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SELENATE UPTAKE THROUGH ROOTS IMPROVES THE TOLERANCE TO CELL DISRUPTORS AND FUNGAL INFECTION IN CANOLA SEEDLINGS AFTER EXOGENOUS SELENIUM APPLICATION

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Certain hyperaccumulator plants can accumulate the Selenium (Se) at high concertation (1000 mg kg^{-1} d.wt.) from the soil available Se sources. It is still unknown whether this Se hyperaccumulation may be used to control the cell disrupter and fungal infection in Aridisoil of Punjab-Pakistan. Additionally, its deterrence and toxic properties also motivated the researchers to use Se in Plants. In this study, canola (*Brassica napus* L) was grown on nutrient culture with low (2 µM; -Se) and high Se (30 µM; +Se) concentrations to test the protection capabilities of Se against the cell disrupters and fungal infections. *B. napus* plants tested with higher Se concentrations (more than 800 mg Kg^{-1}) were observed more protected from turnip aphid's herbivory as compared to the low concentrations (less than 100 mg Kg^{-1}). Similarly, the leaves of test plants with elevated Se concentrations (30 µM) were found more successful to avoid herbivory than the leaves of plants containing low Se concentrations (2 µM). Furthermore, feeding of red legged mites were reduced by 200% with the +Se application as compared to -Se application. Additionally, in another experiment, already established mite population was reduced by 55% with +Se application over -Se application. It was also noted that the leaves of *B. napus* plants contained the 1000 $mgkg^{-1}$dw Se concertation. However, the smaller lesions (8 fold) on the B. aphid leaves were found in +Se treated plants than in -Se applied plants. The experiment resulted concluded that the Se concentration i.e. 500 mg kg^{-1} d. wt. in the plant leave tissues was sufficient to restrict the growth of *A. brassicicola*. In conclusion, Se application can significantly protect the Brassica plants from cell disruptor and fungal infection in both conditions i.e. fresh attacked and already established population. The results of current study would beneficially encourage the plant breeders and researchers to introduce genetically engineered crops which may produce significant amount of selenium in the plant cells.
Keywords: Selenium, insects, fungus, resistance, herbivory, *Brassica napus*

INTRODUCTION

Environment friendly technologies in agriculture sector are always suitable for food security, improving human health and to conserve and rehabilitate the environment despite their high cost. Due to the limited resources, farmers especially from developing countries prefer to adopt the strategies which are low in cost and most effective against insect pests and disease of different crops. Selenium (Se) is a better option as compared to other prohibitive with low cost and environment friendly. Se is beneficial for both animals and human (Seppänen *et al.*, 2003; Bybordi, 2010) and involved in the activation of number of enzymes (Hanson *et al.*, 2003). Furthermore, it has been reported that Se improves plant growth by improving the antioxidant capacity (Hanson *et al.*, 2004) and producing 2-3-fold more biomass of plant (El-Mehdawi *et al.*, 2011).

Previous studies showed that the Se can cause the destructive effects on aphid's herbivory, and hence reduced the aphid population on plants (Freeman *et al.*, 2009; Boyd, 2010; Quinn *et al.*, 2010; Prins *et al.*, 2011; Quinn *et al.*, 2011). In addition, Se affects the feeding site preferences, the host plant selection and acts as antifeedant for larvae of herbivores insects. Plant species differ in their capacity to accumulate and tolerate Se (El-Mehdawi *et al.*, 2012). Se hyper accumulator plants can accumulate more than 0.1% of their dry weight as Se, without showing any symptoms of toxicity (White *et al.*, 2007). Moreover, the plants (*Brassica* spp.) that accumulated Se have defense mechanisms against caterpillars (*Pieris rapae* L.) and aphid (*Myzus persicae* L.) due to both deterrence and toxicity (Hanson *et al.*, 2003; Hanson *et al.*, 2004). Se was found predominantly in the periphery areas such as the margins, epidermis and trichomes resulting in less or no insect's herbivory (Freeman *et al.*, 2009) and helps in removing the stress of plants during attack (Kumar *et al.*, 2012). Moreover, Selenium, Nickle and Zinc hyper accumulation appeared to be protective for the plants against fungal pathogen attack (Hanson *et al.*, 2003; Freeman *et al.*, 2006) and they also reduce the stress against the insects and fungal attack. Accumulation of Se in the various locations of tissues of the plant determines the frequency and intensity of herbivory. In addition, the plant species try to sequester the Se in tissues and organs which are susceptible to herbivores (Hanson *et al.*, 2003).

