	0.075	0.042	0.072	0.045	0.071	0.043	0.073	0.043

CD for fruit type (B/NB) : 0.001
CD for rind, flesh & placenta : 0.002
CD for BE, MID, SE : 0.002
CD for age (5, 15, 25 & DAF) : 0.002

(rind = 0.073; flesh = 0.058 and placenta = 0.051 mg g^{-1}) and 25 days maturity stage (rind = 0.086; flesh = 0.070 and placenta = 0.062 mg g^{-1}).

Within a fruit, amino acid content was the highest in rind (0.056 mg g^{-1} at 5th day, 0.073 mg g^{-1} at 15th day and 0.086 mg g^{-1} at 25th day), followed by flesh and placenta. The amino acid content in flesh and placenta were 0.042 mg g^{-1} and 0.04 mg g^{-1} at 5 days maturity stage; 0.058 mg g^{-1} and 0.051 mg g^{-1} at 15 days maturity stage and 0.070 mg g^{-1} and 0.062 mg g^{-1} at 25 days maturity stage.

In five days old fruit, amino acid content was highest at stalk end (0.049 mg g^{-1}), followed by blossom end and middle portion (0.045 mg g^{-1}). At 15 days maturity stage, the amino acid content in stalk end, middle portion and blossom end were 0.062, 0.061 and 0.058 mg g^{-1} respectively. The stalk end recorded an average amino acid content of 0.071 mg g^{-1} at 25 days maturity stage. The amino acid content at blossom end and middle portion at this stage were 0.075 and 0.072 mg g^{-1} respectively.

4.6.2.3.2 Amino acid content in non bitter fruit

The amino acid content in rind, flesh and placenta of 5 days old fruit were 0.029, 0.023 and 0.021 mg g^{-1} respectively, which was reduced to 0.039, 0.032 and 0.027 mg g^{-1} in 15 days and finally to 0.050, 0.043 and 0.036 mg g^{-1} in 25 days.

Rind of the fruit recorded highest amino acid content at 5th, 15th and 25th day of fruit set (0.029, 0.039 and 0.050 mg g^{-1} respectively), followed by flesh (0.023, 0.032 and 0.043 mg g^{-1} respectively) and placenta (0.021, 0.027 and 0.036 mg g^{-1} respectively).

In general, no significant difference was observed for amino acid content in stalk end (0.023 mg g^{-1}), middle portion (0.025 mg g^{-1}) and blossom end (0.025 mg g^{-1}) of 5 days old fruit. At 15 days maturity also, the amino acid content at stalk end, blossom end and middle portion were on par (0.032, 0.032 and 0.033 mg g^{-1} respectively). In 25 days old fruit, amino acid content at middle region (0.045 mg g^{-1}) was higher than stalk end (0.043 mg g^{-1}) and blossom end (0.042 mg g^{-1}).

Table 39. Polyphenol oxidase activity at different parts of bitter and non bitter fruits at 5, 15 and 25 days after flowering

DAF	5 days after flowering(DAF)						15 days after flowering(DAF)						25 days after flowering(DAF)					
Fruit part	Rind		Flesh		Placenta		Rind		Flesh		Placenta		Rind		Flesh		Placenta	
Fruit type / Time (sec.)	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB
30	0.239	0.235	0.065	0.059	0.021	0.018	0.225	0.206	0.043	0.040	0.017	0.016	0.166	0.152	0.010	0.006	0.013	0.012
60	0.309	0.300	0.089	0.080	0.024	0.022	0.276	0.254	0.059	0.052	0.020	0.019	0.221	0.204	0.015	0.012	0.015	0.013
90	0.354	0.361	0.110	0.098	0.027	0.025	0.323	0.300	0.074	0.064	0.022	0.022	0.264	0.244	0.018	0.013	0.016	0.014
120	0.390	0.394	0.129	0.116	0.029	0.028	0.357	0.336	0.086	0.078	0.025	0.023	0.291	0.267	0.024	0.016	0.018	0.016
150	0.420	0.419	0.143	0.133	0.030	0.029	0.387	0.367	0.104	0.090	0.026	0.025	0.314	0.282	0.029	0.022	0.020	0.017
180	0.438	0.435	0.155	0.145	0.031	0.030	0.406	0.392	0.118	0.101	0.027	0.026	0.340	0.324	0.034	0.027	0.021	0.018
210	0.455	0.450	0.163	0.154	0.032	0.031	0.425	0.413	0.125	0.113	0.028	0.026	0.358	0.333	0.038	0.033	0.021	0.019
240	0.460	0.459	0.170	0.161	0.032	0.031	0.441	0.426	0.134	0.125	0.028	0.026	0.370	0.349	0.044	0.036	0.021	0.019
270	0.491	0.476	0.177	0.168	0.032	0.031	0.450	0.434	0.140	0.133	0.028	0.026	0.379	0.353	0.050	0.041	0.020	0.018
300	0.500	0.475	0.191	0.174	0.031	0.031	0.451	0.435	0.145	0.138	0.028	0.025	0.380	0.357	0.054	0.042	0.020	0.017
330	0.494	0.475	0.191	0.179	0.031	0.030	0.449	0.435	0.145	0.141	0.027	0.025	0.379	0.360	0.054	0.043	0.019	0.016
360	0.491	0.471	0.190	0.178	0.030	0.030	0.445	0.434	0.145	0.139	0.027	0.025	0.377	0.358	0.052	0.042	0.017	0.014

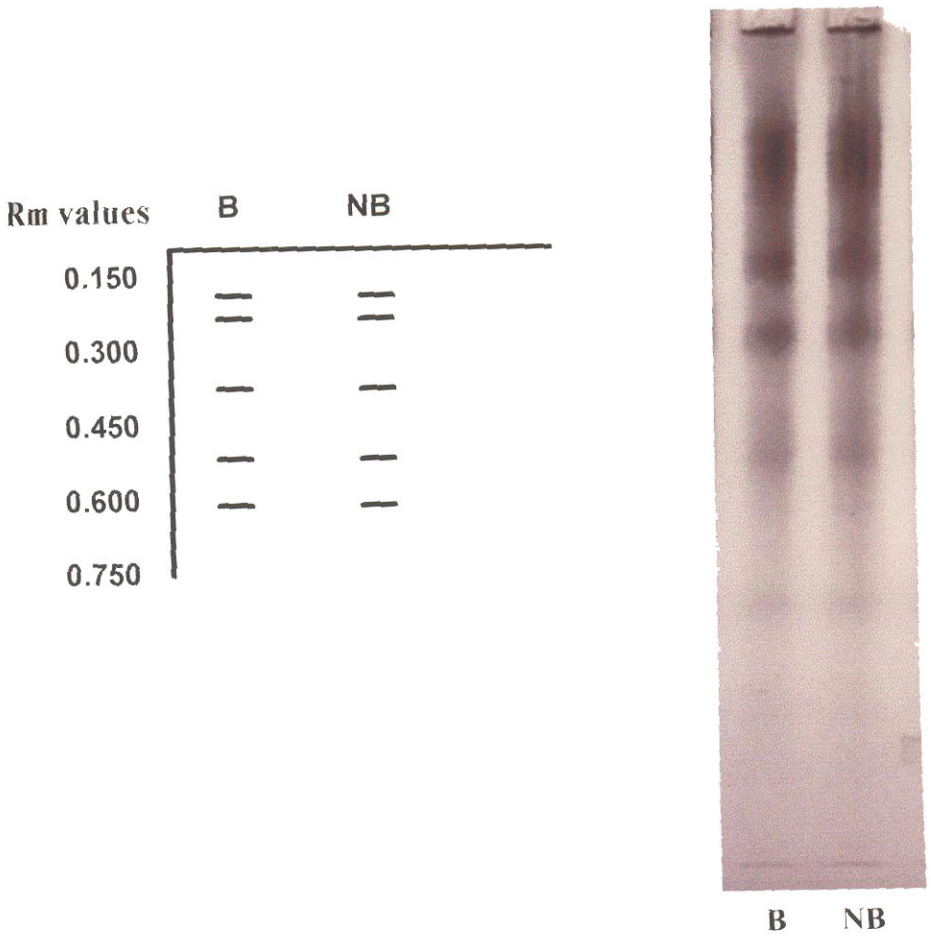


Plate 7. Protein banding pattern of bitter and non bitter seeds

4.6.2.4 Polyphenol oxidase activity

Polyphenol oxidase activity in rind, flesh and placenta of bitter and non bitter fruits at different stages of maturity is given in Table 39. The activity was higher in bitter fruits than in non bitter fruits.

In the case of bitter fruits, the enzyme activity was highest at five days maturity stage (rind = 0.500; flesh = 0.191 and placenta = 0.032) followed by 15 days (rind = 0.451; flesh = 0.145 and placenta = 0.028) and 25 days maturity stage (rind = 0.380; flesh = 0.054 and placenta = 0.021).

