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Promotory effects of Uv-B radiation alone and along with certain plant growth regulators on protease and peroxidase activity of two varieties of *Brassica species*

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Abstract
The aim of this study was to evaluate the appropriate concentrations of plant hormones from (10^{-5}) to (10^{-7} M) concentrations over UV-B damage on *Brassica campestris and Brassica juncea* in case of protease and peroxidase enzyme extraction. Seeds of both varieties were grown in the laboratory for the seed germination & seedling growth with appropriate concentrations of plant hormones for the isolation of enzyme protease and peroxidase. The protease activity was also enhanced in the germinating seeds of both varieties of mustard crops due to the UV-B exposure as compared to the control. When the seeds were supplied with plant hormones *viz*. IAA, Kn & GA_3 along with UV-B treatment, which changed significantly & decreased in *Brassica compestris* & *Brassica juncea* respectively as compared to UV-B exposure alone. A marked increase in peroxidase was noted in inhibited seeds of both the crops due to UV-B radiation recorded in *Brassica compestris* & *Brassica juncea* respectively due to UV-B radiation as compared to control. When the seeds were supplied with plant growth hormones along with UV-B radiation, this effect was altered significantly and a decrease was reported in *Brassica compestris* & *Brassica juncea* as compared to the UV-B exposure only.

Keywords: *Brassica compestris*, *Brassica juncea* protease, peroxidase, IAA, kinetin GA_3 & UV-B exposure

Introduction
Ultraviolet-B radiation (280-315nm) is highly vigorous and can cause damage to a wide range of cellular components such as DNA, amino acids & lipids. Plants because of their sessile nature are potentially very susceptible to UV-B exposure and the increase in UV-B as a result of ozone depletion could have severe consequences. Over the last few decades there has been a substantial amount of studies on the effect of UV-B exposure were showed in the plants. These studies show a large number of responses including changes in growth and development, increase in protective pigment biosynthesis, effects on photosynthesis, DNA damage and cellular changes induced by UV-B exposure such as changes in gene expression. These responses are also variable, both between species and even within varieties of the some species. The effects of UV-B radiations on the plants include reduced biomass allocation, altered biomass allocation and increased flavonoid content (Lavola 1998; Hofmann *et al*. 2001 & Kolb *et al*. 2001) [23, 12, 20]. The growth responses of the plant species to ambient UV-B radiation can be positive or negative (Lakso & Huttuncen *et al*., 1998) [22]. These differences in UV-B responses may be associated with the genetic variation, influence of other factors such as drought and nutrient availability, the presence of protectively features like waxy and reflective layers or a thick epidermis and the presence of UV-B absorbing flavonoids (Correia *et al.*, 2000; Yuan *et al*., 2000; Beiza & Lois 2001) [6, 41].
The effects of UV-B radiation on water relations, leaf development and gas-exchange characteristics in pea (*Pisum sativum*) plants subjected to drought were investigated. Plants grown throughout their development under a high irradiance of UV-B radiation were compared with those grown without UV-B radiation. The UV-B radiation resulted in a decrease of adaxial stomatal conductance by approximately 65%, increasing stomatal limitation of CO_2 up take by 10 to 15%. The growth in UV-B radiation resulted in large reductions of leaf area and plant biomass, which were associated with a decline in leaf cell numbers and cell divisions. UV-B radiation also inhibited epidermal cell expansion of the exposed surface of leaves. Photosynthetic rate and productivity in many plants species can be reduced by increased exposure to UV-B radiation (Teramura & Ziska, 1996) [39]. UV-B induced inhibition of CO_2 assimilation in mature leaves. A reduction in Rubisco activity has been suggested as a cause of the reduced CO_2 assimilation rate in leaves exposed to increased UV-B radiation. A primary cause of the decrease in the light-saturated rate of CO_2 assimilation induced by exposure to elevated UV-B radiation in leaves of oilseed rape has been shown to be a loss of Rubisco (Allen *et al.*, 1997) [1,3], which may also be associated with the loss of activity of other Calvin cycle enzymes (Baker *et al*., 1997) [1,3].
The leaves of *Zea mays* were subjected to different scenarios of ultraviolet-B radiation in a Sun simulator to determine the cellular vitality at the microscopic level and the contents of carbohydrates and photosynthetic pigments. The carbohydrate partitioning was more significantly influenced by UV-treatments. The glucose was decreased under high UV-B. Changes in the photosynthetic pigments were limited to a slight destructive effect of UV-B on the chlorophyll b. (Michael Barsing *et al*., 2000) [29]. Amongst these, there is now a substantial body of evidence, indicating significant effects of UV-B on secondary compounds of the Phenyl propanoid pathway and the enzymes responsible for their synthesis (Tattini *et al.*, 2000; Liakaura *et al.*, 2001), which act as sun-screens, providing protection against UV-B radiation. Interestingly, an enhancing effect of UV-B has also been obtained (over a much longer growing periods) in *Mentha spicata*, for plants growing in natural conditions in a

Mediterranean climate, apparently even under these conditions, supplementary UV-B (given for entire seasons) can lead to enhanced levels of volatiles in the plants (Karou *et al.*, 1998) [19].The concentration of those compounds is thought to reduce the epidermal transmittance and provides greater protection to the leaf mesophyll.