Turnip aphids (*Linaphis erysimi*) and red legged earth mites (*Halotydeus destructor* L.) are extensive herbivores of brassica species worldwide, as well as in the brassica growing areas of Pakistan. The mites seriously damage the crop establishment while the aphids attack the *Brassica napus* L. at middle and lateral stages of crop by sucking the plant cell sap. The aphid honeydew is responsible for the growth and spread of sooty mold fungus on the plant leaves. Moreover, the photosynthetic activities of the affected plant leaves are seriously damaged by these herbivores. Sometime crop fails completely due to sever attack of these insects at seedling establishment and flower and pod formation, which requires sowing the seed again. Two fungus species; *Alternaria brassicicola* L. (brassica species specific leaf pathogen) and *Sclerotinia sclerotioram* L. (stem and root rot of brassica species) are the common disease-causing agents in canola growing areas of Pakistan (Razaq *et al.*, 2011). These phytopathogenic fungal species infect the brassica plants in the cold and dry environmental conditions of the world. Some time, sever attack may lead to the complete failure of crop.

In Pakistan, aphid can feed and cause damage on wide range of crops and plants including cabbage, peas, potato, tomato, beans, vegetables plants, annual flowering plants (e.g., snapdragons) perennials (e.g., lupines) flowering shrubs (e.g., roses, snowball bush) and fruit trees such as apple, pear, crab apple, plum and pine (Aziz *et al.*, 2013). Aphids have major contribution in reducing (75.06 %) the canola production in Pakistan (Razaq *et al.*, 2011). Moreover, aphid have developed the resistance against pesticides used against it (Sarwar *et al.*, 2013). Accumulation of Se in the different body parts of *Brassica* may act as chemosensory for aphids results in least damage by the insect or mammalian herbivores (Prins *et al.*, 2011; Quinn *et al.*, 2011; Kumar *et al.*, 2012) ultimately making plants more attractive to other pollinators (Freeman *et al.*, 2006). Furthermore, Se metabolize selenoamino acids in insects resulting in non-functionality of many proteins and enzymes (Hladun *et al.*, 2013) which restricts the growth and developments of larvae.

The very first study carried out to test the hypothesis that elevated concentration of Se can protect the *Brassica napus* L. from herbivory and fungal infection. No study was carried out on the control of the phytopathogenic fungi by the exogenous application of Se in Pakistan. *Brassica napus* L. is secondary accumulator and has ability to accumulate the Se at higher magnitude by ensuring the elemental defense mechanism against cell disruptors and phytopathogenic fungi. In the current study, two cell disrupters species turnip aphids: *Linaphis erysimi* and red legged earth mites (*Halotydeus destructor* L.); and two *Brassica* host specific fungal species: *Alternaria brassicicola* L. (leaf pathogen) and *Sclerotinia sclerotioram* L. (stem and root rot pathogen) were tested during the experiment. Both these herbivores widely attack on *B. napus* L. crop. Both pests are economically and ecologically important herbivores for *B. napus* L. crop. The results of this study would provide the crucial information to the farmers and agricultural scientist to control the herbivores and fungal infection through alternative means rather than the traditional pesticides which are toxic for environment and human.

MATERIALS AND METHODS

Chemical and biological materials: Sodium selenate (Na_2SeO_4, Sigma-Aldrich, St. Louis, MO, USA) was used as source of selenium for experiment. The nutrient solution composition contained 1 NH_4NO_3, 1 $CaCl_2$, 0.25 KCl, 0.1 $MgSO_4$ (mM), and 10 NaH_2PO_4, 10 Fe EDTA, 1 $MnCl_2$, 1 $ZnCl_2$, 0.1 $CuCl_2$, 0.1 $NaMoO_4$, and 3 H_3BO_3 (µM) (Parker *et al.*, 1991). Half strength Hoagland's nutrient solution was used for fungal infestation experiment (Hoagland *et al.*, 1950). All the chemicals were purchased from Sigma-Aldrich, St. Louis, MO, USA distributer, Faisalabad, Pakistan.

Hybrid seeds of *Brassica napus* L. (Canola, var. Hyola 433) were obtained from the local grains market of Vehari-Punjab, Pakistan (marketed by ICI Agri. Science Division Pvt. Ltd, Pakistan). Acid washed sand was obtained from a Scientific Store, Faisalabad Pakistan and it was washed 3-4 times with distilled water and rinsed with deionized water prior to its use. The cell disrupter insects (*L. erysimi* and *H. destructor*) as well as phytopathogenic fungal species (*A. brassicicola* and *S. sclerotioram*) were taken from the Ayub Agricultural Research Institute (AARI) Faisalabad and Regional Agricultural Research Institute Bahawalpur Pakistan.

***Brassica napus L. culture and selenium treatments*:** The experiment was conducted at the experimental research area, Department of Environmental Sciences, COMSATS University Islamabad, Vehari Campus-Punjab, Pakistan. Experimental units (pots) were arranged in the randomized complete block design to lower down the possible variations in the light (11h/12h L/D) and temperature (20°C).