The enzyme activity of non bitter fruits also decreased with fruit maturity. In the rind, the OD value were 0.476, 0.435 and 0.360 at 5th, 15th and 25th day of maturity respectively. In the flesh, OD value in five days old fruit was 0.179, which decreased to 0.141 by 15th day and to 0.043 by 25th day. Enzyme activity in placenta was 0.031 at 5 days maturity stage; 0.026 at 15 days maturity stage and 0.019 at 25 days maturity stage.

4.6.2.5 Banding pattern of seed protein

Electrophoretic studies of seed protein showed that there was no difference between seeds from bitter and non bitter plants (Plate 7). There existed five bands in both the cases with Rm values 0.155, 0.282, 0.366, 0.500 and 0.683.

4.7 PEST AND DISEASE INCIDENCE

The major pests observed in the field were red pumpkin beetle, and fruit fly. Powdery mildew was noticed as the major disease. The extent of damage observed in both bitter and non bitter types are recorded in Table 40. No significant difference was observed between the two types for the pest and disease incidence.

Table 40. Extent of damage by pests and disease on bitter and non bitter plants

Sl. No.	Name of pest/disease	Sample size	Percentage of infected samples		t value
			Bitter	Non bitter	
1	Red pumpkin beetle	40 seedlings	78.25	77.75	NS
2	Fruit fly	30 fruits	17.99	16.33	NS
3	Powdery mildew	40 plants	88.00	88.25	NS

4.8 WEATHER AND BITTERNESS

Data on various weather parameters viz., maximum temperature, minimum temperature, rainfall and relative humidity during the period of study is furnished in Appendix-II. It was observed that irrespective of weather conditions, throughout the course of the study there was no change in the expression of bitterness. Fruits borne on homozygous bitter plants were always bitter and that on non bitter plants were always non bitter.

Discussion

5. DISCUSSION

Most of the currently cultivated cucurbit varieties have been evolved from bitter wild ancestral species (Schlosser, 1950). Generally, wild forms produce large number of small sized bitter fruits which helps them to thrive adverse conditions. By continuous selection for non bitter and big fruited types, cultivated varieties have now become non bitter. Bitterness is a natural adaptive mechanism of plants to protect itself from animals.

Several studies on genetics of bitterness and effect of environmental factors on expression of fruit bitterness have been conducted in many cucurbits. However no such work has been attempted in oriental pickling melon and hence this study was undertaken with a view to understand the inheritance pattern of bitterness and the chances of reducing bitterness by management practices in oriental pickling melon. The possibility of identifying bitter plants at early stages of growth was also explored into.

5.1 STUDIES ON INHERITANCE OF BITTERNESS

The studies conducted to know the inheritance pattern of bitterness revealed that bitterness in this species is least influenced by environmental factors. The homozygous bitter and non bitter lines developed by continuous selfing were crossed in both directions and the resultant generations viz., F_1, F_2, BC_1, BC_2, F_1^1, F_2^1, BC_1^1 and BC_2^1 were evaluated for bitterness. It was observed that all the plants in F_1 and F_1^1 generations produced bitter fruits. Results of the back cross studies revealed that back crossing with bitter parents produced bitter fruited plants only and that with non bitter parent produced bitter fruited as well and non bitter fruit plants. The F_2 generation also segregated into bitter fruited and non bitter fruited types. The segregating bitter and non bitter lines in F_2 and back cross generations fitted well into the ratio for inhibitory gene action i.e., 13:3. Thus the gene action for bitterness can be explained as digenic, with least influence of environmental factors. This was also evident from the study that irrespective of weather conditions, sources of nutrients, physiological conditions and maturity of the plant, all the fruits borne on bitter plants were bitter and that on non bitter plants were non bitter. In the cultivated non bitter forms of *Cucumis melo*

var. *conomon*, a dominant gene 'N' is controlling bitter principle production. But due to outcrossing with some wild species, a dominant gene (I) having inhibitory action on 'N' might have entered the genome of non bitter forms, thus resulting in increased production of bitter principles in bitter fruits. Though most of the scientists have reported monogenic dominant nature of the gene responsible for bitterness in different cucurbits, occurrence of a suppressor gene controlling bitterness was reported by Rehm (1960) in *Cucurbita pepo*. In water melon, Chambliss *et al.* (1968) proved the presence of a modifier gene for controlling bitterness.

On analyzing the association of different morphological characters with cucurbitacin content, the characters vine length, number of fruits per plant and fruit set percentage were significantly and positively associated with bitterness. In general, bitter types had lesser fruit weight and early maturity. Most of these associated characters usually seen in wild species supports the possibility of transfer of gene responsible for bitterness from wild species to cultivated form.

Results of the path coefficient analysis indicated that selection for non bitter forms can be done through the selection of plants with higher fruit weight, lesser number of fruits and lesser vine length.

The influence of genetic and environmental factors expressed as variability was studied by determining the magnitude of phenotypic and genotypic coefficients of variation, heritability and genetic advance. The trends of above parameters are presented in Fig. 2.

The cucurbitacin content showed very high heritability, genetic advance and coefficient of variation. The characters like vine length, fruit set percentage, number of fruits and fruit weight, were having high heritability and therefore, possibly the selection for non bitter types can be done by taking these characters into consideration.

5.2 EFFECT OF FOREIGN POLLEN ON INDUCTION OF BITTERNESS

In cucurbits, chances of induction of bitterness through metaxenia have been reported (Sheshadri, 1986). In this experiment, efforts were made to know whether

there is metaxenic effect for bitterness in oriental pickling melon. Nine cucurbitaceous species including bitter fruited oriental pickling melon were used in the present study and the results obtained are discussed below.

5.2.1 Evaluation of fruits of F_0 generation

When the pollen from different species were used alone (without mixing pollen from any other species) for controlled pollination, to study their effect on fruit set and fruit qualities in *Cucumis melo* var. *conomon*, it was found that no fruit set was obtained except with *Cucumis melo* var. *callosus* (40%). As evident from *in vivo* pollen germination studies, the pollen of *C. melo* var. *agrestis, Cucumis trigonus*, bittergourd, snakegourd and wild *Luffa* germinated on the stigma of *C. melo* var. *conomon,* but no fruit set was obtained. The species *Trichosanthes lobata* and *T. cucumerina* did not even germinate on the stigma of *C. melo* var. *conomon.* This shows that there exists certain natural mechanism to prevent outcrossing of cultivated *C. melo* var. *conomon* with other cucurbitaceous species (used in the study) except *Cucumis melo* var. *callosus*..

In all the treatments where external pollen grains were mixed with that of oriental pickling melon, fruit set was observed. Fruit size and seed count were more, when the amount of pollen from oriental pickling melon was increased from 75 to 90 per cent. It throws light on the fact that the fruit size and the number of seeds is dependent on availability of compatible pollen grains. This also revealed that oriental pickling melon is fertilized by its own pollen, rather than that of external pollen. This is an adaptive mechanism of nature to prevent free outcrossing.

The non bitter form of oriental pickling melon was pollinated with pollen from different wild and cultivated cucurbitaceous species as per the treatment combinations. The combinations which resulted in fruit set were evaluated for fruit bitterness. All the fruits were found to be non bitter. Even the fruits formed by pollinating non bitter oriental pickling melon with bitter oriental pickling melon were non bitter. Thus the possibility of metaxenia in inducing bitterness in oriental pickling melon can be completely ruled out. This is in accordance with the report of Robinson and Decker-Walters (1997) in *Cucurbita pepo* and against the reports of Seshadri (1986)

in cucumber and bottle gourd. The result of *in vivo* pollen germination studies confirmed that even the germinating bitter pollens could not induce fruit bitterness in F_0.

5.2.2 Evaluation of fruits of F_1 generation of the cross involving pollen from external sources

The only species which was found to be crossable with *Cucumis melo* var. *conomon* was *Cucumis melo* var. *callosus*. Free crossability of the species *Cucumis melo* var. *callosus* with *Cucumis melo* was reported by Robinson and Kowalewski (1978) and Parthasarathy (1980). The F_1 plants of the treatments in which pollen of *Cucumis melo* var. *callosus* was mixed with oriental pickling melon, included both bitter and non bitter fruited forms. Among the F_1s, bitter fruits had smaller fruit size, showing resemblance to *Cucumis melo* var. *callosus* (Plate 4). This F_1 on continuous crossing with non bitter *C. melo* var. *conomon*, may result in fruits with all the characteristics similar to *C. melo* var. *conomon,* but bitter in taste. This proved the possibility of pollen from both the species fertilizing the ovule of oriental pickling melon. All the resultant F_1 plants of the cross with *C. melo* var. *callosus* produced bitter fruits. The F_2 generation of the cross segregated in the ratio 13:3 with 50 to 70 per cent probability indicating the possibility of inhibitory gene action. Similar results were obtained in the cross between bitter and non bitter oriental pickling melon. Since segregation and gene action for bitterness is alike in both the crosses, there is every possibility that the bitter gene got transferred from *C. melo* var. *callosus* to the cultivated oriental pickling melon by natural outcrossing. Thus, in nature, the presence of a few pollen from the wild bitter fruited *Cucumis melo* var. *callosus* on the stigma of oriental pickling melon, may result in bitter fruits. Transfer of bitter genes from wild to cultivated cucurbita was reported by Rymal *et al*. (1984).