It has been well established that the plant growth regulators, influence the growth and development of plants. These chemical substances are able to coordinate growth among different plant parts or different physiological and biochemical processes. The main naturally occurring plant growth hormones viz. IAA, Kn and GA_3 are able to control many of the physiological processes that involved in the plant development. Plant growth regulators have been tried to improve growth and ultimately yield (Patil *et al*., 1987 and Kumar *et al*. 1996) [33, 16], tried various growth regulators to obtain better yield of good quality heads in cabbage and obtained encouraging result. The maturity of the vegetable crops is hastened, due to the application of plant growth regulators (Chnonkar & Jha, 1963) [5]. The phytohormones play a regulatory role in the imposition of seed dormancy, in the release of seed from dormancy, in the reserve mobilizations during seed germination and in the subsequent development, cytokines role in the seed germination (Khan & Tao, 1978) [17]. The overall plant growth was improved by the plant growth regulator treatments as compared to the UV-B treatment only. These treatments significantly, increased all plant growth parameters. The increased vegetative growth of plants nourished and developed in a better manner, than the nourishment to UV-B alone. IAA, Kn and GA_3, which are most important growth regulators and has a profound effect on the crop production, through increase in the stem length, leaf area, flower induction, yield and weight & size of crops. There are numerous studies on the effect of growth hormones on plants (Mishra *et al.*, 1986 and Reis *et al*., 2000) [26, 34]. Some of these studies have shown physiological and growth traits and many have found promotion in these traits in response to increased growth hormone. The impact of growth regulators on various physiological parameters have been worked out by various workers. Mahmud (1983) evaluated the effect of various growth regulators on growth, development and yield of various varieties of oil-seed crops. The treatments of different growth substances have given remarkably encouraging results in promoting seed germination in tomato, bottle gourd, radish, lettuce, watermelon, brinjal, carrot and a number of other vegetables (Swaminathan, 1987) [35]. In intraspecific crosses of *Brassica*, enhanced siliqua and seed production was reported by Sharma *et al*, (1997) [37] due to exogenous application of NAA, GA_3 and Kinetin. Cytokinins have also been reported to release dormancy and enhanced germination. The endogenous cytokinins (Kn) would appear to be key factors in the initiation of the radicle growth. The external application of cytokinins and gibberellins has been shown to substitute for the physiological influence of roots on the growth of de-rooted oat (Jordan & Skoog, 1971) [14] and in the soybean seedlings (Holm & Key, 1969) [9]. Kinetin used as seed treatment or foliar spray individually or in combination, increased the seed yield by 26%, while foliar spray increased it by 43.6% over control. However, maximum favorable effects of kinetin (Kn) were obtained with combined application of seed treatment plus foliar spray, which increased seed yield by 68.6% over untreated plants (Shang *et al*., 2000) [38]. Kinetin (Kn) also favorably affected two important plant processes *viz*. photosynthesis and nitrogen metabolism. Net photosynthetic rate and nitrate reductase activity significantly increased in plant treated with kinetin. The significant increase in content of the total chlorophyll content with kinetin application as also reported by (Khalil & Mandurahi, 1989) [18]. The concentrations of starch, soluble protein and free amino acids were maximum, when kinetin (Kn) was applied both as seed treatment and foliar spray. The anti-oxidative activities of SOD and enzymes increase progressively with flower development, but declined during the advance stage of petal senescence of gladiolus (Hossaian *et al*., 2006) and in rose (Kumar *et al*., 2007) [21].

The Kn application was associated with a high Harvest Index (HI), thereby, indicating partitioning of more photosynthesis towards seeds. Significantly higher seed yield in Kn treated plants also led to higher water use efficiency in spite of lower or comparable water use in control and water treated plants (Blackman & Davies, 1985) [2]. Nagel *et al.,* (2001) [31] have evaluated that cytokinin application plays a significant role in the flower production and exerted a positive effect on the yield of soybean thus increasing the total seed production.

Exogenous level of gibberellins partially substituted for the cold requirement for flower stalk elongation, also implicating the involvement of plant hormone in the processes (Hanks, 1982) [11]. During rapid flower stalk growth in tulips elongation occurs mainly due to cell expansion (Gilford & Rees, 1973) [8]. The cell expansion requires readily available hexose substrates used for increased metabolism and biosynthesis of new material such as cell wall. The gibberellins have been observed to influence the carbohydrate status in the many plant species (Yim *et al.,* 1997) [40]. In elongated tissues, common response to exogenous gibberellins is an increase in acid invertase activity (Miyamoto *et al*. 1993; Wu *et al*., 1993) [23].