B. napus seeds were sown in pots containing acid washed sand (HCl acid; Sigma-Aldrich, St. Louis, MO, USA). The pots were placed in green house for germination and irrigated with distilled water as per seedlings requirement. The canola seed germinated from 6 to 38 days. Before transplanting the seedlings, shoot is allowed to attain 7-8 cm and root 5-6 cm. At this stage, the seedlings were ready for transplantation. The transplanting process was completed during the last week of October 2015. The *B. napus* seedlings were transplanted to 7 kg of acid washed silica sand. Initially, six plants pot^{-1} were shifted but latterly, planting density was thinned to four plants pot^{-1}. The dead/failed plants were replaced from the healthy plant stock during the following weeks of the experiment to ensure the same number of plants pot^{-1}. The concentration of nitrogen and phosphorous in the nutrient solution was examined weekly and maintained the levels throughout experiment. The pH of the nutrient solution was maintained 6.0±0.05 during the whole period of experiment.

The Se concentrations (low to high) were added to the nutrient solution which was started to apply after 28 days of seedlings establishment in the sand culture. Two selenium concentrations (2 µM (-Se) and 30 µM (+Se)) were used.

***Choice and non-choice feeding experiments*:** To explore the distribution of Se concentration in the different vegetative portion of *B. napus* and its subsequent effects on the herbivory and distribution/population of animals, aphids or mites were provided the choice to feed between the low (-Se) and high (+Se) selenium treated plants. One of each -Se and +Se *B. napus* plant was place in the same plastic glass tank (20 L) and 100 animals per plant were inoculated. Separate experiments were performed for each insect species. After 16 days of insect's herbivory (8 and 16 days), percentage of young leaves (top four nodes), medium leaves (ML) (nodes of the middle of the plants) and old leaves (OL) (three bottom nodes) and the leaflets per leaf showing visual signs of aphids were noted. The experiments were repeated four times and the feed rate of the animals (mg g^{-1} f. wt.) was recorded and the number of animals were counted after 8, 16 or 24 days of inoculation.

To determine whether Se concentration rich leaves were toxic to the aphids and mites, non-choice experiment was performed. Four plastic glass tanks (20 L) were used, each was contained four *B. napus* L. plants with -Se treatments and +Se treatments. The plastic tanks were coved with nylon mesh tops (0.2 mm^{-2}) to stop the animals to escape without disturbing the light and gases. Each container was inoculated with 100 animals (collected from *B. napus* plant that were highly infested with the aphids and mites). After 8 and 16 days, the number of animals on each plant were recorded. A separate experiment was performed for each insect species. Leaf (young, medium and old leaves as described in choice experiment) were sampled for Se concentration determination.

Additional to choice and non-choice experiment, another experiment was performed to determine whether the addition of more Se concentrations (60 µM) reduce the established mite population on the plant on not. The -Se treated plants (eight plants) were heavily infested with mites. Four plants were treated with higher selenium concentrations (60 µM) and other four plants with treated with distilled water (control). After 24 days (eight-day interval), the change in mite population was noted. Se concentration was recorded in the youngest leaves.

***Pathogenic fungal infection and selenium tolerance*:** To determine the tolerance of Se in the leaves of plants against fungal infection (n = 4 plants for +Se and -Se treatments with 10-12 leaves/plants), six-week-old *B. napus* L. seedlings were sprayed with *A. brassicicola* spores (suspension at the rate of 5×10^{6}/ml) and the number of lesions per leaf due to fungal infection were measured after 8 d.

To determine the tolerance of Se against the infection of *S. sclerotioram* (stem and root rot of brassica species), *B. napus* L seedlings (n = 16 plants per treatments; L/D 14/10, 25°C) were grown for at least 10 days on agar medium in absence of Se and in the presence of 80 µM Se. The seedlings were weighed and submerged into the fungal spores (suspension at the rate of 5×10^{4}/ml) and then transplanted into a plastic container (250 mL) containing the half strength of Hoagland solution (Hoagland *et al*., 1938). Similarly, the controlled seedlings without Se were transplanted in to separate plastic containers. This experiment was carried over a period of 8 days and the number of lesions per leaf were measured as well as the fresh weight (f. wt.)

To explore the tolerance of both fungal species *A. brassicicola* and *S. sclerotioram* in the form of colonization against different Se treatments, the fungal spores were inoculated on agar medium plates (half strength of Hoagland solution) with different Se concentrations (0, 10, 20, 30, 40, 50, 60, 70 and 80 µM). Each fungal species was cultured in each plate for 10 or 20 days. The diameter of each fungal mycelium was measured. The EC_{50} after 20 days (50% reduction in mycelium diameter) was also estimated.

***Analytical determinations*:** Samples of leaf were oven dried in the warm air at 70±2°C (Wise Ven, Wisd Laboratory Instruments, Korea) until constant weight was achieved. The wet acid-based digestion was done by taking 1 g sample of leaf as explained by Campbell *et al.* (1998) to extract Se from the plant tissues. Animal tissues were also processed by acid-based digestion for Se determination. The distilled water was used for sample dilution (10 mL). The filtrates were analyzed using the atomic absorption spectroscopy (Pohl, 1987).

Se content was determined using an atomic absorption spectrometer equipped with a hydride generation technique (Perkin Elmer). The calibration of the system was done by using the nitric acid (0.5%; v/v). New calibrations were constructed every time before the start of analysis.

