CHAPTER 3

RESULTS

CHAPTER 3: RESULTS

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Medicinal plants selection for preliminary screening of allelopathic potential was done based on their relative importance and utility as a medicinal plant in different pharmaceutical industries. Some of them are acclaimed source of pharmaceuticals and are widely used in the conventional medicinal system, others are used in preparation of Ayurvedic drugs or as alternative medicines. About half of the plants are observed to grow only as wild plants, where as remaining half are under cultivation also. All the eighteen selected medicinal plants were preliminarily screened for their allelopathic potential against radish.

3.1 Preliminary allelopathic analysis: The preliminary analysis was conducted using aqueous extracts bioassay. Aqueous extracts of leaf, stem and root of each medicinal plant were analysed at different application rates against the germination and seedling growth parameters of radish *Raphanus sativus* L. Inhibitory responses manifested by radish are expressed and compared in terms of % inhibition with reference to the respective control. Among the analysed parameters, seed germination and radicle length were most sensitive to medicinal plant aqueous extract treatments, exhibiting distinct responses as compared to parameters like plumule length and seedling biomass.

3.1.1. Aqueous extract bioassays

3.1.1.1. Germination and seedling health

1) *Acalypha indica* L. (Ai): Aqueous extracts of leaf, stem and root exhibited various degrees of inhibition on the analysed parameters of radish and the results of the same are presented in Figure 3.1. Extracts of leaf, stem and root at the lowest concentration rate (i.e. at 0.5 %) inhibited the seed germination upto 5 % as compared to control (Figure 3.1a). Extracts at all the other concentration had no significant inhibitory effect on germination. Radicle length was highly inhibited by the leaf extracts at all

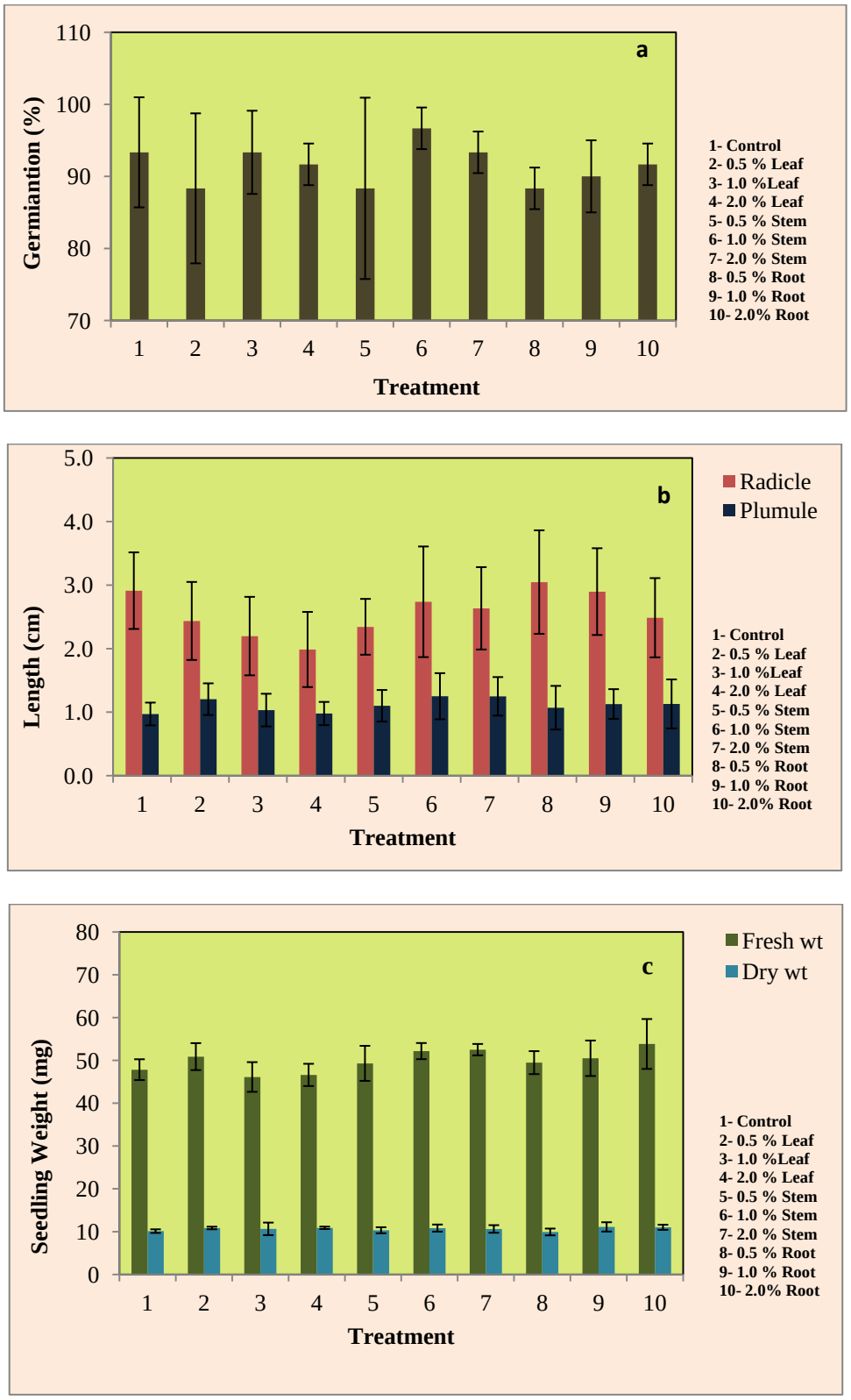
the concentrations and the decrease in length was observed to be concentration dependent (Figure 3.1b). Leaf extracts at 0.5, 1.0 and 2.0 % concentration suppressed the radicle length by 16, 25 and 32 % respectively. The seedling fresh weight was reduced by 4 % and 3 % in treatment with 1.0 and 2 % of leaf extracts (Figure 3.1c). No inhibitory effect was observed for Ai extracts treatment on plumule length (Figure 3.1b) and seedling dry weight (Figure 3.1c) of radish seedling.