The enhanced number of open flowers per spike induced by GA_3 and sucrose treatment can also be attributed to higher petal sugar status (reducing and non-reducing sugars) and higher solution uptake. High petal sugar status and water balance in flowers is suggested to improve bud opening (Halevy & Mayak, 1981) [10]. Gibberellin has the characteristics property to improve the yield, plant height, flower of *chrysanthemum* as shown by Mohariya *et. al.,* (2003) [30]. Pharis and King (1985) [32] observed that gibberellins (GA) play a major role in the development of fruit set. Variations in irradiance, light quality photoperiod and radiant exposure, produced changes in the production of endogenous growth factors, many of these factors are necessary for the success of plant life (Moe & Anderson, 1987) [27]. The study was to evaluate the appropriate concentrations of plant growth hormones concentrations over the UV-B damage on *Brassica campestris and Brassica juncea* in case of protease and peroxidase enzyme extraction. The protease and peroxidase activity was also enhanced in the germinating seeds of both varieties of mustard crops due to the UV-B exposure as compared to the control, when the seeds were supplied with plant hormones with UV-B treatment, which changed significantly & decreased in *Brassica compestris* & *Brassica juncea* respectively as compared to UV-B exposure only.

Study area

The study site was located in Ram Chandra Uniyal Govt. P.G.College, Uttarkashi region of Uttarakhand state. Uttarkashi District is a district of Garhwal division of the Uttarakhand state in Northern India and has its headquarters at Uttarkashi city. Uttarkashi is located at 30.73°N 78.45°E. It has an average elevation of 1,165 meters (4,436 feet) & Most of the topography is hilly. Uttarkashi District town lies high in the Himalaya range and the district contains the source of both the Ganges from Gangotri and Yamuna from Yamunotri rivers, which attract thousands of Hindu pilgrims. The town lies on the main route to Gangotri, has many Hindu temples and is also considered an important Hindu pilgrimage centre. The district is bounded on the North by Himachal Pradesh state, on the Northeast by Tibet, on the East by Chamoli District, on the Southeast by Rudraprayag District, on the South by Tehri Garhwal District and on the West by Dehradun District. Uttatkashi it means North Kashi it's one of the favorite place of Lorad Shiva call this Kashi Biswanaath.

In the present study the protease and peroxidase activity was also enhanced in the germinating seeds of both varieties of mustard crops due to the UV-B exposure as compared to the control, when the seeds were supplied with plant hormones with UV-B treatment, which changed significantly & decreased in *Brassica compestris* & *Brassica juncea* respectively as compared to UV-B exposure only.

Fig 1: Uttarakhand Map

Objective of work

In the present research work, mitigatory impacts of plant growth regulators *viz*. IAA, Kn and GA_3 has been aimed to study on UV-B exposed mustard crops in the laboratory. Though, a number of references are available regarding deleterious or sometimes promotory effects of UV-B on various physiological and biochemical parameters as evidenced from different worker. But no studies are available to assess the synergistic impact of UV-B and plant growth hormones. Therefore, present study was being proposed to evaluate the individual effects of UV-B exposure in the combination of some plant growth regulators such as IAA, Kn & GA_3 concentrations to mitigate or enhance the some biochemical parameters of the two *Brassica species* such as *Brassica compestris* and *Brassica juncea* respectively.

Methodology

Table 1: General experimental design

Treatments		UV-B	IAA			Kn			GA_3			IAA+UV-B			Kn + UV-B			GA_3+UV-B	
Concentration	Control	(3-hrs)	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}

Extraction of protease enzyme

Protease enzyme was extracted in the laboratory from the germinating seeds of different treatments of both the varieties of mustard crops. One gram of germinated seeds was homogenized in chilled Tris-HCl buffer, centrifuged at 5000 rpm for 5 minutes and then supernatant was used as enzyme source. The volume of supernatant was made 25 ml by adding Tris-HCl buffer. All the operations were carried out at 4 to 5^0C as was noted by Sadasivam and Manickam *et al.,* (1996) [36]. Protease activity in extracted material was measured by modified method of Green and Neurath (1954). One ml of extracted material in Tris-HCl buffer, extracted earlier and preserved at 4^0C, one ml of protein solution and one ml of Tris-HCl was incubated at 40^0C, for 1 hrs. One ml of TCA was added to the above and kept in freezer for 3 hrs. After that, the whole solution was centrifuged to get clear supernatant. In supernatant solution, 1 ml of 1.5 NaOH was added in a separate volumetric flask and final volume was made to 10 ml with distilled water. One ml aliquot of the above solution was mixed with 5 ml. alkaline copper tartrate solutions and then incubated for 10 minutes at 40^0C and then added one ml of Folin-phenol reagent. After 30 minutes the absorbance of the solution was read at 600 nm. A calibration curve was also prepared following the above

method and utilizing standard amino acids. The released amino acids were measured through comparisons of assayed and standard curves.