***Statistical analysis*:** The experiment was carried out using the randomized complete block design in the green house. All the plants had similar growing conditions i.e. light and temperature during the whole period of experiment. All the experimental data were statistically analyzed by determining the significance of variance using the Statistix 9.1 V software.

The standard error of the Se treatments means (+Se and -Se) were determined, and to compare the concentration of Se in the leaf samples was done by using the t test at α 0.05 probability level for all the text as well as figure legends.

RESULTS

***Effects of Se on the herbivory of B. napus by aphids*:** During this study, it was noted that the Se accumulation in the tissues of plants significantly protected the Brassica plants from Turnip aphids. Both non-choice and choice experiment were conducted with -Se and +Se concentration. Aphid population number was inversely correlated with the applied Se concentrations and Se concentration in the plant leaves (Fig. 1A-F). At + Se treatments, dead aphids were found, due to toxicity of Se in the tissues of feeding aphids. The data for

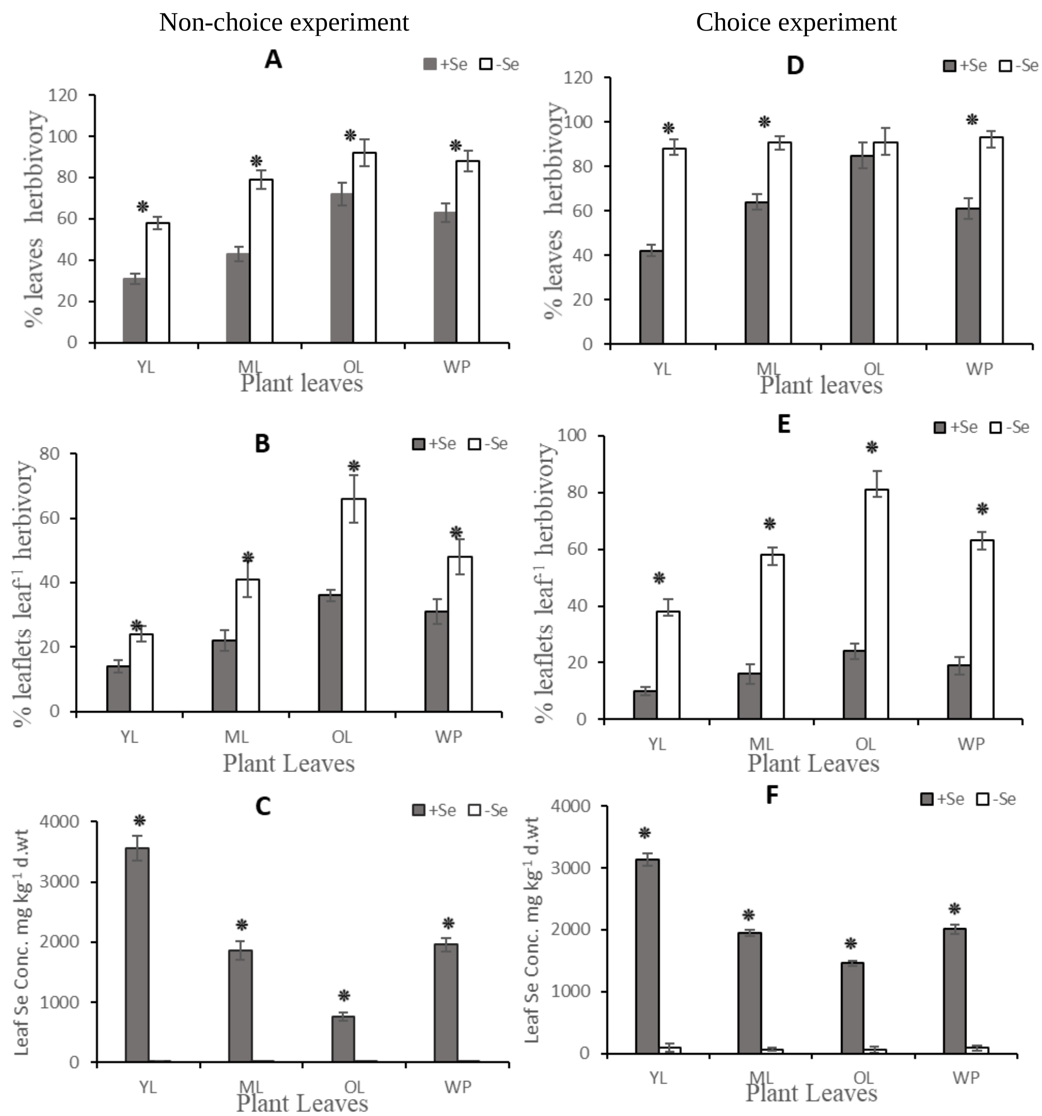

Figure 1. Elevated selenium reduces aphids' herbivory on *B. napus* leaves in both non-choice and choice experiment (A-F). In non-choice experiment, Aphids were allowed to feed on only low Se (-Se) or high Se (+Se) plants and leave herbivory of young leaves (YL), medium leaves (ML), old leaves (OL) and whole plants (WP) was quantified in term of percentage (A) and similarly % leaflets leaf^{-1} herbivory (B). The Se concentration in YL, ML, OL and WP with -Se and +Se plant leaves in non-choice experiment is shown in C section. The choice experiment where aphids were given choice between -Se and +Se plant leaves (D-F). Aphid herbivory in term of percentage in YL, ML, OL and WP in case of leaves herbivory (D) and similarly for leaflets leaf^{-1} was quantified (E). The Se concentration in YL, ML, OL and WP with -Se and +Se plant leaves in choice experiment is shown in F section. Values are represented in ± Standard error bar while an asterisk value (□) above the bar pairs represent the significant difference between the -Se and +Se selenium treatments.