2) *Adhatoda vasica* (L.) Nees (Av): Figure 3.2 shows effect of Av extracts on the analysed parameters of R adish. Only 2 % reduction in 0.5 % of leaf and stem extracts was observed, higher concentration of root extracts (i.e. 2 %) showed inhibition by 6 % but others parameters were not affected (Figure 3.2a). Plumule length was suppressed by 8 % owing to treatment with 1 % of leaf extracts (Figure 3.2b). The leaf, stem and root extracts, failed to bring considerable suppression of radish radicle length and reduce the seedling dry weight at all the applied concentrations (Figure 3.2b and 2c) rather an increase was found in these parameter.

3) *Aerva lanata* (Linn.) Juss. ex Schult (Al): Aqueous extracts of leaf , stem and root at all the applied concentrations failed to suppress the radish seed germination and seedling growth parameters such as radicle and plumule length (Figure 3.3a and 3.3b). Seedling biomass was reduced owing to treatment with leaf aqueous extracts applied at 0.5 % where the biomass was reduced by 7 % and stem extracts applied at 0.5 % and 1.0 % where in the biomass was reduce by 11 and 4 % respectively with reference to the control.

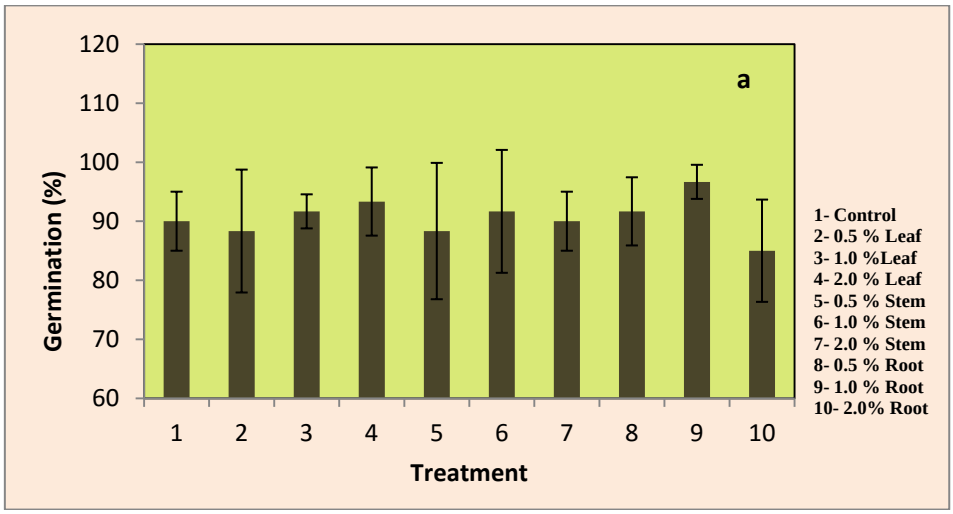
4) *Andrographis paniculata* (Burm.f.) Nees (Ap): Out of three plant part extracts, leaf aqueous extract was the most inhibitory affecting seed germination, plumule