Extraction of peroxidase enzyme

The extraction of crude enzyme was carried out as followed for the protease activity as was observed by Sadasivam and Manickam *et. al.,* (1996) [36].One ml aliquot of enzyme extract prepared earlier and preserved at 4^0 A was mixed with 7 ml distilled water, 2 ml benzidine solution, 2 ml of 6% H_2O_2. The optical density of the solution was measured after 1 minute by spectrophotometer using quartz cuvettes at 610 nm. The activity measured was expressed in Δ.O.D. (difference) as was identified by Mahely and Chance *et al.,* (1967).

Observations and results

Protease enzyme

The uniformly seeds of the two varieties of mustard crops were selected and soaked in the distilled water for 6 hrs, 12 hrs. and 24 hrs. respectively. After some time these presoaked seeds were spread in the different Petridishes as (A, B, C, D and E). Two Petridishes for each crop were kept as control condition, i.e. neither exposed to UV-B and nor plant growth regulators, another two Petridishes were irradiated to 3 hrs. daily UV-B exposure only and nine sets of Petridishes were added with IAA, Kn and GA_3, along with UV-B radiation (3 hrs daily), for the each variety of mustard seeds. After giving the different treatments to seeds, the development of protease activity was measured and compared. In the seeds of the *Brassica compestris* (Table 1.1 & Fig.1.1), the effect of UV-B exposure alone and along with different plant growth regulators on the protease activity was studied. After imbibitions of seeds in water, there was a considerable rise in the activity of protease enzyme. Data obtained from UV-B exposed Petridish, showed a marked promotion and recorded as ca. 115%, 124% & 120% respectively at the 6 hrs, 12 hrs. and 24 hrs. as compared to control petriplates. The maximum inhibition was reported in seeds soaked in Kn and a reduction of ca.11%, 15%, and 19% was reported at the 6 hrs, 12 hrs. and 24 hrs. respectively, as compared to the individual treatment of UV-B exposure. Protease analysis of seeds of Petridishesh IAA & GA_3 plant growth regulators was showed slight inhibition of protease activity as compared to the individual treatment of UV-B.

In *Brassica juncea* (Table 1.2 & Fig.1.2), the effect of UV-B exposure alone and along with some plant growth regulators such as IAA, Kn and GA_3 on the protease activity. After imbibitions of seeds in water, there was a considerable rise in the activity of protease enzyme. The result was obtained from the Petridish-A (control) and recorded as ca. 8.77±0.070, 9.727±0.090, and 11.289±0.038 at the 6 hrs, 12 hrs. and 24 hrs. respectively. UV-B exposed Petridish was showed a marked promotion and increase by ca.114%, 122% and 139% at the 6 hrs, 12 hrs. and 24 hrs. respectively, as compared to control (A). The Petridish-C showed a rise of ca. 11%, 5%, 6% at the 6 hrs, 12 hrs. and 24 hrs. respectively as compared to individual treatment of UV-B exposure. Petridish-D showed a rise of ca. 22%, 17%, 2% at the 6 hrs, 12 hrs. and 24 hrs. respectively as compared to individual treatment of UV-B exposure. Petridish-E also showed a rise of ca 40%, 13%, 31% at the 6 hrs, 12 hrs. and 24 hrs. respectively as compared to individual treatment of UV-B exposure.

Peroxidase enzyme

The effects of UV-B exposure alone and along with plant growth regulators such as IAA, Kn and GA_3 on the peroxidase activity were investigated on *Brassica compestris PT-303* and *Brassica juncea PR-15* during the study of seed imbibitions. Table 1.3 & Fig. 1.3 (Brown sarson) showed that there was a considerable rise in peroxidase activity in control and noticed as ca. 0.42±0.02, 0.44±0.03 and 0.48±0.005 at the 6 hrs, 12 hrs. and 24 hrs. respectively. Petridish-B (UV-B only), showed a rise in peroxidase activity and it was reported as ca. 68%, 123% and 108% at the 6 hrs, 12 hrs. & 24 hrs. respectively as compared to control. Petridish-C showed a rise of ca. 3%, 41%, 24% at the 6 hrs, 12 hrs. and 24 hrs. respectively as compared to individual treatment of UV-B exposure. Petridish-D showed as ca. 2% 12%, 37% at the 6 hrs, 12 hrs. and 24 hrs. respectively as compared to over UV-B treatment. Petridish-E was showed a rise of ca. 49%, 42%, 24% at the 6 hrs, 12 hrs. and 24 hrs. respectively as compared to over UV-B treatment.