non-choice experiment showed that the fraction of leave with aphids' herbivory was significantly higher in the low Se treatments compared to higher Se treatments. Moreover, overall the younger leaves (YL) were less affected with herbivory by aphids than the older leaves in both -Se and +Se treatments (Fig. 1A). Similarly, fewer leaflets leaf^{-1} was affected by aphids' herbivory on +Se as compared to -Se treated plants (Fig. 1B). YL from +Se plants contained approximately 2-fold (3559 mg kg^{-1} d. wt. for YL) and 5.5-fold (662 mg kg^{-1} d. wt. for OL) higher Se concentration than ML and OL of the same *B. napus* L. plants (respectively). Similarly, leaves treated with -Se did not contain a Se concentration higher than 8 mg kg^{-1} d. wt. of same plants (Fig. 1C).

In the choice experiment, when the aphid population were given the choice to feed on +Se and -Se treated *Brassica* plants, higher population of aphids were record on -Se treated plants. Moreover, the results depicted that the aphid population of both species showed a significant preference over their colonization on -Se plants. In addition, more aphid herbivory was recorded on the leaves and leaflets with -Se treatments than the leaves and leaflets with +Se treatments (Fig. 1D). Similar results for + Se treatments was observed as in case of non-choice experiment where YL was suffered with less aphid's herbivory then the ML and OL (Fig. 1F). Similar to non-choice experiment, YL treated with +Se contained significantly higher Se concentrations than the ML and OL of the same plants (i.e. 3116 mg kg^{-1} d. wt. for YL, 1949 mg kg^{-1} d. wt. for ML and 1458 mg kg^{-1} d. wt. for OL) (Fig. 1F).

earth mites (*Halotydeus destructor*) population of mites was decreased over the period. On the other hand, the

In non-choice experiment, surprisingly the population of mites was increased during the initial 8 days of the experiment, and then decreased as the leaves of plants attained the specific toxic levels of Se (Fig. 2A) whereas the plants with -Se treatments showed the 600% growth in the population of mites over the period of 24 days (Fig 2A).

As we have seen that the results showed that +Se plants were contained many fold higher Se concentration in their leaves than the -Se plants while the leaves of -Se plants contained 92 mg kg^{-1} d. wt. for YL and around 50 mg kg^{-1} d. wt. in the ML and OL of the same plants (Fig. 1F).

***Effects of Se on the herbivory of B. napus by mites*:** Non-choice and choice experiments were carried out to explore the effect of Se on the herbivory of mites; red legged When the mites were given the choice to feed on either -Se or +Se treated plants, they were colonized more in -Se plants (Fig. 2B). The negative effects of +Se was already seen in a few days of experiment (first 8 days) when the population of mites was reduced by approximately 200% over the period of 24d (Fig. 2B).

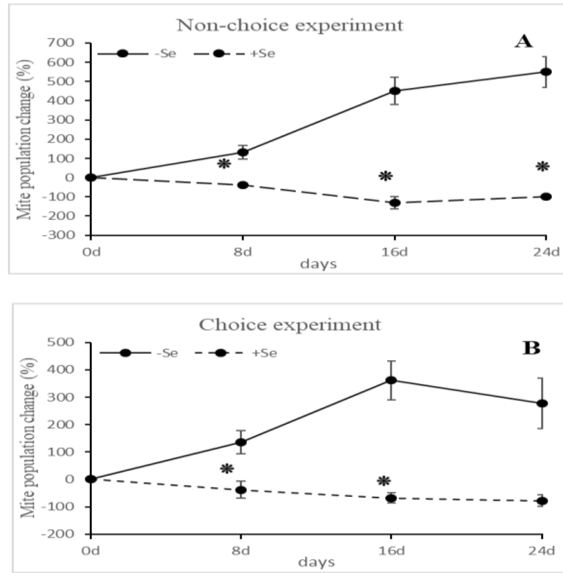

Figure 2. Change in mite population feeding on -Se and +Se treated *B. napus* plants. Percentage (%) change in feeding population in mite population in non-choice experiment (A) and in choice experiment (B). Error bars indicate standard deviations and asterisk indicate significant differences between the -Se and +Se treatments