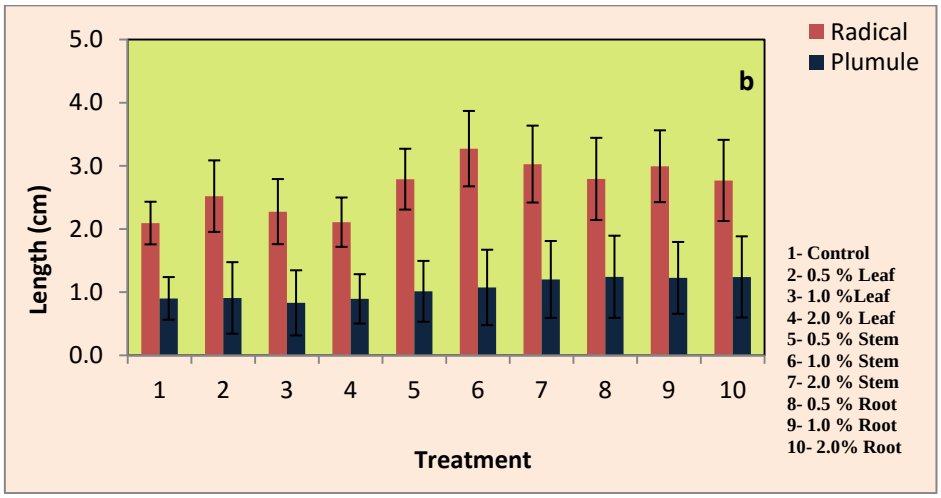
Figure 3.1: Effect of leaf, stem and root aqueous extracts of *Acalypha indica* L. on germination and growth parameters of Radish

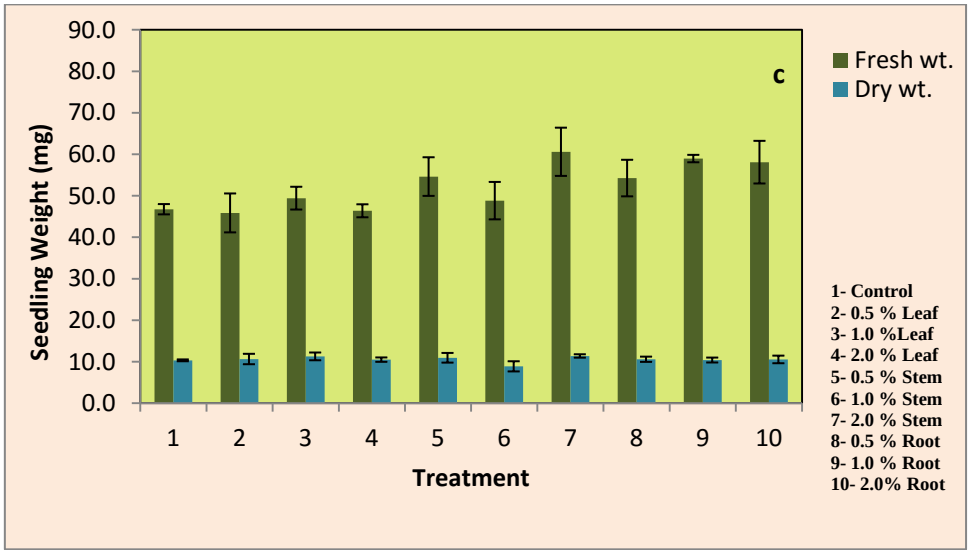


(Note: Bars represent mean ± Std)

Figure 3.2: Effect of leaf, stem and root aqueous extracts of *Adhatoda vasica* (L.) Nees on germination and growth parameters of Radish

