Abstract

The *Brassica* crops occupy a place predominance in the oil seed map of India. They are the second largest source of edible oil next only to ground nut. The major portion of seeds is being utilised for extraction of edible oil though a considerable amount of seed is being used for direct domestic consumptions after for preparing pickles, flavouring curries, cosmetics, and for various other pruposes. Seeds of oleiferous brassicas have nutritive value. The cake left after oil extraction, known as rapeseed meal. (RSM) is a rich source of proteins and is used as animal feed and manure.

Though glucosinolates, a class of natural product available in all brassicas, have long been known as antinutritional and goitre inducing compounds, but very recently some enzymatically degraded products out of them have been observed to show anticancer, antimutagenic and antioxidant activity to induce free radical scavenging properties of cell.

Mustard crops are generally cultivated in winter under rainfed conditions. But erratic and limited rainfall seriously limits their production. The need for a higher and more stable yield under rainfed condition has received considerable attention in recent mustered improvement programme.

During the last fee decades a number of cultivars of *Brassica* species have been released. But it is quite apparent that coverage under existing high yielding varieties is still unsatisfactory. We do not yet have a range of cultivars to suit all environmental conditions prevailing in India.

As Darjeeling district, situated in northern part of West Bengal, India also belongs to rainfed areas, there is enough, scope to cultivate oilferous brassicas for economic development in in the region. In view of the acute shortage of edible oil in West Bengal, efforts are under way to introduce *Brassica* sp in Darjeeling plains and adjoing areas.

During survey it appears that cultivations of mustard is very sporadic innature in the district. Local cultivators believe that cultivation of the existing species in Darjeeling district is not profitable as the quantity of this crop is neither that high nor expected too. It has also been observed that a considerable number of cultivars of *Brassica* are being utilised in southern part of West Bengal but in the plains of Darjeeling district and adjoining areas, the cultivation is limited to only yellow sarson type. *Barassica campestris* L.CV YSB-9 and *B.*

B.campestris L. Cv NC-1. Sarson rai, *B. juncea* (L) Czern cv B-85 is also being utilised. In the region. The crops are grwon mainly in the rabi season from September-October to February-March. Owing to their nature and capacity to thrieve well under poor conditions of moisture and soil fertility, they are generally, raised as rainfed without the application of fertilizer resulting in low average yield in the region. Production of seeds in oleiferous brassicas has also been observed to be affected by the attack of key pest *Lipaphis erysimi* Kalt, an aphid. Commonly found in West Bengal.

Thus it is felt necessary to select better adapted cultivars at the sametime having with aphid resistant capacity for their commercial utilisation in the plains of Darjeeling district, West Bengal.

With this background it has been the objective of the present work to select a cultivar of *Brassica* suitable for Darjeeling plains out of various cultivars available in West Bengal and to study the cultivar from botanical chemical, and agronomy point of view for its purposeful utilisation in the region and the results and observations of which have been represented in nine chapters.

Chapter - I deals with the review of literature related to various aspects of the oleiferous brassicas. Chapter II deals with survey on utilisation of some cultivars of *Brassica campestris* L. and *B. juneca* (L) Czern as field crops in ecologically different regions in India, their organoleptic features with special interest on their adaptibility and productivity of oil in the plains of Darjeeling conditions. All together 12 different cultivars of *B. campestris* L. and *B. juneca* (L) Czern have been taken into consideration in connection with adaptibility study in the ecological condition of Darjeeling district, West Bengal. These are *B.campestris,* YSB-9, NC-1, B-54, C-3 and *B. juneca* B-85, white glossy, T-6342, RW-85-89, RW-85,B-85-glossy, RC-781 and sharma 85-89. Authentic seeds have been procured from Pulse and Oil Seeds Research Station, Govt. of West Bengal, Berhampur (24° 50'20" N, 88° 46' E) and have directly been sown to the experimental plot, Centre for life sciences, Dept. of Botany, NBU (26°25' N, 86°25' E). Out of these *B. campestris* B-54, B. Juneca RW-85-89 and sharma 85-89 have been observed to show good performance in various growth parameters. But *B.campestris* B-54 has been selected to be the best on the basis of early flowering nature (18-DAS) with the understanding that life cycle of the selected cultiver can be repeated under-prolonged cold climate available in the district for maximum productin of oleiferous seeds. In Darjeeling