Table 1.4 & Fig. 1.4, exhibited that peroxidsae enzyme activity was continuously increased in case of *Brassica juncea PR-15*, when subjected to individual treatment of UV-B. This peroxidase activity was reported in control as 0.525±0.04, 0.590±0.09, and 0.598±0.08 at the 6 hrs, 12 hrs. and 24 hrs. respectively. UV-B (3 hrs. daily), exposed Petridish (B), was studied and recorded an increase of ca. 105%, 106% & 113% at the 6 hrs, 12 hrs. and 24 hrs. respectively as compared to control (A). The Petridish-C studied and recorded as increase of ca. 7%, 23%, 21% at the 6 hrs, 12 hrs & 24 hrs respectively as compared to over UV-B treatment. Petridish-D showed an increase of ca.7%, 30%, 36% at the 6 hrs, 12 hrs & and 24 hrs. respectively as compared to individual treatment of UV-B exposure. Petridish-E showed an increase of ca. 9%, 34%, 44% at the 6 hrs, 12 hrs & and 24 hrs. respectively as compared to over UV-B exposure.

Table 2: Protease activity as affected by UV-B radiation (3 hrs. daily), individually and in combination of IAA, Kn and GA_3 during seed imbibitions in *Brassica compestris PT-303*

Stage	A	B	C	D	E
Dry	4.560±0.05	-------	-------	-------	--------
6-hrs.	8.690±0.06	10.070±0.80	13.089±0.80	9.080±0.80	11.790±0.80
12-hrs.	11.740±0.07	14.630±0.60	17.560±0.230	12.480±0.40	14.090±0.49
24-hrs.	13.870±0.08	16.660±0.03	18.650±0.650	13.540±0.50	16.430±0.43

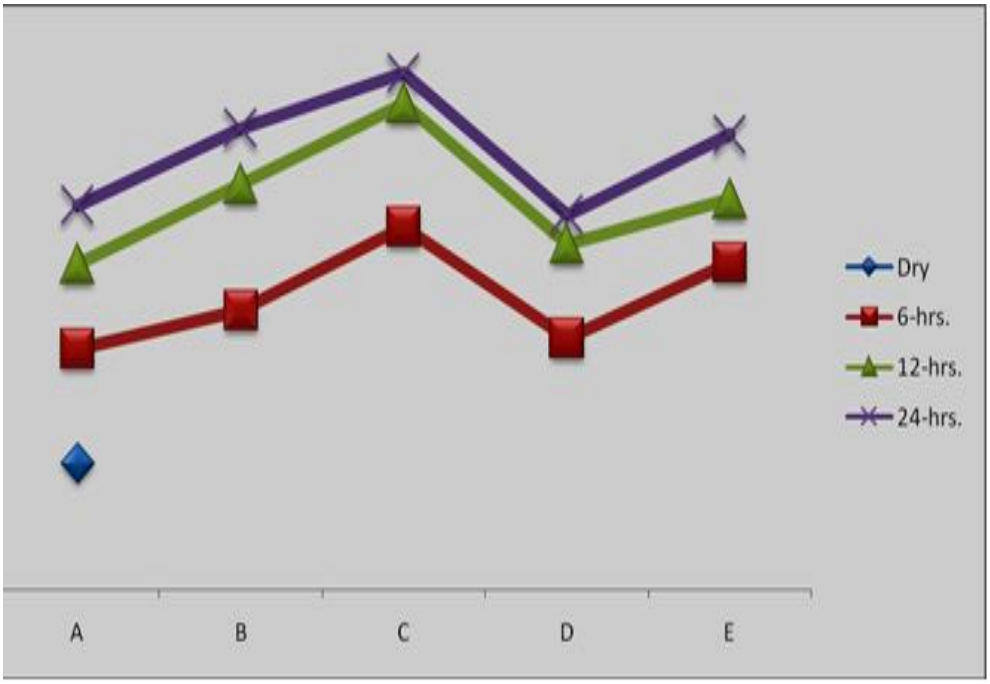

Fig 2: Protease activity as affected by uv-b radiation (3 hrs. daily), individually and in combination of iaa, kn and ga_3 during seed imbibitions in *Brassica compestris pt-303*

Table 3: protease activity as affected by uv-b radiation (3 hrs. daily), individually and in combination of iaa, kn and ga_3 during seed imbibitions in *Brassica juncea pr-15.*

