In conclusion, it can be said that milk thistle (*Silybum marianum* L. Gaertn.), a severely spiny, shrub-like plant belonging to Asteraceae family, can be easily grown and cultivated in land-locked areas of India, including in sandy-loamy soils such as that found in Bithoor, near Kanpur (U.P.).

The treatment, viz., plant stand and a combination of N, P, K fertilizers, vermi compost, supplemented with several macro and micro nutrients, proved to be highly efficacious in enhancing the morphological traits of milk thistle. These traits included height of the plant before flowering and at harvesting; plant canopy, number of branches, leaf length and width; number of capsules per plant and capsule diameter; dry weight per plant and 100-seed weight.

With regard to the yield in the form of 100-seed weight, the plant geometry of 60 cm × 60 cm in conjugation with fertilizers treatment of NPK and vermi compost proved most beneficial where a weight of 4.1 g was obtained as compared to 1.7 g in control plots (F₀S₀). In the spacing of 60 cm × 60 cm without fertilizers, the 100-seed weight was 2.3 g. This indicates the importance of both fertilizers and spacing in the seed yield. The silymarin yield in these F₁S₂ plots was 6.799% as per the HPLC data.

The results of HPLC have shown that, under conditions of adequate nutrition and medium spacing, the silymarin content is 6.799%. When compared with the results of other workers whose experiments were not based on Mediterranean region plants, the results of the work under consideration were about double to four times that of these workers.
(Hammouda, 1993; Tumova et al., 2006). It can be concluded that the plant stand and the nutrients supplied might be instrumental in enhancing the silymarin content. However, it must be emphasized that more work is required, especially with regard to the silymarin yield, before any conclusion can be drawn.
This thesis is a study of the effect of nutrients and crop geometry on the growth parameters, seed weight and silymarin content.

The climatic conditions played important role in the germination and growth of the *S. marianum*. During the year 2003-2004 the average temperature, average dew point, average humidity, average sea level pressure, average visibility, average wind velocity and precipitation were 18° C, 11° C, 69%, 1015 hPa, 2 km, 5 km/h and 0.00 mm, respectively, on the day of sowing (December 2003). The fog had also reported on the same day (Table 3.1). The sufficient germination has been reported in the experimental year.

During the next experimental year 2004-2005 the average temperature, average dew point, average humidity, average sea level pressure, average visibility, average wind velocity and precipitation were 18° C, 10° C, 65%, 1017 hPa, 3 km, 5 km/h and 0.00 mm, respectively, on the day of sowing (December 2004) (Table 3.1). The day was foggy and the sufficient germination has been reported in the year 2004.

In the next experimental year 2005-2006 and 2006-2007 the crop was sown but the germination was inadequate. The temperature in the months of sowing (November and December) in these years was comparatively higher than the other experimental years and no fog was recorded in these years (Table 3.1).

During the year 2007-2008 the average temperature, average dew point, average humidity, average sea level pressure, average visibility, average wind velocity and precipitation were 18° C, 9° C, 67%, 1020 hPa, 2 km, 3 km/h and 0.00 mm, respectively, on the day of sowing (December 2007).
The day was foggy and the sufficient germination has been reported in the year 2007 (Table 3.1).

As already stated in chapter III, four types of plant stand, viz., broadcasted ($S_o$), 30 cm × 30 cm ($S_1$), 60 cm × 60 cm ($S_2$) and 120 cm × 120 cm ($S_3$), were combined with two types of fertilizer treatments. The control plants were not supplemented with any fertilizers ($F_o$) while the supplemented plots contained vermi compost, potassium, phosphorus and urea as basal dressings ($F_1$). Foliar sprays of micro and macro nutrients were also used in the $F_1$ plots. Hence eight combinations were available: $F_oS_o$, $F_oS_1$, $F_oS_2$, $F_oS_3$, $F_1S_o$, $F_1S_1$, $F_1S_2$ and $F_1S_3$. The results obtained from these combinations indicate that the height of the plant before flowering and at the time of harvesting, the capsule diameter and the dry weight per plant were maximum in the $F_1S_1$ plots.