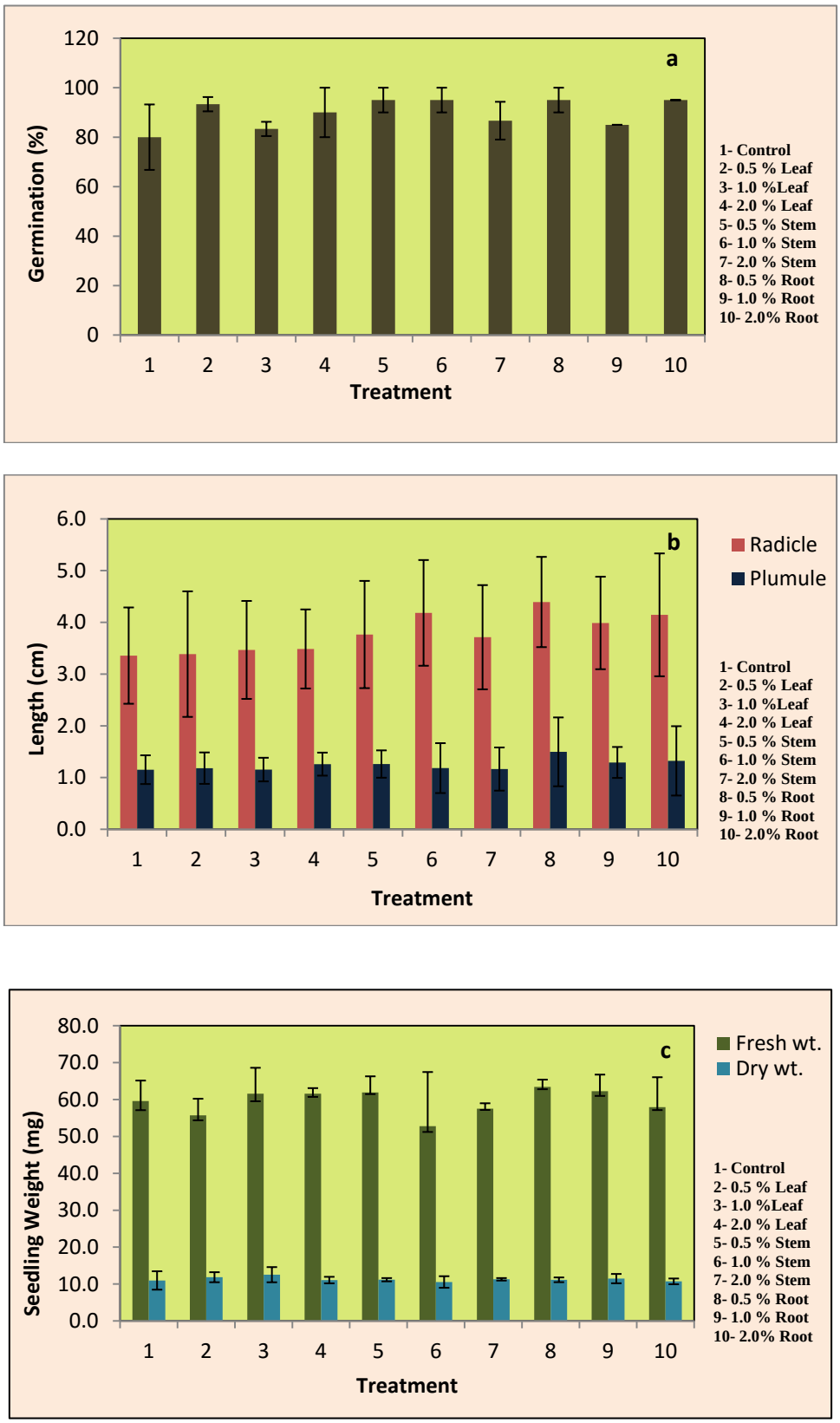






(Note: Bars represent mean ± Std)

Figure 3.3: Effect of leaf, stem and root aqueous extracts of *Aerva lanata* (Linn.) Juss. ex Schult on germination and growth parameters of Radish