Stage	A	B	C	D	E
Dry	0.502±0.05	----------	----------	----------	--------
6-hrs.	0.526±0.04	0.556±0.05	0.597±0.09	0.599±0.09	0.610±0.10
12-hrs.	0.590±0.09	0.630±0.03	0.780±0.08	0.820±0.07	0.850±0.05
24-hrs.	0.598±0.08	0.680±0.08	0.825±0.02	0.930±0.03	0.980±0.98

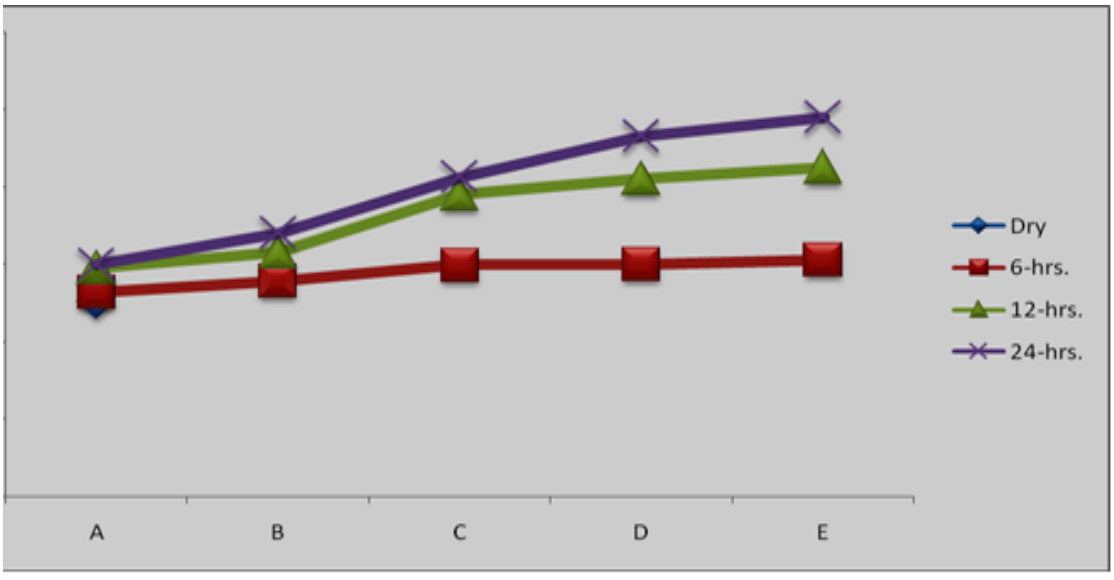

Fig 3: Protease activity as affected by UV-B radiation (3 hrs. daily), individually and in combination of IAA, Kn and GA_3 during seed imbibitions in *Brassica juncea PR-15.*

Table 4: Peroxidase activity as affected by uv-b radiation (3 hrs. daily), individually and in combination of iaa, kn and ga_3 during seed imbibitions in *brassica compestris pt-303.*

Stage	A	B	C	D	E
Dry	0.405±0.04	-------	--------	---------	---------
6-hrs.	0.425±0.02	0.291±0.069	0.297±0.07	0.294±0.02	0.290±0.03
12-hrs.	0.445±0.03	0.549±0.052	0.775±0.067	0.620±0.01	0.780±0.08
24-hrs.	0.485±0.05	0.520±0.030	0.649±0.050	0.715±0.052	0.649±0.06