In Kerala, occurrence of *C. melo var. callosus* is limited, hence chances of outcrossing is very meager. However, once this gene is transferred to cultivated forms, due to its dominant nature, may spread very fast.

5.3 SOURCE EFFECT OF MANURES ON BITTERNESS

The study on source effect of manures was taken up based on the general belief among farmers that application of different organic manures and urea will result

in bitterness of fruits in oriental pickling melon. Hence, different organic manures along with chemical fertilizers were included as treatments and their effect on bitterness and yield characters were recorded. The results obtained are discussed hereunder.

5.3.1 Effect of source of nutrients on fruit bitterness

Evaluation of fruit bitterness, after the application of different sources of manures showed that source of nutrients did not influence expression of fruit bitterness. The expression of bitterness was not altered even in the treatment where no fertilizer or manure was applied. Thus the possibility of modification of bitterness in oriental pickling melon fruits, by chemical fertilizers, poultry manure, goat manure, farmyard manure, groundnut cake, neemcake and the insecticide furadan was ruled out. Even change in soil pH by addition of lime did not alter the expression of bitterness as evidenced by T_7 (Chemical fertilizers + lime) and T_8 (Groundnut cake + lime).

5.3.2 Effect of source of nutrients on yield and yield attributes

The highest yield during both the seasons was recorded for the treatment with poultry manure in bitter as well as non bitter lines. Increase in yield with application of poultry manure was reported by Abusaleha and Shanmugavelu in bhindi (1988), Prezotti *et al.* (1989) and Silva and Vizzotto (1989) in tomato and Prasanna (1998) in brinjal. The role of N in favouring growth and development through organic sources has been already proved by Sorin and Tanaka (1991). The chemical fertilizers release the nutrients immediately after application and is exhaustive. The nitrogen released from poultry manure is readily available to plants and uric acid contained in the poultry manure, with 60 percent of N in the ammoniacal form can be efficiently utilized by the plants (Smith, 1950).

A thorough perusal of data showed that in bitter plants, high yield was mainly contributed by number of fruits and in non bitter plants by average weight of fruits. The number and size of fruits were significantly influenced by the various

sources of nitrogen. Number of fruits per plant was highest for poultry manure during both the seasons which was on par with chemical fertilizers during first season. High weight of fruits was noticed for non bitter plants receiving poultry manure during both the seasons. Increase in fruit size and fruit number with application of pigeon manure was reported by Attia and Nassar (1958) in watermelon. High decomposition rate and low lignin to nitrogen ratio (Prashant, 2002) of poultry manure also added the easy availability of nutrients to plants.

High content of bioavailable form of P in poultry manure (Prashant, 2002) along with K and Ca also contributed to high yield. The solubilizing effect of decomposing organic matter on release of P was reported by Nagarajah *et al.* (1989). The low cost and high returns makes poultry manure the best treatment with benefit cost ratio 1.85. In general, the organic manures improved the soil physical characters and thereby enhanced the availability of micro and macro nutrients. It can also be assumed that the released nutrients have positively influenced the various critical physiological processes leading to high yield in oriental pickling melon.

Drastic reduction in yield, number of fruits, number of female flowers, fruit set percentage, fruit weight and fruit size for treatments receiving no fertilizer / manure showed the poor fertility status of the soil used for raising the crop. Lowest yield, reduced number of fruits per vine and small fruit size from plants receiving no fertilizer / manure was also observed by Nirmala *et al.* (1999).

5.4 EFFECT OF PRUNING ON FRUIT BITTERNESS

Pruning and leaf thinning are operations usually conducted for effective partitioning of dry matter to produce more number of good quality fruits. In vegetables, pruning is adopted mainly in green house crops like tomato and cucumber, with a view to improve plant stature and yield. Recently, pruning operations are being done in cucurbits for high, early and uniform fruit production. The yield may increase or decrease with the extent of severity of pruning and leaf thinning. In the present study, severe pruning and thinning operations were done to find out its' effect on fruit bitterness. The effect of various treatments adopted on yield and yield attributes were also studied and the results obtained are discussed here under.

The expression of fruit bitterness did not vary with treatments, i.e., all the fruits borne on bitter plants were bitter and those on non bitter plants were non bitter. Thus the present study lead to the conclusion that fruit bitterness in oriental pickling melon cannot be managed by pruning or thinning operations. However, Kano *et al.* (1997) reported that in cucumber, production of bitter fruits were more on primary laterals, for the various pruning treatments adopted.

In general, yield, plant vigour and other yield attributes varied with the different pruning and thinning operations. It was evident that pruning failed to impart any significant positive effect on yield, number of fruits, number of female flowers, fruitset percentage, fruit weight, fruit length, fruit breadth, days to first female flower opening, duration of the crop, fruit cavity size and number of branches.

Significant reduction in yield was noticed due to the severe pruning treatments adopted. When all the primary and secondary branches were removed, a reduction upto 90 percentage was noticed in yield. This is because, in oriental pickling melon, fruits are mainly produced on primary and secondary branches. Reduction in photosynthetic efficiency of plants due to the severity of pruning might also have contributed to poor yield. In normal cases, during pruning, development of fruits near the point of cutting will be enhanced due to removal of competing sinks (Shishido *et al.*, 1990). But, in the present study, since entire branch, which bear the female flower primordia were removed, there was no sink to effectively utilize the photosynthates. Similar reports of reduction in yield due to unjudicious pruning are available in watermelon (You Tiao *et al.*, 1996), bottle gourd (Damato *et al.*, 1998) and tomato (Salinas *et al.*, 1994). In contrast, Young Hah *et al.* (1995), Aurin and Rasco (1988) and Mougou *et al.* (1991) reported increase in yield by pruning in cucumber, smooth gourd and muskmelon respectively.

In the case of leaf thinning operations, no significant effect was noticed for all the characters studied including yield. Even when 50 percentage of leaves were removed, the reduction in yield was not significant. This may be due to efficient translocation and utilization of photosynthates, produced by the existing leaves. This

was in accordance with the findings of Humphries and Vermillion (1994) who reported that removal of 50 per cent of leaves did not significantly affect the yield in cucumber. Lack of effect of leaf pruning on yield in tomato was reported by Salinas *et al.* (1994).

5.5 BITTERNESS IN RELATION TO AGE OF THE PLANT.

Formation of different metabolic products may change with the physiological condition of the plants. Hence to find out whether change in bitterness is occurring in oriental pickling melon with respect to plant vigour and age, the present study was undertaken. The results obtained are discussed below

The results showed that all the fruits borne on bitter plants, irrespective of age of the plant, were bitter. Similarly all the fruits borne on non bitter plants were non bitter. This was contradictory to the report of Kano *et al.* (1997) where fruits formed on young vigorous plants were bitter and that on old less vigorous plants were non bitter. The present study indicated that bitterness is a genetic character least affected by the physiological condition as evidenced from pruning and thinning studies. Moreover, increased plant vigour and more number of fruits in bitter plants compared to non bitter plants, pointed out the possibility of transfer of gene for bitterness from wild types to cultivated forms, since these characters are usually associated with wild species.

5.6 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF BITTERNESS AND ITS EARLY DETECTION

Considering the possibility of linkage between the genes governing different morphological characters and the genes for bitterness, the seeds, seedlings and plants from homozygous material were studied in detail to facilitate identification of bitter and non bitter plant at any stage of growth. Also the biochemical parameters like cucurbitacin, total phenol, total free aminoacid and poly phenol oxidase activity were estimated for both bitter and non bitter fruits. Analysis were done for rind, flesh and placenta from the stalk end, middle portion and blossom end of fruit separately at 5^{th}, 15^{th} and 25^{th} day of fruit set. Electrophoretic study of seed protein was also conducted. The results obtained are discussed here under.

Though not found to be a key character in differentiating the two, many morphological characters were different for the bitter and non bitter homozygous materials used for the study. The seasonal effect on quantitative traits cannot be ruled out and a general idea relating to the morphological characters of bitter and non bitter plants is presented hereunder.