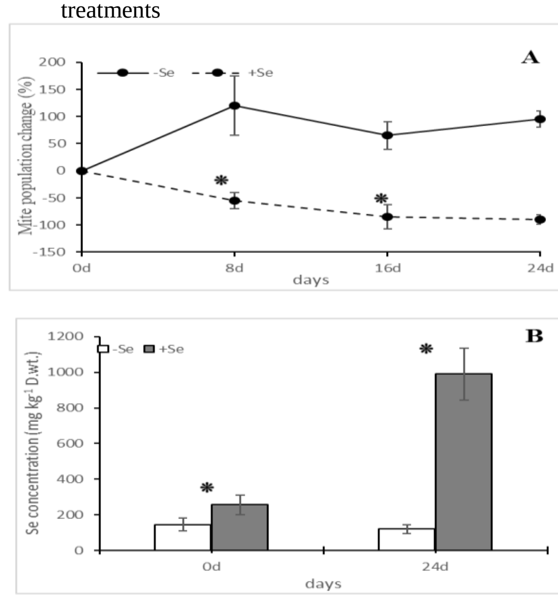

Figure 3. Elevated dose of Selenium (Se) added to already established mite population to reduce its population growth on *B. napus* plants. Percentage (%) change in feeding population in mite population over the period of 24 d with low Se and high Se treatments (A). Se concentration of *B. mapus* plants at beginning and end of experiment (B). Error bars indicate standard deviations and asterisk indicate significant differences between the -Se and +Se treatments

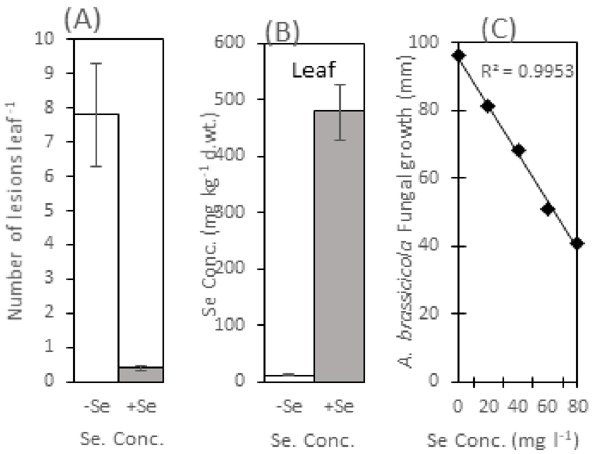

Figure 4. Susceptibility of Brassica napus plants by fungal infection Sp. *Alternaria brassicicola*. Number of Lesions leaf $^{-1}$ on B. napus plants treated with -Se and +Se treatments ($p<0.02$ for both ±Se treatments) after 8 d fungal infection sp. A. brassicicola (A) and leaf Se concentration of the same plant (B), Growth in the colony of A. brassicicola after 10 d on agar medium containing different Se treatments

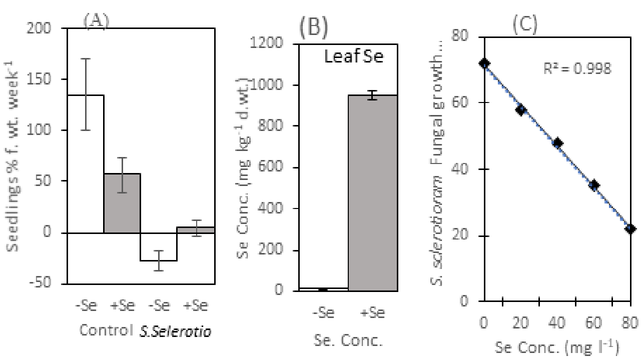

Figure 5. Susceptibility of Brassica napus plants by fungal infection Sp. *Sclerotinia sclerotioram*. Seedling growth of *B. napus* ± Se and ± *S. Sclerotioram* L. ($p<0.04$ for both ± Se treatments), Se concentration in the seedling shoot (B), Growth in the colony of S. *Sclerotioram* L. after 20 d on agar medium containing different Se treatments.

In the subsequent experiment, it was determined if the further addition of Se concentration reduced the already established population of mites. All the plants (8 plants) were already infested with mite population. Half of the *B. napus* plants were treated with +Se concentration and remaining half plants were given only distilled water.

Results showed a reduction of 55% on the mite population when the plants are treated with +Se treatments, while, almost 110% increase in mite population was in case of -Se treatments over the same period (8 d). After 24 d, the mite population was decreased by 95% on +Se plants but its reduction percentage was gradually decreased as compared to 16 d of mite population, while on the other hand, the population of mites was increases by 55% in -Se plant (Fig. 3A). Prior to start of experiment, plant leaves contained the Se concentration ranging 100-250 mg kg^{-1} d. wt. but after the completion of experiment, +Se plant leaves of *B. napus* contained roughly 1000 mg kg^{-1} d. wt. while -Se plant leaves had approximately 100 mg kg^{-1} d. wt. (Fig. 3B).