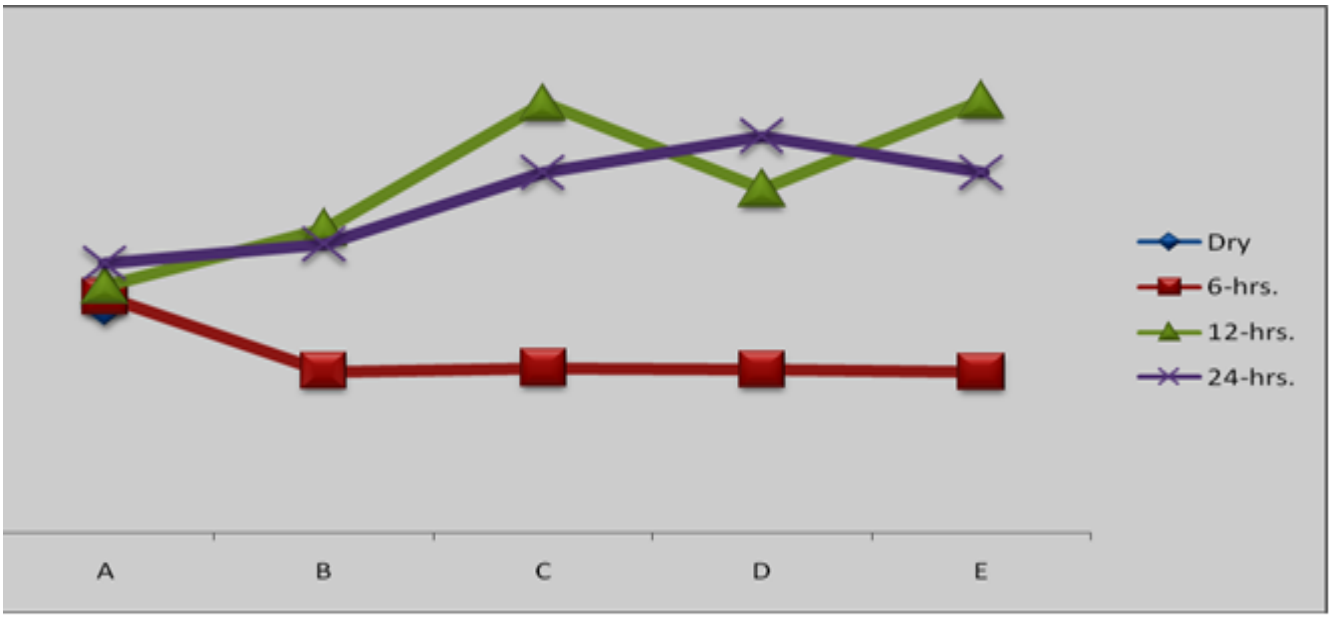

Fig 4: Peroxidase activity as affected by uv-b radiation (3 hrs. daily), individually and in combination of iaa, kn and ga_3 during seed imbibitions in *brassica compestris pt-303*

Table 5: Peroxidase activity as affected by UV-B radiation (3 hrs. daily), individually and in combination of IAA, Kn and GA_3, during seed imbibitions in *Brassica juncea PR-15*

Stage	A	B	C	D	E
Dry	0.502±0.05	-------	--------	---------	---------
6-hrs.	0.526±0.04	0.556±0.05	0.597±0.09	0.599±0.09	0.610±0.01
12-hrs.	0.590±0.09	0.630±0.03	0.780±0.08	0.820±0.07	0.850±0.05
24-hrs.	0.598±0.08	0.680±0.08	0.825±0.02	0.930±0.03	0.980±0.98

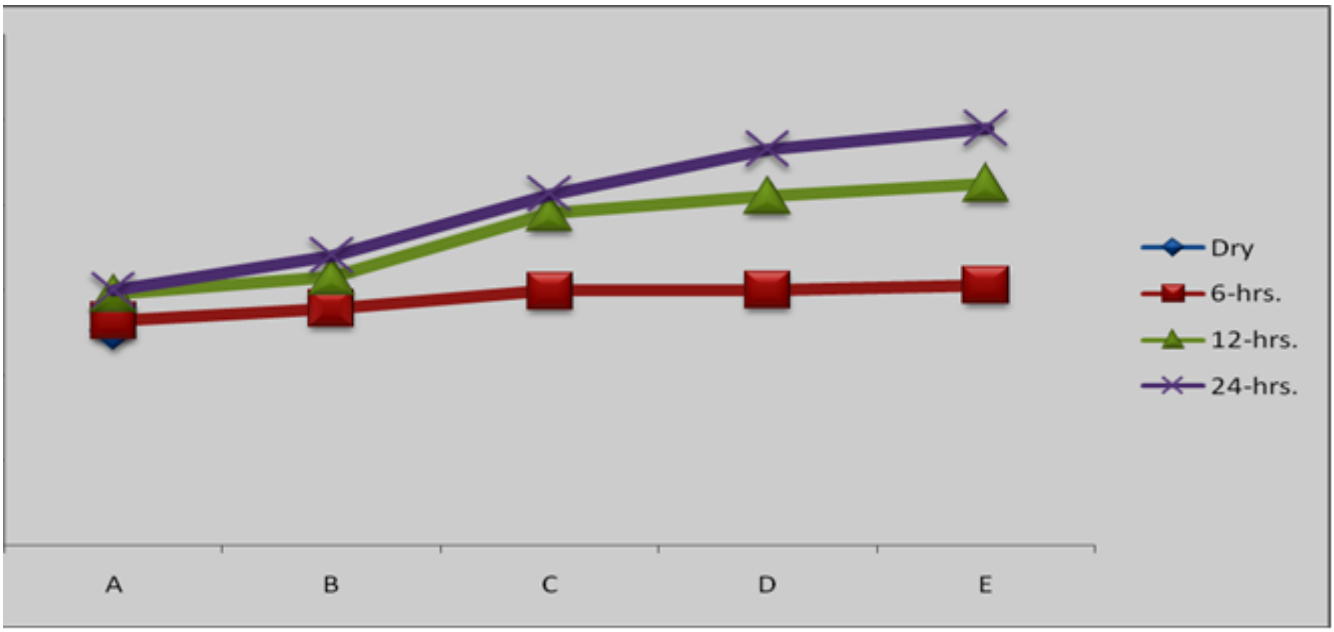

Fig. 5: Peroxidase activity as affected by uv-b radiation (3 hrs. Daily), individually and in combination of iaa, kn and ga_3, during seed imbibitions in *brassica juncea pr-15***.**