In general, the size of seeds from bitter fruits were smaller than that from non bitter fruits, as indicated by length of seed, breadth of seed, thickness of seed and weight of hundred seeds. Eventhough the seeds were smaller, speed of germination (9.75) and germination percentage (88) were higher for bitter seeds. Small seed size and high seedling vigour are considered to be characters of wild species. This supports the result that genes responsible for bitterness reached non bitter forms of cultivated *Cucumis melo* var. *conomon* possibly from the wild relative *Cucumis melo* var. *callosus,* as was concluded from the crossability studies. The fruits and seeds of *Cucumis melo* var. *callosus* are smaller than the cultivated oriental pickling melon.

Even though hypocotyl length (10.37 cm) and radicle length (3.49 cm) were higher for bitter seedlings, other seedling characters like cotyledonary leaf shape, hairiness and pigmentation were similar for both bitter and non bitter forms. High phenol content in bitter plants compared to non bitter plants was reported by Kannaiyan and Purushothaman (1973) in muskmelon. Increased seedling vigour, earliness and production of more number of fruits in bitter plants can be attributed to high content of phenol, which is having a hormonal action on growth (Mcclure, 1979).

Comparative evaluation of fruit characters revealed that total number of female flowers, and fruit set were higher in bitter plants than in non bitter plants. The higher phenol content in bitter plants might have a positive response in the flowering and fruit set. Also, bitter plants were of short duration and exhibited higher vine length and produced large number of small sized fruits.

For early detection of bitter and non bitter plants, the seeds as such and the seedlings were evaluated organoleptically. The dried mature seeds were non bitter in the case of both bitter and non bitter types. But on germination the bitter forms started

expressing bitterness. Initiation of synthesis of bitter principles with the onset of seed germination and its increase rapidly in roots and cotyledons upto six days was earlier reported by Rehm (1960). The radicle, the cotyledons and the hypocotyl regions were also bitter for seedlings raised from bitter fruits. Under field conditions, testing of radicle and hypocotyl region is practically impossible since it will result in the destruction of the seedling. Hence testing of the part of cotyledonary leaves is sufficient for the early detection of bitter seedlings. A detection of a part of the cotyledonary leaves will not hinder further development of the plant and this method can be widely adopted under field conditions where a mixture of both the bitter and non bitter plants may be present.

The data revealed that unit content of cucurbitacin in bitter fruits was higher than that in non bitter fruits at all stages of fruit maturity. The percentage content of phenol, aminoacid and polyphenol oxidase activity were also higher in bitter fruits than in non bitter fruits. The bitter principle cucurbitacin is present in both bitter and non bitter fruits and the trend in change of different biochemical constituents, viz. cucurbitacin, phenol, amino acid and polyphenol oxidase, due to age and fruit part was similar in both the cases (Figs.13 to 17). Hence this can be considered as the character of the variety selected for study. Outcrossing of cultivated species with wild species might have resulted in increased production of bitter principles, which is supported by the results obtained from studies on gene action and studies on crossing of oriental pickling melon with the wild cucurbit *C. melo* var. *callosus*.

The present study revealed that irrespective of bitter and non bitter fruits, cucurbitacin was the highest in the placenta, followed by flesh and almost nil in the rind. At 15 days of maturity, the placental portion contained 0.249 and 0.176 units g^{-1} of cucurbitacin, flesh contained 0.221 and 0.137 units g^{-1} of cucurbitacin and rind contained 0.077 and 0.052 units g^{-1} of cucurbitacin in the case of bitter and non bitter fruits. The phenol content was present at varying rates with a high concentration in the rind. Reports showed that the concentration of cucurbitacin in different species may be varied in placenta and flesh. Rehm *et al.* (1957) and Jaworski *et al.* (1985) observed

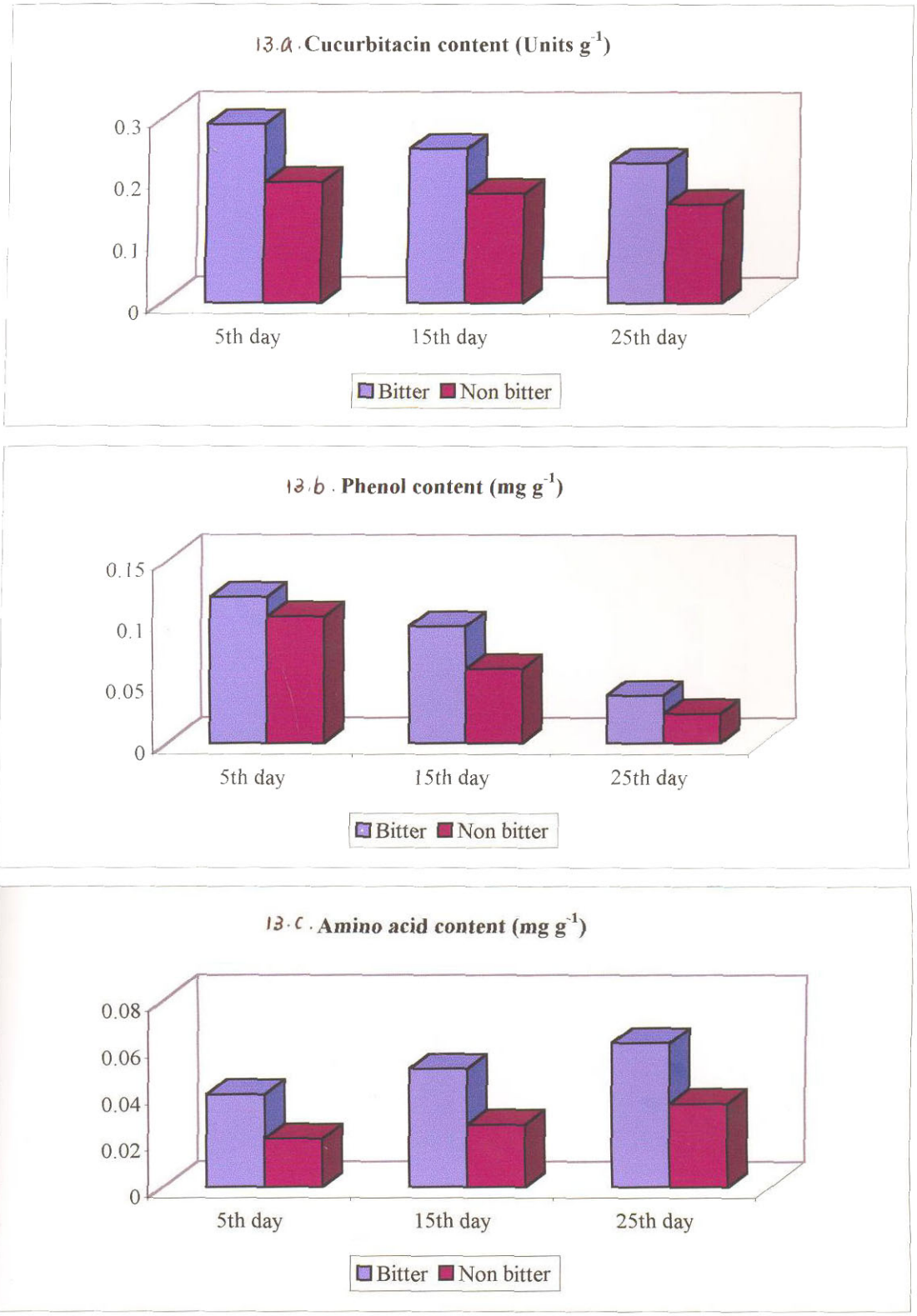


Fig. 13. Change in cucurbitacin, phenol and amino acid content with fruit maturity