condition, *B. campestris* B-54 have shown better growth performance specially in relation to height (86cm), leaf no per plant (929), total dry wt. of leaves per plant (2.72gm) total leaf area per plant (5250 cm^2), no of primary (8) and secondary branches (8) per plant. Besides the cultivar has shown total no of flower per plant (19) total no of pods per plant (8) total to of seeds per pod (17) and weight of 100 seeds (3.50gm). Oil content in seeds has been estimated to become 46 percent. As relatively higher values of oil content in *Brassica* always associated with early flowering type of cultivar, *B.campestris* B-54 has been considered suitable for the district. From survey and locally available information it appears that all most all the cultivars taken into consideration are commonly utilised in different ecological condition of different states in India, specially in Punjab, Bihar, UP, MP, Assam and West Bengal but *B.campestris* B-54 is generally uncommon to Darjeeling district W.B. through it is widely cultivated as field crop in Punjab UP and Assam. Some organoleptic features of seeds of different cultivars have been worked out with a view to utilising the feutures for identificatin of seed samples collected from different sources in the district. The seeds of *B.campestris* B-54 having acidic taste, pungent odour, characteristic light bluish black colour, nearly round shape with 2-2.5 cms in diameter, rugose surface of seed coat and presence of pale spot on hilum have helped to realise that *B.campestris* B-54 uncommon in Darjeeling district.

Chapter III deals with the comparative study on aphid (*Lipaphis erisimi* Kalt) incidence on all the cultivars grown in different ecological condition of northern and southern part of West Bengal to select suitable strains of *Brassica* for Darjeeling condition. In this respect experiments have been conducted growing all the 12 cultivars at two research centres, one in North Bengal University, Camcus, Darjeeling district and the other at Pulse and Oil Seeds Research Station, Berhampur situated in southern part of West Bengal. OUt of all the cultivars, *B.campestris* B-54 has been shown to be affected with *L. erysimi* Kalt in minimum percentage (46) of plants in Darjeeling district as compared to that (86) in southern part of West Bengal. When the average number of aphid per twig of a single plant has been taken into consideration, *B.campestris* B-54 has shown the minimum value in Darjeeling district (55) as compared to that in southern part of the state (184). All these observations also support better resistant capacity of *B.campestis* B.54 against aphid infestation in Darjeeling condition out of all the cultivars so far taken into consideration..

Chapter IV deals with isolation, purification and characterisation of phytosterol and glucosinolates from the seeds of *B.campestris* B-54 grown in Darjeeling plains. Chemical investigation has been carried out following conversional phytochemical method. 1kg. of seeds of the cultivar has been dried and made to powder. The powdered material has been subjected to soxhlet extraction first with petroleum ether followed by methanol. The concentrated petroleum ether extract has directly been colum chromatographed over alumina and eluted with different solvents starting from nonpolar to polar one and their mixtures. After elution with petroleum ether : Benzene (3:1, 1:1 and 1:3) and Benzene a considerable amount of sitosterol has been isolated. Another phytosterol, cholesterol has also been isolated from Benzene : Chloroforms (3:1 and 1:1) fraction. Both of them have been identified after comparing melting point, paper chromatographic behaviour in different solvents and characteristic peaks of IR spectrum of the isolated chemicals with those of authentic samples. The methanolic fraction has been concentrated to small volume and an appreciable amount of crystals has been obtained in solvent mixture of methanol : water (7:3) being kept in a refrizerater for 15 days. The methanol soluble crystals have been subjected to column chromatography over alumina previously washed with dil H_2SO_4. Crystals obtained after elution with methanol has been subjected to paper chomatography. The paper shows the characteristic brown spot of glucosinolate after dipping in ammonical $AgNO_3$ solution followed by spraying with 5% NaOH in methanol. As the methanolic solution does not show any absorption of UV in the range 200 to 400 nm, it is suggested to contain hydrocarbon side chains as a part of the molecule. IR spectrum shows the charactaristic peaks (λmax): 3550 (OH), 2820 (CH), 1440 (CH_2), 1360 (CH_3) 1160 (SO_4), 1100 (C=N), 1050, 1010 (S-O), 800, 700 cm^{-1} and which suggests the glucosinolate nature of the isolated natural product. Glucosinolate nature of the compound has further been confirmed after studying the enzynatic degradation products of the isolated chemical.The enzyme myrosinase has been isolated from the crude extract of seed with the help of phosphate buffer activated with ascorbic acid at ph.7 and activity has been performed after being incubated at 35°c for 45min. The degraed products have further been purified with the help of column chromatography over alumina. The waxy solid obtained after elution with petroleum ether and petroleum ether : Benzene (3:1, 1:1) has been subjected to paper chromatography in different solvent mixturese. The spots have been observed to become positive to ammonical $AgNo_3$ solution.