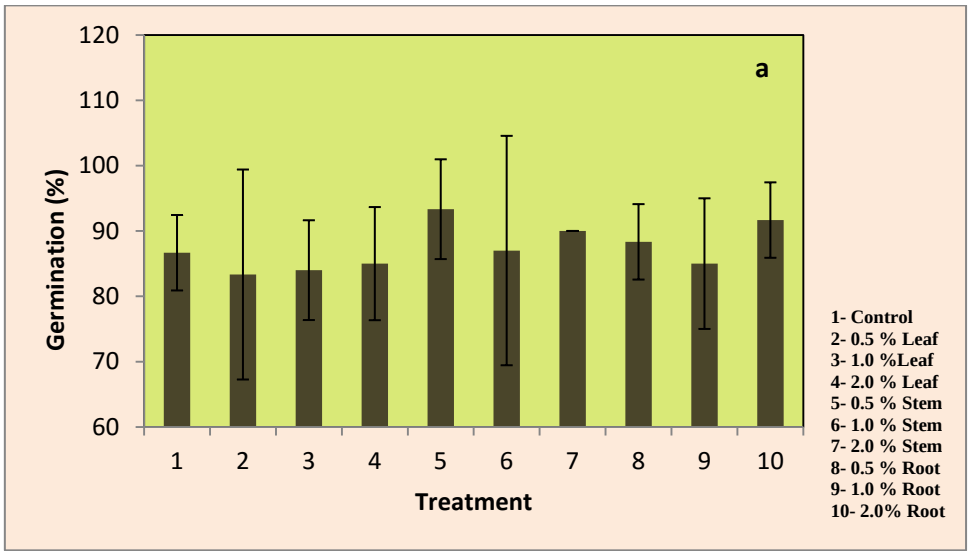
(Note: Bars represent mean ± Std)

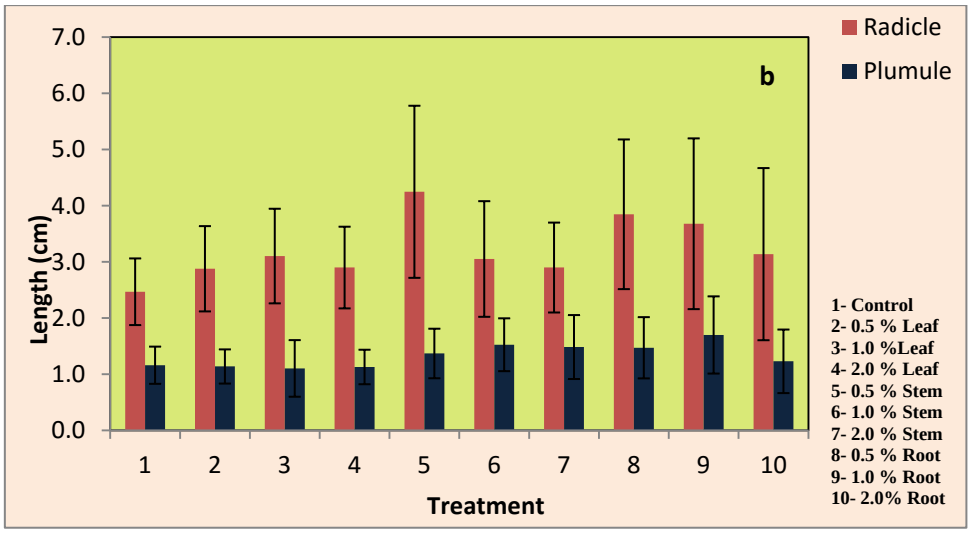
length and seedling fresh weight (Figure 3.4). Leaf extract at 0.5, 1.0 and 2 % concentration suppressed the germination by 4, 3 and 2 % with respect to the control (Figure 3.4a) hence the least germination was observed for extracts applied at lowest concentration. Plumule length was least in leaf extracts applied at 1.0 % concentration and was reduced by 5 % as compared to the control (Figure 3.4b). Seedling fresh weight was reduced by 9% as compared to the control which was observed for treatment with 0.5 % of leaf extracts (Figure 3.4c). No effect was observed for stem and root aqueous extracts. And all the extracts at the applied rates had no effect on radicle length (Figure 3.4b) and seedling dry weight (Figure 3.4c).