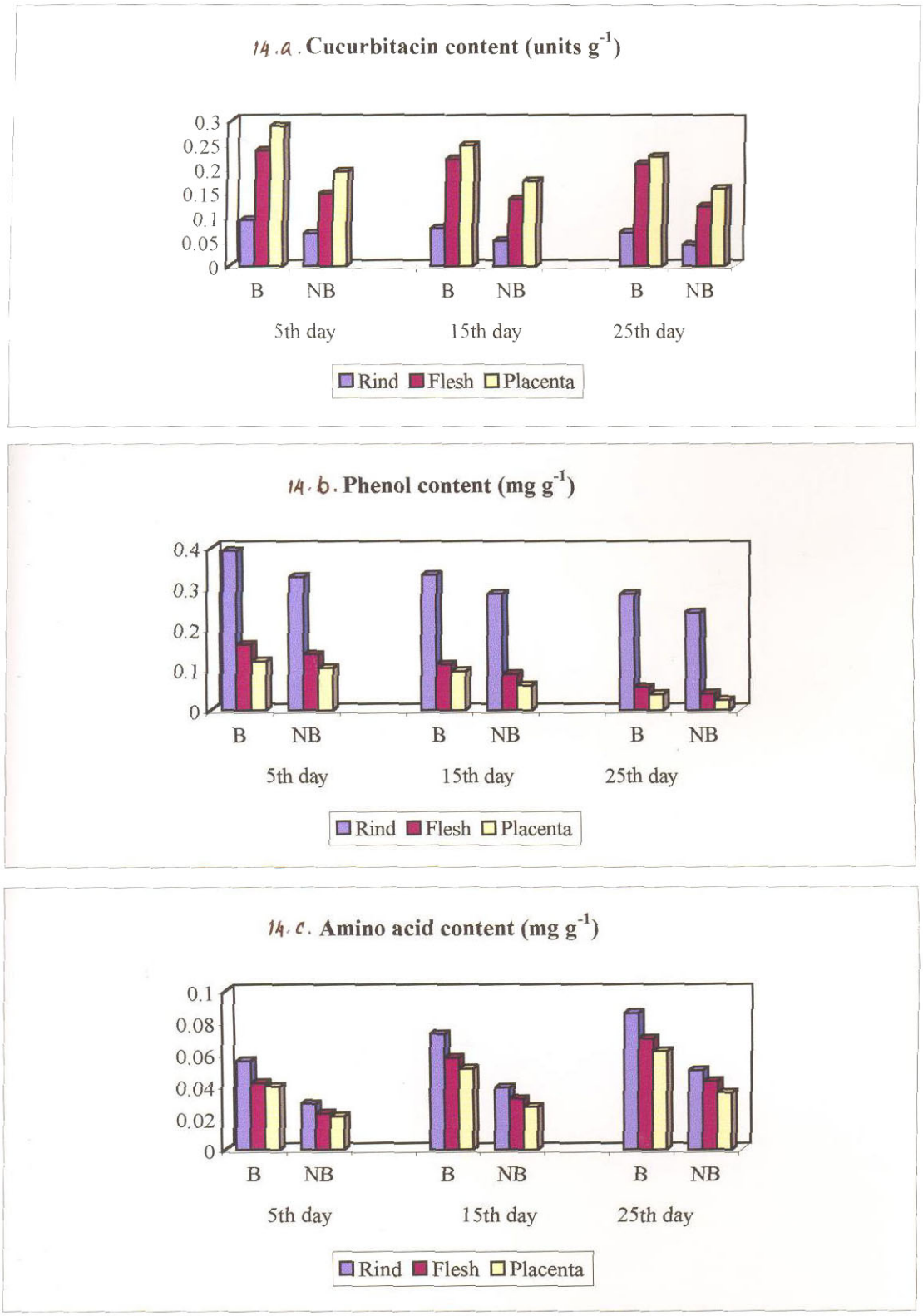


Fig. 14. *Content of cucurbitacin, phenol and amino acid in rind, flesh and placenta of fruits*

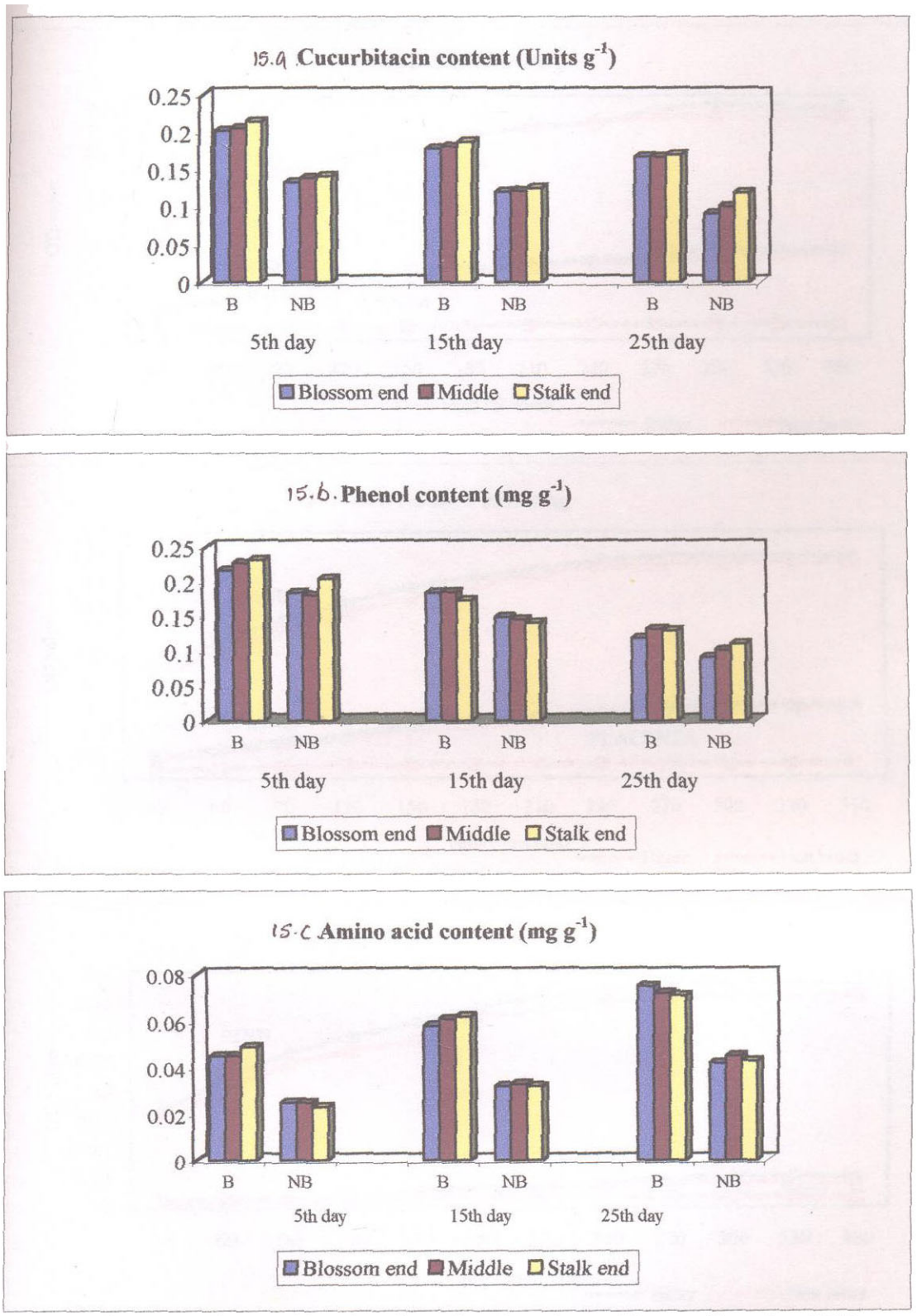


Fig. 15. Content of cucurbitacin, phenol and amino acid at stalk end, middle portion and blossom end of fruits