***Pathogenic fungal infection and selenium tolerance*:** Two fungal species *Alternaria brassicicola* (brassica species specific leaf pathogen) and *S. sclerotioram* (stem and root rot of brassica species) were used to explore the Se dependent susceptibility to *B. napus* plants. The number of lesions per leaf on +Se were approximately 8-fold less than the number of lesions on the leaves of those plants that are treated with +Se concentration (Fig. 4A). Hence, the Se increased the tolerance in the plants, and thus protected the leaves of *B. napus* plants from the infection of *A. brassicicola*. The Se concentration in the leaves of +Se treated plants were *c.*800 mg kg^{-1} d. wt. (Fig. 4B). Now, to test whether the presence of Se in the leave tissues was likely to be toxic for *A. brassicicola*, an experiment was performed in which fungus was grown on agar medium with different Se concentrations. The EC_{50} (50% fungal growth inhibition at specific Se concentration) was measured i.e. *c.* 65 mg L^{-1} Se (Fig. 4C). Therefore, Se concentration in the plant leave tissues (500 mg kg^{-1} d. wt.) is sufficient to restrict the growth of *A. brassicicola*.

In the second fungal inoculation experiment, the plants of *B. napus* were infected with *S. sclerotioram* (stem and root rot of brassica species) to determine the tolerance to Se. Black/brown spots on stem and root was noted while the roots of some plants are damaged and turned in to blackish strip with in the stem. The symptoms of fungal attack were decreased significantly as the concentration of Se was increases as data indicated in EC_{50}. Control seedlings grown and gained fresh weight by roughly 60% and 140% when the plants were treated with +Se and -Se respectively (Fig. 5A, B). In the control treatments, no fungal inoculum was applied and f. wt. of *B. napus* seedling was reduced by 28% in the absence of Se concentration (-Se) while the seedlings gained fresh weight by 5 % as treated with the Se (+Se). The results suggested that *B. napus* seedlings were poor in growth and gained less fresh weight in the presence of Se than the control. Meanwhile, in the presence of fungal infection, the Se treated seedlings lost less fresh weight than that of control seedlings (Fig. 4A).

To investigate whether the Se concentration accumulated in the leaves of young *B. napus* seedlings *c.* 950 mg Se kg^{-1} d. wt. was the probable reason of toxicity to the *S. sclerotioram.* The fungus spores were grown on agar plates as in case of 1st fungal infection experiment. The EC_{50} was around 50 mg L^{-1} Se (Fig. 4C). Thus, the Se concentration in the leave tissues of seedings *c.* 950 mg kg^{-1} d. wt. was inhibited the growth of fungal specie *S. sclerotioram* (Fig. 4C).

DISCUSSION

The results of this study showed that the accumulation of Se in the tissues of *B. napus* plants can protect the leaves and leaflets of plants from the herbivory of cell disrupter insects i.e. aphids (*Linaphis erysimi*) and mites (red legged earth mites (*Halotydeus destructor*)). These cell disrupters avoided to attack on high Se treated leaves when provided the choice between -Se and +Se leaves. Moreover, the Se rich leaves also increased the tolerance against the two-brassica specific fungal species *A. brassicicola* and *S. sclerotioram* than the leaves of control treatments (-Se). These results are in line with those of past studied investigated the protective effect of Se in the plants and concluded that elevated Se accumulation in the tissues of plant leaves protects plant vegetative portion from the herbivory of many animals i.e. sucking and chewing insects, arthropods, mammalians, fungal diseases (Hladun *et al.*, 2013; De La Riva *et al.*, 2017; Dhillon *et al.*, 2017; Rosenfeld *et al.*, 2017; Shelby *et al.*, 2017). According to our best of knowledge, this first comprehensive study to determine the protective effects of high Se accumulation in the *B. napus* plants against the cell disrupter and phytopathogenic fungal species in the tropical condition of Pakistan. The study results provided the conclusive evidence that the elevated Se in the plant tissues deterred and protected the *B. napus* plants from two ecologically pervasive and economically crucial herbivores.