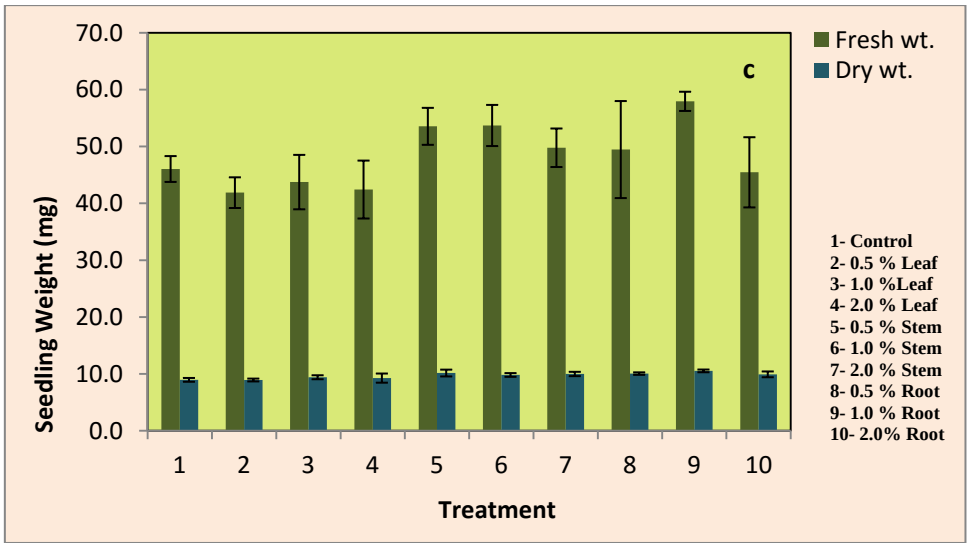
5) *Asparagus racemosus* Willd. (Ar): The plant root aqueous extracts were the most and highly allelopathic extracts of all the analysed extracts from Ar. Leaf extracts at 0.5 % concentration suppress the germination (Figure 3.5a), radicle length and plumule length (Figure 3.5b) and fresh weight (Figure 3.5c) by 4 %, 9 %, 10 % and 2 % respectively, in comparison to the control. The same parameters (Figure 3.5 a, b, c) in 2 % of root extract treatments were inhibited by 16 %, 18 %, 16 % and 3 %. The root extracts at 2 % concentration reduced the seedling fresh weight by 44 % as compared to the control. Leaf and stem extracts at 2 % inhibited the germination by 5 % in comparison to control (Figure 3.5a).

6) *Artemisia annua* L. (Aa): Aqueous extracts of leaf, stem and root were highly suppressive to the germination and growth of radish seedling, however the inhibitory effects were not observed to be concentration dependant. Leaf extracts at 1 % concentration was most inhibitory to all the studied parameters except dry weight. The seed germination was reduced by 27 %, radicle and plumule length were reduced by 43 and 34 % and fresh weight was reduced by 28 %, as compared to the respective controls (Figure 3.6). However leaf aqueous extracts at the other applied rates that are**,**

Figure 3.4: Effect of leaf, stem and root aqueous extracts of *Andrographis paniculata* (Burm.f.) Nees on germination and growth parameters of Radish