IR spectrum of the waxy solid shows the absorption peaks (λmax) : 3200 (OH) 2230, 2120 (-N=C=S), 2060, 1440 (CH_2) 1360 (CH_3) 1100 (C=N)700, 600 cm^{-1} and which are characteristic for isolthiocynate. That isolated glucosinolate contains glucose as sugar component has been confirmed after observing similar behaviour of enzyme hydrolysate containing sugar with that of authentic glucose in paper and Thin layer chromatography and after developing coloured spots with different reagents charactaristic for glucose. The SO_4^{--} part of the glucosinolate has also been confirmed with the heavy white ppt. obtained after the treatment of enzyme hydrolysate with Barium chloride. It has further been confirmed after the treatment of the enzyme hydrolysate with lead acetate to obtain lead slphate dissolved in ammonium acetate.

An waxy granular residue has been Isolated during column chromatography of the hydrolysate obtained after enzyme activity on isolated glucosinolate from methanolic extract of seeds of *B.campestris* B-54. After being eluted with Benzene chloroform (3:1, 1:1) the waxy granular solid has been further purified from chloroform-ethanol mixture kept in the refrizerater for seven days. The IR spectrum of the solid shows characteristc peaks (λmax): 3400 (NH), 2820 (CH), 1685 (C=C), 1440 (CH_2) and700cm^{-1}. The most interesting feature of the compound is that the spectrum does not show the characteristic OH absorption peak at 3200 cm^{-1} or 3350 cm^{-1} as has been found in the IR spectra of isothiocynate and glucosinolate respectively. On the contrary the IR spectrum shows absorption at 3400 cm^{-1} which is probably due to NH and not for OH. Besides IR spectrum also shows no absorption peak for C=N (1100cm^{-1}) as is found in isothiocynate or glcosinolate. The characteristic peaks at 1200cm^{-1} 1040cm^{-1} may be considered due to (C-N) and (C-O) respectively. On the basis of these characteristic peaks the degraded product is supposed to be a thione like compound produced due to cyclization of isothiocynate structure and which is a common occurrence in *Brassica* sp. showing degradation of glucosinolate. Further work has not been done due to paucity of isolated chemical products. More work is needed in connection with identification of glucosinolate and its degraded products in the seed of *B.campestris* B-54.

The presence of sitosterol, cholesterol, the glucosinolate and its degraded products are being reported first time to occure in the seed of *B.campestris*B.54.

A new and rapid spectrophotometric method has been worked out for quantitative estimation of glucusinolate in the seeds of *B.campestris.* B-54 and

has been represented in Chapter-V. The method is based on the principle that glucosinolate in pure form produce characteristic colour in presence of ammonical $AgNO_3$ solution. 1gm of dry seeds crushed into powder has been extracted with 70% methanol. The concentrated extract has been subjected to column chromatography over alumina previously washed with dil H_2SO_4. The subfraction obtained after being eluted with Methanol : water (1.3) has been observed contain glucosinotate. 1ml of hot water extract of glucosinolate mixed with 1ml of ammonical $AgNO_3$ (1%) develops characteristic colour after being heated 1-2 minutes at 100°c when the reaction mixture is kept at room temperature (22±°C) for 30 min. The obserption maxima has been worked out to be 414nm. The colour remains stable up to 8 hours. The standard curve prepared with the solvent 10^0ppm to 10^3 ppm obeys Beers law. The proposed method may be considered for the first time to report and has several advantages. Glucosinolate may be determined from a minimum abount of 5-500mg of powdered seeds. It takes a very small duration of time to estimate the chemical. During estimation no enzymatic degradation is required as done by previous authors. The purification of glucosinolate by column chromotography is a new approach. With the help of this method, seeds of *B.campestris* B-54 grown in Northern and Sourthern part of West Bengal have been estimated to contain glucosinolate content of 0.64% and 0.44% respectively.

Chapter VI deals with observation on the effect of time of sowing on growth and yield of *B.campestris* B-54 in the plains of Darjeeling conditions. In this respect October sowing of seeds has been observd to show the best growth performance for all the parameters though November sowing of seeds swhos more or less the same growth performances as those observed in connection with October sowing seeds. From the investigation, it has also been recomended that sowing of seeds may be continued to January in the plains of Darjeeling condition to achieve appreciable amount of productivity of seeds. Cultivar fails to produce any seed during the period of sowing of seeds in the month of March, April and May.