The choice experiments demonstrated that turnip aphids and red legged mite population fed more preferably on -Se plants than +Se plants. While in the non-choice experiments the turnip aphids and mites avoided the herbivory on the leaves containing +Se concentrations. In addition, -Se concentration in the leave tissues were suffered to more aphid's herbivory than Se-rich leaves. Moreover, the study results showed that aphid population preferred OL and ML which had less Se accumulation than the YL, contained higher Se concentration. A higher attack on aphids on the OL indicated that Se was moved to new young tissues than the old plant tissues. This is in line with Pilon-Smits *et al.*(2009), who reported that Se is accumulated in the plant tissues as organic forms and transported through phloem in to newly developing tissues. Similarly, Dhillon *et al*. (2017) noted that the cell disrupters (cabbage looper) avoided to feed on *B. juncea* plants continuing high Se concentration *c.* 465 mg kg^{-1} d. wt. than low or with Se. Surprisingly, very low turnip aphids and mites herbivory was noted on the 1st node of ML and OL as compared to subsequent nodes of ML and OL both in case of -Se and +Se concentration but this difference was more visible in case of +Se concentration (data not shown). But as we moved away from the last node of YL/ML towards the first node of ML or OL, the turnip aphids and red legged mite population was steadily increased. This showed the plants sequestered Se more in their YL than the ML and OL. It was might be due to chemosensory action of Se on the insects, which may restrict the insect population growth and herbivory. The chemosensory organs were might be the mouthparts or body. By using the Se chemosensory cues, insects choose the plant/plant parts for herbivory or find out the more damaged part of plant. Moreover, further studies are required on male and female (at individual level or in combination) preferences/chemosensory mechanism and chemosensory organs against Se in plants. In case our study, variation in insect's herbivory was also seen on various parts of plants. It might be due to chemosensory action of elevated Se. These results are supported by the findings of Kitajima *et al.* (2002), plants may be transported and accumulated more Se in to their younger leaves, may be due to higher photosynthetic rate than the older leaves, and ultimately, plants were able to protects its more valuable parts from these herbivores. Interesting results in non-choice studies were seen, where no significant difference in turnip aphid's herbivory on YL of +Se and -Se leaves but more turnip aphids' herbivory was noted on -Se on ML and OL than +Se contained ML and OL (Fig. 1C, E). Thus, YL are more attractive source of foods for herbivores. Hence, these resulted concluded that higher concentration of Se serves as barrier/protection wall against the insect's herbivory, and may be the effective tool for other herbivores that dislike the availability of Se in the plant tissues. These results fit in to past study that YL are rich sources of nutrients compared to OL (Norghauer *et al.*, 2016). Therefore, it may be best food choice for these herbivores.

The growth and propagation of *Brassica* host specific pathogen *A. brassicicola* (leaf pathogen) and *S. sclerotioram* (stem and root rot pathogen) was restricted by the application of Se. The results of this study presented here explained that high Se concentrations in the tissues of *B. napus* plant leaves increased the tolerance in fungal infested plants, and thus protected the plants from infection compared to no or low Se concentrations. The significantly fewer number of lesions were recorded on the +Se treated plants over -Se treatments plants. It is possible that the accumulated concentration of Se in the plant materials could be high enough to reduce the growth of fungus. In addition, it is also proved from fungus growing agar medium experiment where increasing level of Se badly reduced the growth of fungus (Fig. 4C). From the past studies, for example, Nickle (Ni) accumulation in the plant tissues could protect the plants from microbial infection. Moreover, plants of *Alyssum spp*. had shown the resistance against the fungal infection (*Pythium* spp) as seedlings were treated with Ni. In addition, the negative correlation between the Ni concentration and plant resistance was recorded (Ghaderian *et al.*, 2000). Almost related results were noted by Boyd *et al.* (1994) and stated that growth and attack of some leaf fungal pathogens (*A. brassicicola* and *Erisyphe polygoni*) was restricted by the accumulation of Ni. Similarly, the number of lesion and growth of fungus spp. *A. brassicicola* and *Fusarium* sp. on the leaves of *Brassica juncea* was correlated with the degree of Se concentration in the tissues of plants (Hanson *et al.,* 2003). Thus, elevated concentration of toxic elements (i.e. Se and Ni etc.) proved a valuable tool to protect the plants from pathogenic fungal infections.

During the experiment, we have noted the evaporation/temperature may also help towards the accumulation of Se in the plant tissues, so, this question should be explored and very important for the plants grown in the subtropical environmental conditions like Pakistan where temperature touched 50°C in summer.

***Conclusion*:** First time in Pakistan, interaction of the herbivores and fungal with the crop plants are being studies through the exogenous application selenium. Our results indicated that accumulation of Se in to tissues of *Brassica napus* plants protected the plant leaves from two cell disrupter herbivores turnip aphids (*Linaphis erysimi*) and red legged earth mites (*Halotydeus destructor*). In addition, Se also produce the tolerance and resistance in the plants against fungus species *A. brassicicola* (leaf pathogen) and *S. sclerotioram* (stem and root rot pathogen). So, Se may use as natural pesticides for agricultural crops. The bioaccumulation of Se in to food chain due to accumulation of Se in the plant tissues may cause toxicity and needs to further investigation. On the other hand, the Se bioaccumulation and bioconcentration in the food chain may be decreased as the avoidance of herbivores probability towards Se rich plants are increased. It was also observed that accumulation of Se is different in different parts of plants, they may leave other plant tissues vulnerable to insects' herbivory. The applied Se treatments protected the *B. napus* plants from herbivory and fungal infection. More studies are required for its application on other agricultural and horticultural crop of Pakistan. More studies are required for its application on other agricultural and horticultural crop of Pakistan. This study provides the base for the agricultural scientists of Pakistan to protect the crops through alternative ways. However, more studies are still need about the non-accumulator and hyper accumulator species in the lab as well as in the field and the chemosensory effect of selenium on insect population. In the same way, bioaccumulation of Se in the future generation of insects also needed a comprehensive study to under the role of Se for sustainable control of insect. Moreover, the soil pH and atmospheric environmental conditions would affect the Se intake by plant roots in subtropical conditions of Pakistan and needs to be further investigated.

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