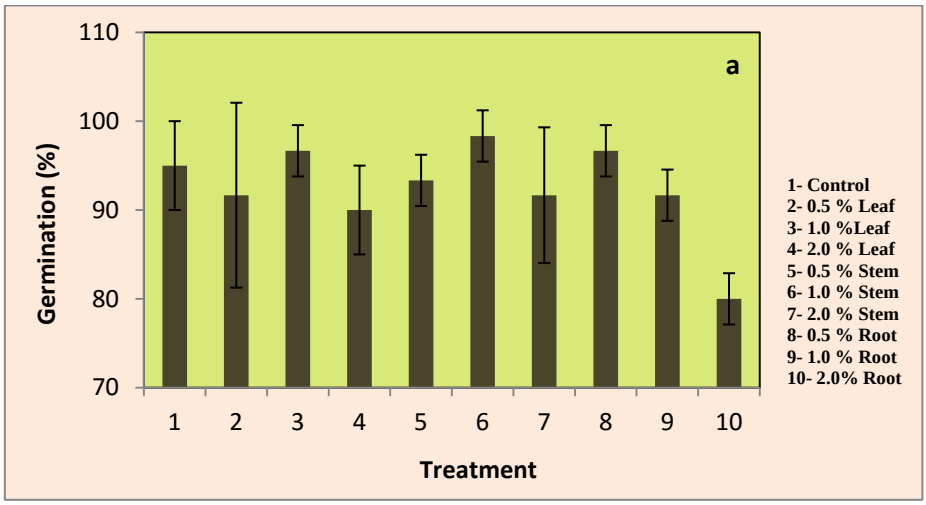


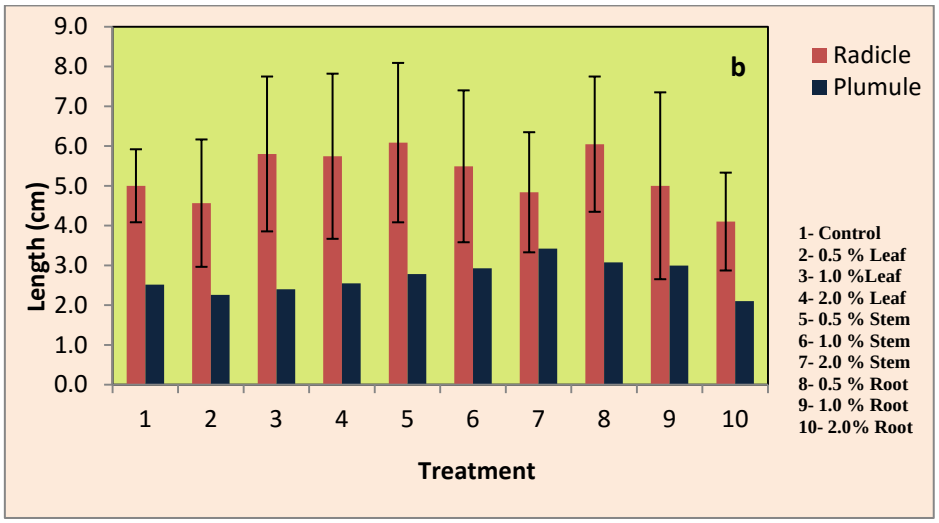


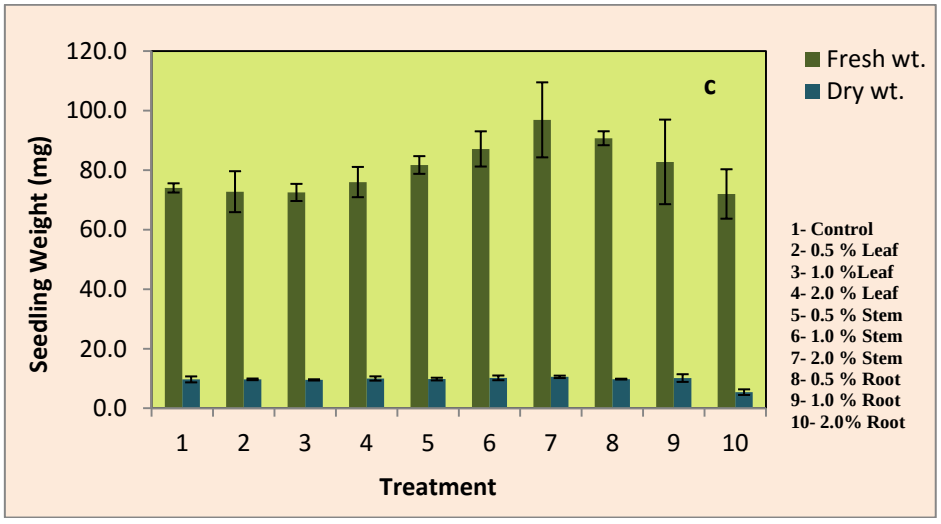


(Note: Bars represent mean ± Std)

Figure 3.5: Effect of leaf, stem and root aqueous extracts of *Asparagus racemosus* Willd on germination and growth parameters of Radish