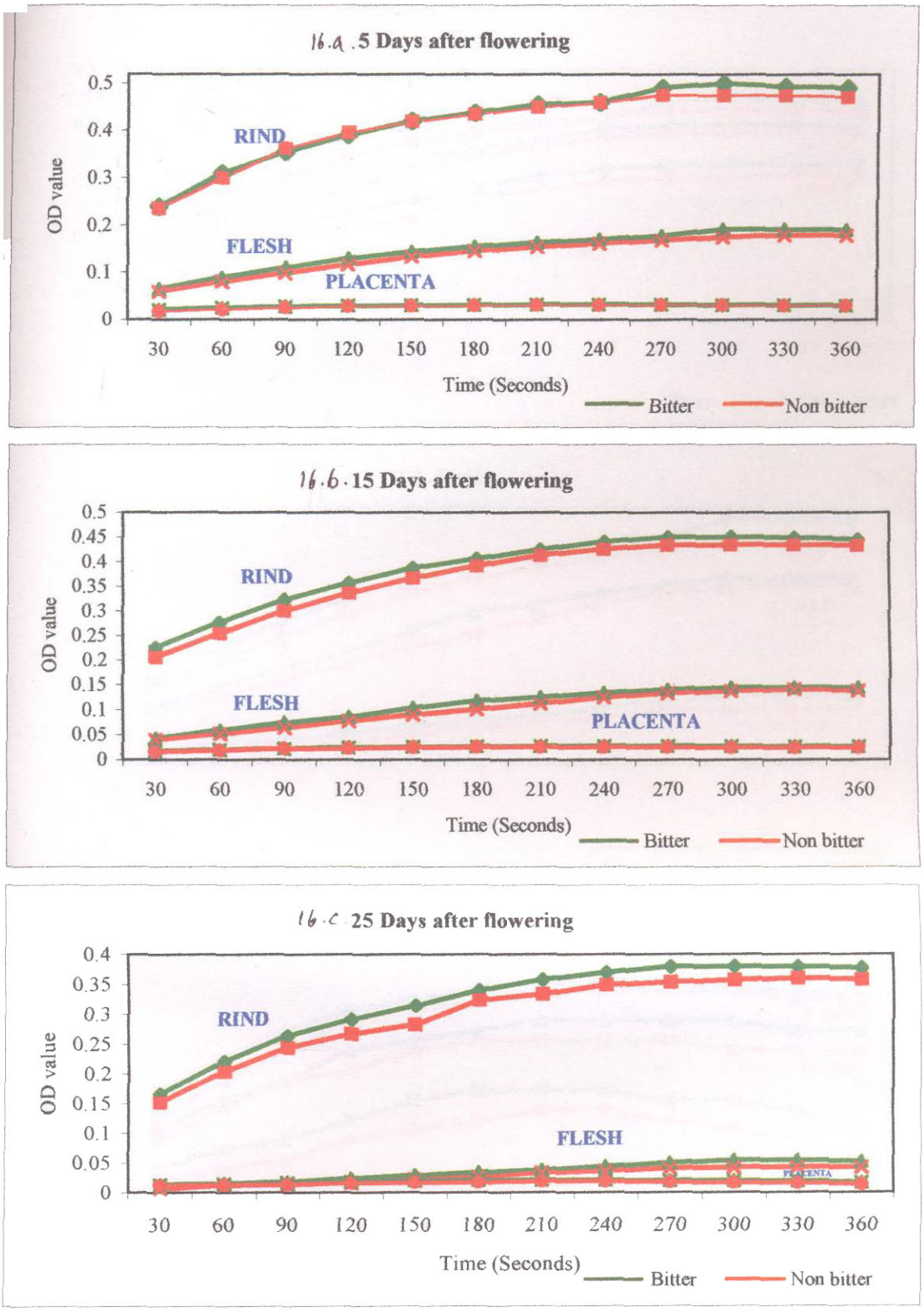


Fig. 16. Polyphenol oxidase activity in rind, flesh and placenta of fruits at different stages of maturity

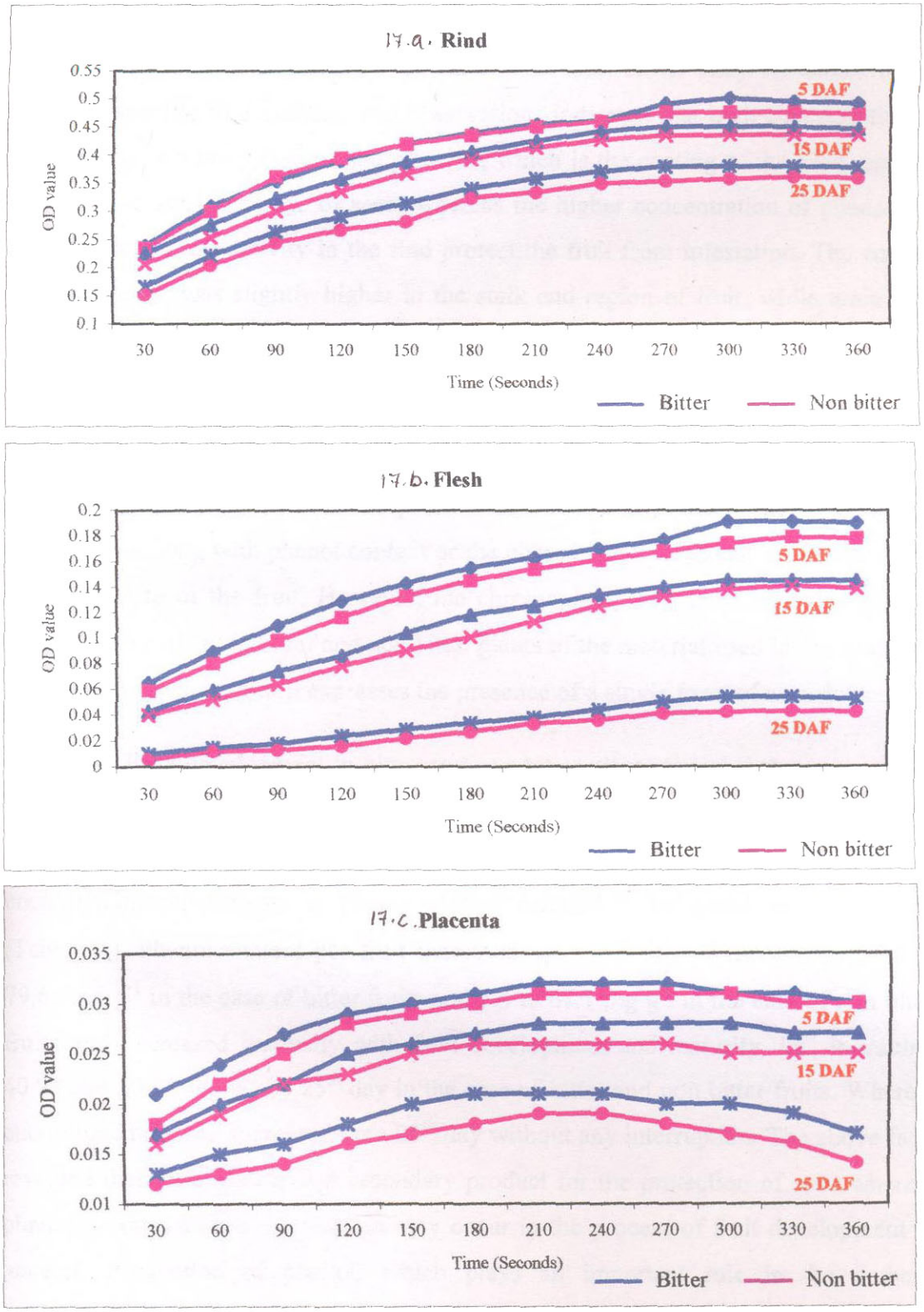


Fig. 17. Polyphenol oxidase activity at different stages of maturity in different fruit parts

that in *Cucurbita mixta* and *Cucumis sativus*, placental region was the most bitter and in *Cucumis humifructus*, placental region was the least bitter. They concluded it as a character specific to a species. The observations indicated that high concentration of cucurbitacin and bitter taste in the placenta, which is the coating of the seed, provide protection to avoid damage of seed; whereas the higher concentration of phenol and polyphenol oxidase activity in the rind protect the fruit from infestation. The content of cucurbitacin was slightly higher in the stalk end region of fruit, while aminoacid and phenol content did not show much variation.

The presence of cucurbitacin and phenol were observed in both bitter and non bitter fruits, but at a higher rate in bitter fruits. The reports of different forms of cucurbitacin and varying rates of phenol content revealed that either the form of cucurbitacin along with phenol content or the phenol itself played an important role in the bitter taste of the fruit. However, the chromatographic studies revealed that the cucurbitacin content in bitter and non bitter plants of the material used in the study had only one spot which in turn expresses the presence of a single form of cucurbitacin.

Fruit development in bitter and non bitter plants revealed that phenol and cucurbitacin content of fruits at different stages of development may have a direct influence on fruit weight in biometrically opposite direction i.e., increase in cucurbitacin and decrease in phenol content resulted in increased weight of fruits (Table 41). Phenol content per fruit increased upto 15^{th} day of fruit set i.e., 5.4 to 79.68 mg g^{-1} in the case of bitter fruits and 5.4 to 67.1 mg g^{-1} in the case of non bitter fruits and decreased gradually with fruit development and maturity, i.e., it reached 40.95 and 39.60 mg g^{-1} by 25^{th} day in the case of bitter and non bitter fruits. Whereas cucurbitacin content increased upto 25^{th} day without any interruption. The above facts revealed that cucurbitacin is a secondary product for the protection of seed whereas phenol production and degradation may occur in the process of fruit development in general. Production of phenol, which plays an important role in the defence mechanism of plants during immature stages, decreases with maturity. The percentage cucurbitacin and phenol content showed a decreasing trend with fruit maturity. This

Table 41. Change in phenol and cucurbitacin content with fruit maturity

No. of DAF	Weight of fruit (g)		Phenol (%)		Phenol (mg/fruit)		Cucurbitacin (%)		Cucurbitacin (units/fruit)	
	B	NB	B	NB	B	NB	B	NB	B	NB
5	45 (0.00)	52 (0.00)	0.012 (0.00)	0.010 (0.00)	5.4 (0.00)	5.4 (0.00)	0.020 (0.00)	0.019 (0.00)	12.99 (0.00)	10.17 (0.00)
15	830 (18.4)	1100 (21.2)	0.009 (0.75)	0.006 (0.60)	79.68 (14.8)	67.1 (12.4)	0.025 (12.5)	0.018 (0.9)	206.39 (15.58)	193.6 (19.3)
25	1050 (23.3)	1650 (31.73)	0.004 (0.33)	0.002 (0.20)	40.95 (7.58)	39.6 (7.33)	0.022 (11.0)	0.016 (0.80)	237.65 (18.3)	262.35 (25.8)