Though mustard is generally consider as winter crop in Darjeeling district, but during the month of winter rainfall in this region is scarce. Moreover soil in the region specially in the plains has been observed to be of sandy loam type having low water holding capacity. Thus irrigation of water is felt necessary for a higher yield of seeds under such an adverse situation. But loses of nitrogen

from cropland after irrigatin, specially in a sandy loam soil of concern from the stand point of fertilizer use efficiency as well as potential water pollution. As it is accepted that nitrogen is the most important nutrient for Indian mustard in term of its requirement of the rate of its application and total cost management for plant respons much attention has been given to study the combined effect of irrigation and nitrogen or growth and yield attributes with the consideration of application of maximum number of splitdoses of nitrogen and of instalments of water irrigation during investigation Chapter VII deals with the part of work involving the application of six levels of irrigation freatment adopted separately in different plots during the growth period of *B.campestris* B-54. These are separately combined with one to seven levels of N-fertilizer treatment in split doses applied at different time intervals. Out of the investigation it has been observed that application of five levels of irrigation at 12 DAS, 20 DAS, 30 DAS, 40 DAS and 50 DAS to check water strees and five levels of N-fertilizer at 15 DAS, 25 DAS, 35 DAS, 45 DAS and 55 DAS to avoid salt strees become optimum to show maximum productivity of 16.60 q/ha of seed in the plains of Darjeeling condition.

The study of seed germination behaviour of any economically important crop is very essential specially when raising of large number of seedlings are necessary inconnection with its large scale cultivation. But storage of seeds in Darjeeling condition due to high humidity becomes a problem. Seeds of *B.campestris* B-54 has been observed to show decline in germination percentage during storage in laboratory condition and attain nonviability after several years of storage. As a result, much of stored seeds are becoming useless. Investigation has been carried out to identify methodology of chemical treatment by which invigoration of nonviable seeds may be possibel.

Chapter VIII deals with invigoration of non-viable seeds of *B.camprestris* B.54 due to treatment with vitamins for their commercial utilisation, in Darjeeling condition. On the basis of rating category of relative stages of nonviability of stored seeds with the help of TTC test showing degree of redness of seeds caused by formozan due to triphenyl tetrazolium chloride has been observed. Maximum of 93 percent of germination has been shown by the seeds immediately after their harvest when these are completely viable. Gradually they attain nonviability during their storage in laboratory condition and have shown one percent of seed germination after five years of storage. Several vitamins like

riboflavin, ascorbic acid, pyridoxine hydrochloride, nicotinicacid have been observed to show invigoration of seed germination for all types of seeds stored for a duration from one to five years. Maximum of 75 percent of germination of seeds stored for two years has been observed due to treatment of nicotinic acid (10^{-5}M) as compared to control (58 percent). Similarly ascorbic acid (10^{-7} M) stimulates germination of seeds (58 percent) stored for three years when the control value becomes 35 percent. It has been recomended that seeds of *B. campestris* B-54 stored maximum of 2 to 3 years are suitable for their commercial utilisation being pretreated with suitable vitamin.

Inter cropping of various plants is considered beneficial due to economic advantage in any region. In Darjeeling district cultivation of wheat coincides with that of mustard. As *B.campestris* B-54 shows characteristic of early flowering type, there is possibility to grow wheat immediately after the harvest of mustard plant. But from several traials of cultivation it has been observed that the growth and maintenance of wheat in the same plot previously utilised for cultivation of the same cultivar, become difficult due to some inhibitory activity of crop residue of *B.campestris* B-54 showing plant to plant interaction or in other words allelopathic effect. Chapter IX deals with investigation on allelopathic effect of seeds and crop residue of *B. campestris* B-54 with special empasis on isolation and identification of chemical inhibitor from crop residue and seeds of the cultivar. Water extract of crop residue has been observed to show complete inhibition of lettuce seed germination and maximum of 30 percent of wheat seed germination as against 100 percent seed germination in control. During phytochemical analysis of the crop residue, ferulic acid, a phenollic acid inhibitor has been isolated from $NaHCO_3$ subfraction of methonolic extract of the crop residue. Ferulic acid has also been observed to show similar inhibitory activity as that of water extract of crop residue of *B.campestris* B-54 and this has been verified with the bioassay test of lettuce and wheat seed germination. Ferulic acid has been identified after comparing m.p. 170-127°C, the co-chromotography and co-IR spectrum of the isolated chemical with those of authentic ferulic acid showing charactaristic absorption peak (λmax) : at 3310 (OH), 3250, 1680 (C=O), 1600, 1520 (aromatic C=C) 1430, 1360, 1320, 1290 (C-O), 1010, 870, 780 (O-subtituted benzene) 750, 720 and 690 cm^{-1} Glucosinolate isolated from seeds at 1000 ppm and 500ppm. has shown 30 and 60 percent respectively of lettuce seed germination and 40 and 55 percent respectively of wheat seed germination as against control (100 percent) for both the cases of treatment of glucosinolate.