However, the total percentage increase between the height before flowering and at the time of harvesting was maximum in $F_oS_o$, $F_oS_2$ and $F_1S_2$, the enhancement being about 500%. It has also been seen that the height of the plants is more in the $S_1$ plots, whether supplemented with fertilizers or not. It appears that, at this spacing of 30×30 cm, the individual plants compete with each other for space, and sunlight and, hence, grow rapidly upwards in search of sunlight. In the case of plots supplemented with the fertilizers potassium, phosphorus, nitrogen and vermi compost as well as with other micronutrients and macronutrients, the plants, probably, photosynthesize at a higher rate, thereby, requiring more sunlight.

The plant canopy, on the other hand, is maximum in the $S_3$ plots. This can be attributed to more space that is available for the spread of the plant.
When the plots are supplemented with fertilizers, there is a 27% increase in the canopy at the same spacing.

Ram et al. (2005) have shown that the plant canopy is inversely proportional to the plant height. The results in the present study indicate the same trend. This is to be expected because, if there is ample space for the plant to grow and spread, it does not need to grow higher in search of sunlight. The plant canopy has attained a maximum of about 89.0 cm on an average, in the F₁S₃ plots while the F₀S₃ plots had a maximum canopy of about 70.0 cm on an average.

The number of branches per plant is also a function of the spread of the plant and is directly proportional to the canopy. However, the increase in the number of branches in plots supplemented with fertilizers was 130% more than in the unsupplemented plots. A similar relationship has been shown between number of branches and plant canopy by Ram et al. (2005). This luxuriant growth of the plant can be directly attributed to both the factors taken in the study, viz., supply of appropriate nutrients and adequate spacing for the more-or-less limitless growth of the plant. The effect of spacing can be demonstrated by the 40% to 85% increase in the plant canopy diameter and the over 20% increase in the number of branches from S₀ to S₃. The effect of fertilizers shows an increase of about 25% in plant canopy and about 200% increase in number of branches from F₀ to F₁. This appears to be the first report regarding the combined effect of fertilizers and spacing on plant canopy and number of branches.

The increase in canopy can be partially attributed to the leaf length. The maximum leaf length has been recorded in F₁S₃ plants where an average
length of about 68.0 cm was obtained. The WHO monograph on selected medicinal plants (1999) has shown that the leaf length is from 25.0 to 50.0 cm. In the present study, the combination of fertilizers and adequate spacing has proved to be potent enough to give an increase of about 36% over the length shown in the WHO monograph. A similar increase was seen in the leaf width, as well. While it is 12.0 to 25.0 cm in the WHO monograph (1999), the present study has revealed that the width of the leaf has reached a maximum of around 40.0 cm on an average, again in the F1S3 plots. These results also indicate a positive correlation among plant canopy, number of branches, leaf length and leaf width. An additional feature of the leaf that shows a positive correlation to the other characters is leaf colour. The F1S3 plants had a dark green, glossy colour with prominent white variegated markings and hard, pointed spines.

Morazzoni and Bombardelli (1995) have described the leaves as green and glossy with white or variegated markings. The dark green colour of the leaves indicates the formation of a large amount of chlorophyll for which the foliar sprays may be partially responsible. The requirement for iron and magnesium, required in the synthesis of chlorophyll, is probably met through the components of foliar spray. It is also likely that, due to the presence of vermi compost, potassium, nitrogen and phosphorus, the rate of photosynthesis has increased.

The ratio of leaf width to leaf length is in the range of 1:1.8 to 1:2.5. The WHO monograph (1999) has shown this relationship to be about 1:1.2. The wider leaves in the present study may be instrumental in increasing the surface area of the leaf, thereby, again, aiding photosynthesis. The wider leaves also ensure that the spines are more in number. The spines, of course, protect the plant against animals and birds but may also act to
reduce the rate of transpiration. The milk thistle plant is a plant of Mediterranean origin but has survived well in the sandy loamy soil characteristic of Bithoor where the crop was cultivated. Since the water leached downwards, the conditions of some water scarcity were probably created. Under these circumstances, the spines might be helpful in allowing the plant to conserve water.