Figures in parenthesis indicate the fold increase of different characters
DAF - Days after flowering

Table 42.Change in phenol, cucurbitacin and amino acid content with maturity

No. of DAF	Weight of fruit (g)		Phenol content (mg/fruit)		Cucurbitacin (units/ fruit)		Amino acid (mg/fruit)	
	B	NB	B	NB	B	NB	B	NB
5	45 (0.00)	52 (0.00)	5.4 (0.00)	5.4 (0.00)	12.99 (0.00)	10.17 (0.00)	1.8 (0.00)	1.09 (0.00)
15	830 (18.4)	1100 (21.1)	79.68 (14.8)	67.1 (12.43)	206.39 (15.0)	193.6 (19.3)	42.33 (23.0)	29.70 (27.3)
25	1050 (23.3)	1650 (31.73)	40.95 (7.58)	39.6 (7.33)	237.65 (18.3)	262.35 (25.8)	65.10 (36.2)	59.4 (54.5)

Figures in parenthesis indicate the fold increase of different characters
DAF - Days after flowering

Table 43.Change in phenol content/fruit and polyphenol oxidase activity at different stages of fruit maturity

No. of DAF	Rind				Flesh				Placenta			
	Phenol content (mg/fruit)		PPO activity after 30'		Phenol content (mg/fruit)		PPO activity after 30'		Phenol content (mg/fruit)		PPO activity after 30'	
	B	NB	B	NB	B	NB	B	NB	B	NB	B	NB
5	17.64	17.06	0.239	0.235	7.29	7.12	0.065	0.069	5.4	5.4	0.021	0.018
15	276.39	315.7	0.225	0.206	93.79	96.80	0.043	0.040	79.68	67.1	0.017	0.016
25	300.30	397.65	0.166	0.152	58.8	66.00	0.010	0.006	40.95	39.6	0.013	0.012

DAF - Days after flowering
PPO - Poly phenol oxidase

was in accordance with the reports of Rehm *et al.* (1957) that in *Coccinea adoensis* and *Acanthoscyos horrida*, bitterness decreased with ripening.

Aminoacid is considered to be the precursor of secondary metabolites. Accumulation of free aminoacids during fruit development stages was observed in this study (Table 42). The possibility in the degradation of phenolics to aminoacids as well as organic compounds cannot be ruled out. Studies of Jayaraman *et al.* (1982) in banana supports this. Even though phenolics showed a decreasing trend, in the course of fruit development, the cucurbitacin content per fruit was increasing which shows that the mechanism of cucurbitacin of production may be independent of the normal trend of fruit development. Aminoacid accumulation was double the quantity of cucurbitacin produced in both bitter and non bitter fruits. The content of aminoacids were higher in bitter fruits than non bitter fruits. Findings of Kano and Goto (2003) supported this result.

The activity of polyphenol oxidase showed a decreasing trend with maturity and also in rind, flesh and placenta of both bitter and non bitter fruits (Table 43). On considering the phenol content per fruit, it was the highest on the 15^{th} day and in flesh a placenta, which decreased later. Continuous increasing trend was observed for phenol content in rind of the fruit. However polyphenol oxidase activity showed a continuous decreasing trend for rind, flesh and placenta. This shows that PPO activity was independent of total phenol. The electrophorogram of protein also supported the same by showing similar banding pattern for both bitter and non bitter seeds. Since seed is the miniature plant, and the banding pattern of seed protein is strictly governed by genetic factors, there is every possibility to show a similar trend in plant as well as fruit. The activity at different intervals for rind, flesh and placenta also supported the protein banding pattern by way of showing same rate of increase in activity. The other factors which supported similar banding pattern of protein are similar trend in change of cucurbitacin, aminoacid and phenol content.

5.7 PEST AND DISEASE INCIDENCE

No significant difference was observed between bitter and non bitter plants for the preference/non preference by red pumpkin beetle and fruitfly. This was in

contradiction to the reports of Eben *et al.* (1997) and Nayar and More (1998), who opined that cucurbitacins, the bitter principles, acted as feeding attractants for red pumpkin beetles. However, results of the present study was in confirmation with the findings of Dhillon (1993), who did not establish any relationship between cucurbitacin and pest incidence.

Kannaiyan and Purushothaman (1973) observed that in varieties resistant to diseases like wilt, the cucurbitacin content was high. However in the present experiments no significant difference was observed among the two plant types with respect to susceptibility to powdery mildew.

5.8 WEATHER AND BITTERNESS

From the results obtained, it is evident that bitterness in oriental pickling melon is not affected by environmental conditions. This is supported by the digenic control of bitterness as evidenced from inheritance study. During the course of experiments sources of nutrients and physiological conditions of the plant were modified under varied weather conditions. But there was no change in bitterness of fruits confirming that weather parameters have no influence on this character. This was in contrast to the findings of Kano *et al.* (2003) who observed increased bitterness at lower temperatures and Attard and Spiteri (2003) and Swaider *et al.* (1983) who observed increased bitterness at higher temperatures.

The salient conclusions drawn from the study conducted to characterize bitterness in genetic, physiologic and management contexts are as follows.

Bitterness being a qualitative character under digenic control is least modified by environmental conditions. Even the pollen from wild/cultivated cucurbitaceous species could not induce bitterness in the resultant fruits (F_0) of oriental pickling melon, thus eliminating the possibility of metaxenia. However the possibility of transfer of bitter genes from the wild species *Cucumis melo* var. *callosus* has been obtained, suggesting natural flow of bitter genes from this species to cultivated forms. Physiological modifications of the plant by pruning of branches and

thinning of leaves did not alter the expression of bitterness. Application of different sources of nutrients including urea or fresh cowdung and alteration in soil pH by application of lime did not alleviate/promote bitterness. Irrespective of the season and weather conditions bitter plants produced only bitter fruits at all the stages of growth of the plant. Many of the morphological and biochemical characters differed between the two types. For early detection of bitter seedlings organoleptic evaluation of a part of the cotyledonary leaves can be resorted to.

Being a character of dominant nature, special attention should be paid by the seed producers to rouge out the bitter plants before flowering to reduce its perpetuation under field conditions. In future, the characterisation of bitter and non bitter plants at molecular level can be attempted.

Summary

6. SUMMARY

The present investigation entitled "Factor analysis of bitterness in *Cucumis melo* var. *conomon* Mak." was conducted in the Department of Olericulture, College of Horticulture, Vellanikkara during 2000-2003 with the objective of characterising bitterness and its persistence in genetic and management contexts.

To find out the inheritance pattern of bitterness in oriental pickling melon, homozygous bitter and non bitter lines were developed by continuous selfing of both bitter and non bitter types. These homozygous lines were used to produce F_1, F_2, BC_1, BC_2 and their reciprocals and were raised in the field during May 2002. On evaluation, it was found that all the F_1 plants produced bitter fruits. The segregation pattern in F_2 and BC generations fitted well into the ratio 13:3 (bitter : non bitter) with 80-95 per cent probability on χ^2 test. This indicated that bitterness is governed by an inhibitory gene. This was also evident from the fact that irrespective of weather conditions, all the fruits borne on bitter plants were bitter and that on non bitter plants were non bitter. Phenotypic and genotypic coefficients of variation were high for bitterness (32.89 and 32.83 respectively). High heritability (99%) and genetic advance (52.86) were also observed.

Effect of foreign pollen on induction of bitterness in oriental pickling melon was studied. Pollen grains from *Cucumis melo* var. *agrestis, Cucumis melo* var. *callosus, Cucumis trigonus, Trichosanthes lobata, Trichosanthes anguina, Trichosanthes cucumerina, Momordica charantia* and *Luffa cylindrica* (wild), were utilised for the study. The resultant fruits (F_0 generation) of mixed pollination with these species were found to be non bitter in all the cases. Pollen grains from all these species except *Trichosanthes lobata* and *T. cucumerina*, germinated on the stigma of *Cucumis melo* var. *conomon*, but failed to induce bitterness in fruits. It was observed that the only species which could induce successful fruit set on crossing with the cultivated oriental pickling melon was *Cucumis melo* var. *callosus*. Though the F_0 fruits of this cross were non bitter, all the F_1s were bitter and F_2 segregated in the ratio 13:3 with 50-70 per cent probability. This indicated the possibility of transfer of bitter

gene by natural outcrossing between *Cucumis melo* var. *callosus* and oriental pickling melon. The pollen grains from widely cultivated and bitter species like bittergourd could not induce bitterness in non bitter oriental pickling melon. This completely rules out the possibility of metaxenia in inducing bitterness in oriental pickling melon. Inspite of germination of external pollen grains as confirmed by the *in vivo* studies using Leitz Dialux epifluorescence microscope, it was conclusively proved that no metaxenic effect of bitterness was imparted by pollen from other species.