Discussions

This study showed that the considerable rise in protease and peroxidase activities in the germinating seeds as compared to pre-existing enzymes in the seeds. The experimental data showed that the enhancement of protease activity upto ca. 115% at 6 hrs; 124% at 12 hrs. & 120% at the 24 hrs. respectively in the UV-B exposed geminating seeds of *Brassica compestris* as compared to control condition. When the crop was subjected to combined treatment of UV-B along with Kn (10^{-5} M), the most mitigatory effects was found and which lowered the activity of protease upto 15% at the 6 hrs; 11% at the 12 hrs & 19% at the 24 hrs. respectively as compared individual treatment of UV-B exposure. Other growth regulators have showed the slight inhibition as compared to UV-B exposure alone. When the crop was exposed to UV-B only, showed a considerable rise

in the peroxidase activity and reported as ca. 68%, 123% & 108% at 6 hrs, 12 hrs. & 24 hrs. respectively as compared to control. When the crop was subjected to UV-B along with PGRs such as IAA, showed a rise of ca. 3% at 6 hrs, 41% at 12 hrs and 24% at 24 hrs; Kn of ca. 2% at 6 hrs; 12% at 12 hrs; 37% at 24 hrs. and GA_3 of ca. 99% at 6 hrs; 42% at 12 hrs; 24% at 24 hrs. respectively as compared to individual treatment of UV-B exposure.

In case of *Brassica juncea*, exposed to UV-B radiation, a marked promotion was showed and increased by ca 114%, 122% and 139% at 6 hrs, 12 hrs & 24 hrs. respectively in terms of protease activity as compared to control. When the crop was subjected to combined treatment of UV-B along with PGRs such as IAA, showed a rise of ca. 11%, 5%, 6%; Kn of ca. 22% 17%, 2% & GA_3 of ca. 40%, 13%, 31% at 6 hrs, 12 hrs and 24 hrs. respectively as compared to individual treatment of UV-B exposure. When crop exposed to individual treatment of UV-B exposure, the peroxidase activity was studied and recorded an increase of ca. 105%, 106 & 113% at 6 hrs, 12 hrs & 24 hrs. respectively as compared to control. When crop subjected to combined treatment of UV-B along with PGRs *viz*. IAA, showed a rise of ca. 7% at 6 hrs, 23% at 12 hrs, 21% at 24 hrs; Kn of ca. 7% at 6 hrs, 30% at 12 hrs, 36% at 24 hrs and GA_3 of ca. 9% at 6 hrs, 34% at 12 hrs, 44% at 24 hrs. respectively as compared to individual treatment of UV-B exposure.

Conclusions

The present study was concluded that to investigate the protease activity was also enhanced in the germinating seeds of both varieties of mustard crops due to UV-B exposure. A rise of ca.115% after 6 hrs; 124% after 12 hrs. and 120% after 24 hrs. respectively in *Brassica compestris* as compared to control. In *Brassica juncea*, a rise of ca.114%, 122% & 139% was noticed after 6 hrs; 12 hrs. & 24 hrs. respectively as compared to control. When the seeds were supplied with plant growth hormone along with UV-B treatment which changed significantly and a decrease of ca. 11%, 15%, 19%; 22%, 17%, 2%; 40%, 13% 3% at 6 hrs, 12 hrs. & 24 hrs. reported in *Brassica compestris* and *Brassica juncea* respectively as compared to UV-B exposure alone. A marked increase in peroxidase was noted in inhibited seeds of both the crops due to UV-B radiation. An increase of ca. 68%, 123%, 108% was recorded in *Brassica compestris* and ca. 105%, 113%, 106% in *Brassica juncea* after 6 hr, 12 hr and 24 hr of imbibitions respectively due to UV-B (3 hrs. daily) radiation as compared to control. When the seeds were supplied with plant growth hormones along with the above treatment, this effect was altered significantly and a decrease of ca. 3%, 41% and 24% with IAA at 6 hr, 12 hrs and 24 hrs; with Kn ca. 2%, 12%, 37% at 6 hrs, 12 hrs and 24 hrs and with GA_3 ca. 42%, 42%, 24% at 6 hrs, 12 hrs and 24 hrs. respectively was reported in *Brassica compestris*. In case of *Brassica juncea*, a decrease of ca. 7%, 23%, 21% with IAA; ca. 7%, 30%, 36% with Kn and ca. 9%, 34%, 44% with GA_3 at the 6 hrs 12 hrs & 24 hrs respectively as compared to UV-B exposure only.

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