The number of capsules per plant follows the same pattern as the plant canopy and is maximum in the F$_1$S$_3$ plots. This is an expected outcome because, in these plots, the number of branches is also highest. It is evident that the greater the number of branches, the greater would be the capsule number. H.S. Hadi et al. (2008) have reported that the average number of capsules per plant is 10.4 whereas, in the present study, this number has reached an average of nearly 40 capsules per plant in F$_1$S$_3$. Under normal circumstances, this would mean that whatever nutrients were absorbed from the soil would be distributed among these many capsules but, with the enhanced nutrient doses provided to the plant in this study, they appear to be more than adequate for the plant.

At the same time, the capsule diameter was also found to be much higher than that shown in the WHO monograph (1999) which records 2.5 to 4.0 cm diameters. The present study has recorded capsule diameters of about 18.5 cm in the F$_1$S$_1$ plants. Initially the inflorescence is a bright purple colour, quite similar to the report in WHO monograph (1999) where it has been described as a red-purple colour. However, as the flowers mature the inflorescence changes into a brownish colour and becomes a capsule. The duration from the opening of the buds to the dehiscence of the fruits varies from 15-20 days. There is no known report on the time duration from anthesis to dehiscence of fruits. As such, there is no way of
comparison between the Mediterranean plants and the plants grown in India. Even so, the entire crop is a short duration crop of 3 to 4 months duration in which case the reproductive phase of 15 to 20 day appears to be quite reasonable.

The dry weight per plant has also been recorded. This has shown an unusual trend. When fertilizer doses were added, the plant showed the maximum dry weight at the 30x30 cm crop geometry but, when no fertilizers doses were added, the dry weight per plant increased as the spacing increased from S_o to S_3. In the presence of fertilizer supplements, the plants showed a sharp increase from about 105 g in S_o to about 130 g in S_1 but then started showing a negative trend, decreasing to about 118 g in S_2 and 113 g in S_3. Since the height of the plant showed a parallel trend, before flowering as well as at harvesting, the dry weight is probably partially due to this increase in height. Although, the plant canopy, leaf length and leaf breadth do not follow this trend but a combination of the height and the girth have probably resulted in storage of a large part of photosynthate in the form of dry weight.

Another character studied was the 100-seed weight. In this case, the highest 100-seed weight was recorded in the S_2 plots, both with and without fertilizers. In the presence of fertilizers, the 100-seed weight was almost double that of the weight recorded without fertilizers. For example, the 100-seed weight in F_oS_o was about 1.7 g while that in F_1S_o was about 3.65 g. In F_oS_2, this weight was 2.3 g whereas, in F_1S_2, it was 4.1 g. This indicates that the seeds were able to accumulate almost twice the amount of nutrients when the plants received adequate fertilizers.
The effect of nutrients has been clearly seen and discussed so far. In most cases the application is in the form of basal dressings wherein the fertilizer doses are determined on the basis of the plot size and the plant density. However, the doses get diluted due to irrigation and much of the fertilizer is leached downwards due to the downward movement of the water used in this irrigation.

There have been several reports on the use of foliar sprays of both macro as well as micronutrients (Kuepper, 2003; Wojcik, 2004) as a more viable alternative to basal dressings. One of the reasons cited for the success of foliar sprays is that many nutrients have limited mobility and, hence, are unable to move through the roots to the leaves. Therefore, in the present study, the basal dressings were supplemented with foliar sprays.

The foliar sprays consisted of Aquafer 19:19:19 where N, P and K were in the ratio of 19:19:19 although nitrogen is in different forms. The nitrogen in ammoniacal form was 4.5% by weight, in nitrate form 4.0%, urea 10.5%; phosphate as P$_2$O$_5$ minimum 19% by weight; potassium as K$_2$O minimum 19% by weight. This was complemented by a mixture of micro and macronutrients, MS-24.

Plant cells are capable of active transport of ammonium ions which may be found in substantial amounts in acidic soils (Buchanan et al., 2000). However, the sandy-loamy soil in Bithoor is slightly alkaline; hence, the ammonium form of nitrogen may be much lower. The foliar spray, in this context, has proved to be more suitable because, in that case, the availability of ammoniacal-nitrogen is not dependent on soil pH. Another form is nitrate-nitrogen which is, in fact, a major source for plants (Buchanan et al., 2000). Nitrate can be taken up by the epidermal and
cortical cells of the root but can also occur in leaves (Buchanan et al., 2000). So, the foliar application can be of immense value to the plant. Nitrate is stored in roots, shoots and leaf midribs (Buchanan et al., 2000).