Studies on the effect of different sources of nutrients like chemical fertilizers, poultry manure, goat manure, farmyard manure, ground nut cake, neem cake and the insecticide furadan on changing the expression of bitterness revealed that the nutrient source and insecticide have no influence in imparting bitterness. The fruits borne on bitter plants were bitter and that on non bitter plants were non bitter. Cultural operations like addition of lime for changing soil pH also had no influence on fruit bitterness.

Management practices like severe pruning and leaf thinning operations which modifies plant vigour and physiology did not alter the inherent nature of the plants to produce bitter and non bitter fruits. Investigations on bitterness in relation to physiological age of the plant revealed that it has no influence to change the bitter character of the fruit by manifesting inherent nature of the plants.

Various morphological and biochemical parameters were studied to characterise the bitter and non bitter plants. The morphological characters of the seed, viz. seed length, seed breadth, seed thickness and hundred seed weight were compared and was found to be significantly different between the two types. The bitter seeds showed early germination and exhibited higher seedling height indicating the closeness of the bitter type to its wild relatives. However, cotyledonary leaf shape, hairiness and pigmentation of seedlings exhibited no variation in both the types. Among the different plant characters, vine length, days to first female flower, number of female flowers, percentage of fruit set and number of fruits per plant were significantly high for bitter plants. Yield per plant was not significantly different between bitter and non bitter types.

The biochemical constituents like cucurbitacin content, total phenol and total free aminoacids were higher in bitter fruits. Cucurbitacin content, phenol content and polyphenol oxidase activity decreased with fruit maturity, while aminoacids showed an increasing trend in both types. Cucurbitacin content was the highest in placenta, followed by flesh and rind. Results of thin layer chromatographic studies of cucurbitacin extract confirmed higher concentration of cucurbitacin in the placenta followed by flesh and rind. The phenol content, aminoacid and polyphenol oxidase activity showed a decreasing trend from rind to placenta in both the types. In general, cucurbitacin and phenol content were more at stalk end of the fruit than middle portion or blossom end. Electrophoretic studies of seed proteins showed similar banding pattern for seeds from bitter and non bitter fruits. These results lead to the inference that the biochemical characters expressed by both the types are specific to the species used in the study.

Eventhough the mature dried seeds from bitter and non bitter fruits were non bitter, the radicle, hypocotyl and cotyledonary leaves of seedlings from bitter fruits were bitter. Hence under field conditions, identification of bitterness is possible at seedling stage by organoleptic evaluation of a part of the cotyledonary leaves, which will not hinder further development of the plant.

The investigations on factor analysis of bitterness in *Cucumis melo* var. *Conomon* conducted to probe into the factors influencing the expression of bitterness in fruits revealed the purely genetic nature of the character with least influence of external climatic and management practices. The possibility of identification of bitter seedlings at cotyledonary stage is of great practical utility in expelling the bitter plants from the lot at an early stage itself.

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* Originals not seen

Appendices

APPENDIX-I

Average nutrient content of manures used in the experiment

Material	Nutrient content (%)		
	N	P_2O_5	K_2O
1. Poultry manure	1.35	1.6	0.8
2. Goat manure	1.00	-	-
3. Farmyard manure	1.00	0.5	1.0
4. Groundnut cake	7.0	1.5	1.5
5. Neem cake	5.0	1.0	1.5

APPENDIX - II

Monthly weather data during the experimental period (2000-2002)

Month	Temperature (°C)		Relative humidity mean (%)	Mean sunshine hours	Total rainfall (mm)	Number of rainy days
	Maximum	Minimum				
January 2000	32.9	23.2	60	286.0	0.0	0
February 2000	33.3	22.8	67	248.2	4.6	1.0
March 2000	35.6	23.9	67	299.5	0.0	0
April 2000	34.0	24.6	74	216.6	67.9	3.0
May 2000	33.7	24.4	72	263.4	117.2	8.0
June 2000	29.6	22.8	86	98.6	602.2	21.0
July 2000	28.8	21.9	82	147.5	354.0	15.0
August 2000	29.1	22.6	87	96.0	518.8	19.0
September 2000	30.7	23.0	81	178.0	198.1	10.0
October 2000	30.7	22.7	80	174.1	262.2	10.0
November 2000	33.3	23.1	66	202.3	41.3	5.0
December 2000	30.4	22.0	59	244.3	11.2	2.0
January 2001	32.6	23.2	56	8.0	0.0	0
February 2001	34.5	22.9	67	8.0	12.2	1.0
March 2001	34.9	24.0	69	8.2	4.4	0
April 2001	34.2	24.7	75	6.5	243.1	8.0
May 2001	32.3	24.5	81	6.4	192.6	22.0
June 2001	28.4	23.1	87	1.9	676.2	23.0
July 2001	29.0	22.7	85	2.4	477.7	19.0
August 2001	27.5	23.1	87	3.6	253.2	21.0
September 2001	30.8	23.2	79	5.3	200.9	6.0
October 2001	30.1	23.0	81	4.7	215.8	8.0
November 2001	31.6	23.1	72	6.2	115.8	6.0
December 2001	29.5	22.2	60	8.1	0.0	0.0
January 2002	32.8	22.7	62	8.1	0.0	0.0
February 2002	34.3	23.0	50	8.1	0.0	0.0
March 2002	36.3	24.1	63	8.2	16.3	2.0
April 2002	35.0	24.8	71	7.8	50.8	4.0
May 2002	32.6	24.5	87	5.8	308.4	12.0
June 2002	30.0	23.3	86	2.7	533.5	22.0
July 2002	29.8	23.1	84	3.4	354.2	21.0
August 2002	28.9	22.9	86	3.1	506.6	19.0

FACTOR ANALYSIS OF BITTERNESS IN *Cucumis melo* var. *conomon* Mak.

By
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ABSTRACT OF THE THESIS

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ABSTRACT

The research project entitled 'Factor analysis of bitterness in *Cucumis melo* var. *conomon* Mak.' was carried out in the Department of Olericulture, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, during the period 2000-2003. The objective of the study was to characterize bitterness and its' persistence under varying genetic, physiologic and management contexts. The material selected was Mudicode, which is a well accepted non bitter variety. Homozygous bitter and non bitter lines obtained by continuous selfing for four generations was utilised for the study.

The bitter and non bitter parental lines were crossed and F_1, F_2, BC_1, and BC_2 populations were generated. The χ^2 test for F_2 and backcross generations with the ratio 13:3 (bitter:non bitter) showed the presence of inhibitory gene action for bitterness suggesting the possibility of introduction of inhibitory gene from wild bitter forms to the cultivated non bitter types. This is supported by the studies of the cross between oriental pickling melon and *Cucumis melo* var. callosus. The F_1 of this cross produced small bitter fruits with characteristics similar to the bitter parent and the segregation pattern in F_2 generation revealed the presence of inhibitory gene action in the ratio 13:3.

To find the metaxenic effect of bitterness, several bitter wild and four cultivated cucurbitaceous species including the bitter type of Mudicode Local were used as pollen source on non bitter oriental pickling melon. The resultant fruits of these crosses were non bitter, thus ruling out the possibility of metaxenia.

The study on effect of different sources of nutrients on bitterness revealed that the application of chemical fertilizers or organic manures viz., farmyard manure, neem cake, groundnut cake, poultry manure and goat manure will not modify bitterness in fruits of oriental pickling melon. Application of lime or furadan also did not change expression of bitterness.

The change in physiological condition of the plant by pruning operations also did not alter the innate nature of bitterness.

Similarly, the age of the plant did not show any relation with the existence of bitterness in oriental pickling melon.

Comparison of morphological characters of bitter and non bitter plants showed that seeds from bitter fruits were smaller having more initial seedling vigour. Also, the fruits borne on bitter plants were more in number with smaller size, indicating the closeness of bitter plants to wild relatives.

It was observed that the seeds are always non bitter irrespective of its origin from bitter and non bitter plants. But the cotyledon, hypocotyls and radicle of seedlings will remain bitter. Hence organoleptic evaluation of a part of the cotyledon, which will not hinder further development of the plant can be used as a method for identification of bitterness at an early stage.

Results of biochemical analysis revealed that the content of cucurbitacin, phenol, aminoacid and polyphenol oxidase activity was higher in bitter fruits compared to non bitter fruits. The bitter principle was the highest at placental region followed by flesh and rind. In general, stalk end of the fruit was more bitter than blossom end of the fruit. The results also revealed that bitterness decreased with fruit maturity. This was substantiated using thin layer chromatography. Electrophoretic studies of seed protein showed no difference in banding pattern for bitter and non bitter seeds, indicating the closeness of the two types.