Another major nutrient of the basal dressing, potassium, is the most abundant cellular cation. It plays a major role in maintaining the osmoticum of the cell and is also responsible for activating over 50 plant enzymes. It also participates in several metabolic processes including photosynthesis. Much of this potassium is passed on to the xylem vessels for long-distance travel to leaves (Buchanan et al., 2000). This calls for an expenditure of energy so, if potassium can be absorbed directly by leaves through foliar application, the energy flow could probably be better regulated. The accumulation of the photosynthate in the form of vegetative growth - both in height and in canopy- as well as dry matter, clearly indicates the beneficial effects of this nutrient.

Phosphorus is another major nutrient which has been supplied as basal dressing and also in foliar applications. According to Buchanan et al. (2000), about 25 to 40 KJ is required to transport 1 mol of inorganic phosphate into root cells, i.e., the amount of energy derived from the hydrolysis of 1 mol of ATP. Phosphorus has a high sorption to soil particles due to which it is the macronutrient least available to the plant roots (Buchanan et al., 2000). Hence, supplementing basal dressings of phosphorus with foliar applications has proved to be a good strategy.

Although the exact chemical composition of vermi compost is not known, it is a generally accepted fact that the earthworms are able to breakdown mostly waste plant debris into a mixture of simpler inorganic substances as well as monomers or dimers of constituents of macromolecules such as
amino acids, monosaccharides, fatty acids, nucleotides etc. These components are taken up through the roots and are easier to be reabsorbed into macromolecules in the plant and may be partially responsible for the gain in dry weight and increase in height, leaf length and leaf width.

Besides these major macronutrients, other macronutrients and micronutrients have also proved to be more efficacious when supplied through the leaf route, so that they are absorbed directly through the stomata.

Geneva *et al.* (2008) have specifically shown the efficacy of foliar applications of fertilizers on the development, seed yield and silymarin content of milk thistle, although they have also used a growth regulator. In another report, too, Geneva *et al.* (2008) have discussed how seed yield and quality of milk thistle could be improved with foliar fertilisation and a growth effector. In the present study, no growth factor or effector was used although this, apparently, did not show any limiting effect on the growth parameters or morphological characters of milk thistle.

High performance liquid chromatography (HPLC) was performed on the seeds of plants of the F$_1$S$_2$ plots on account of the fact that these plants showed the maximum seed weight and the active principles are all present in the seed. Since the other morphological traits did not show the same trend, it was felt that the high seed weight might be due to the presence of the active principles.

H.S. Hadi *et al.* (2008) have reported a 1000 seed weight of about 25 g whereas, in the present study, this varied from about 17 g in F$_0$S$_0$ to about 40 g in F$_1$S$_2$. 
There are several reports regarding the silybin content and silymarin content. Omer et al. (1993) have shown that potassium has a profound effect on the percentage of silybin in seeds while nitrogen has no effect, whatsoever. Omidbaigi and Nobakht (2001) have shown that silymarin is 9.92% and silybin 33.58% of the injected volume. Tumova et al. (2006) have demonstrated 1-4% of silymarin flavonoids, while H.S. Hadi et al. (2008) have determined the silymarin content to be 7.711%.

The WHO monograph (1999) on milk thistle has reported that the silymarin content may vary from 1.5 to 3.0%.

Valtcho et al. (2006) have found that the silymarin content is 3.52%.

In the present study, it was found that the silymarin content was 6.799% in the seeds of $F_1S_2$ plots. Although this is lower than the content determined by Omidbaigi and Nobakht (2001) and by H.S. Hadi et al. (2008), it is still higher than that reported by other workers (Tumova et al., 2001; Valtcho et al., 2006). The report by Omidbaigi and Nobakht was based on the work done in Tehran, Iran while that of H.S. Hadi et al. (2008) was based on the experiments conducted at Italy. Since both these places are near the Caspian sea and Mediterranean sea, respectively, their climate is of the Mediterranean nature from where the milk thistle plant has originated. So, it is only natural that these plants would have higher silymarin content.