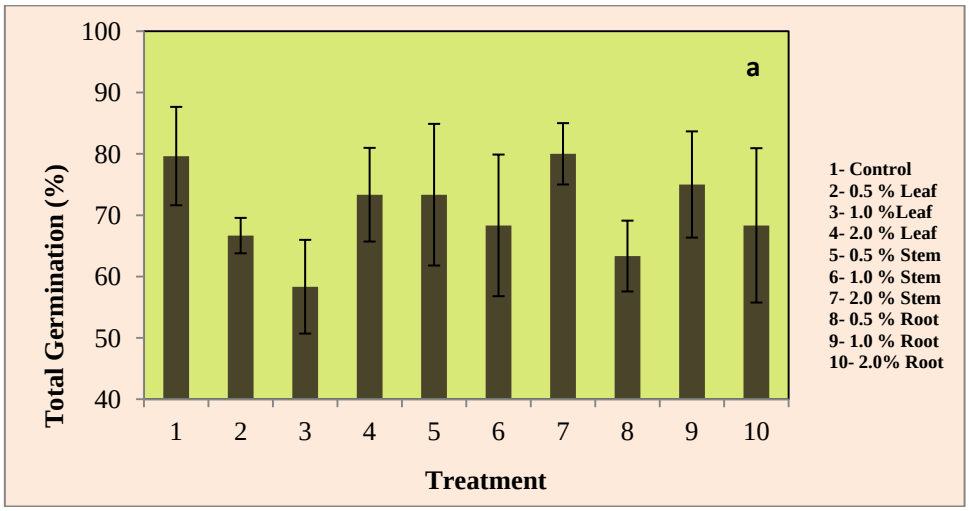


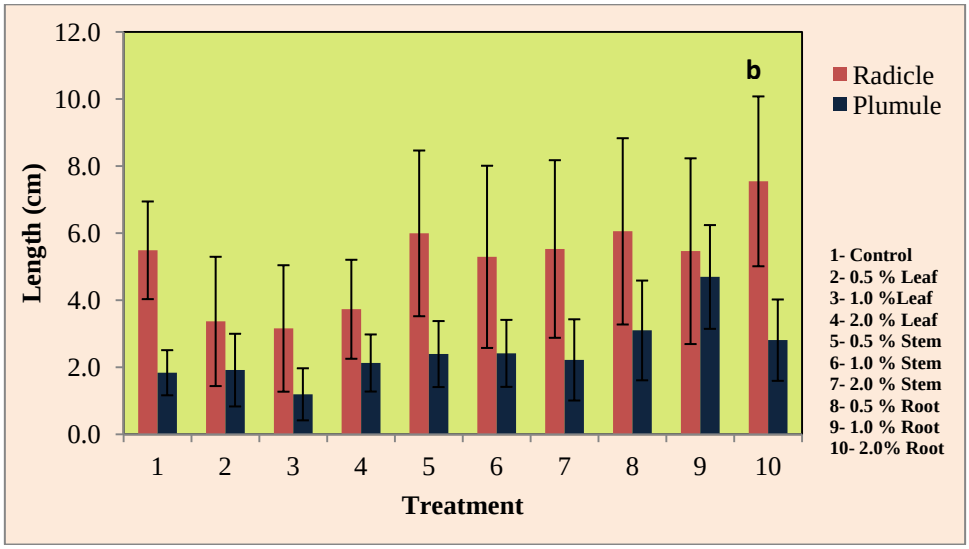


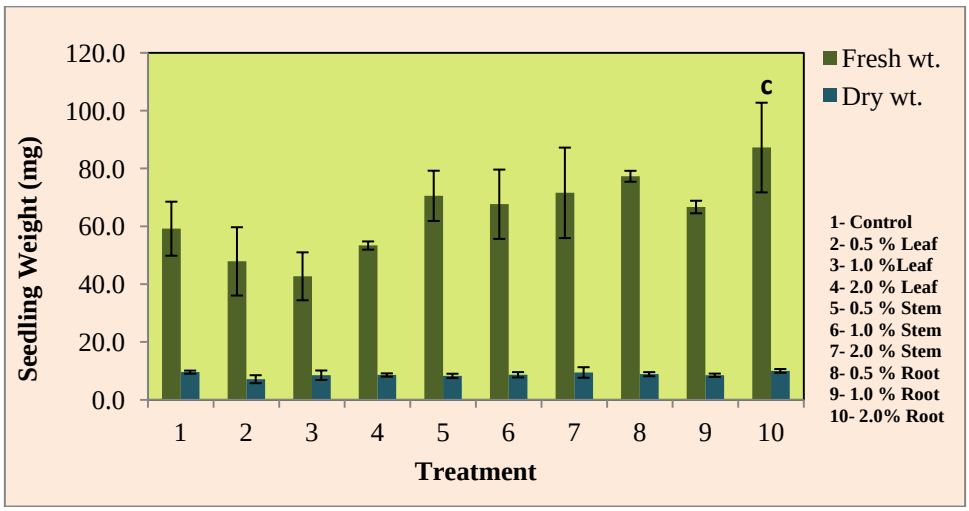


(Note: Bars represent mean ± Std)

Figure 3.6: Effect of leaf, stem and root aqueous extracts of *Artemisia annua* L on germination and growth parameters of Radish







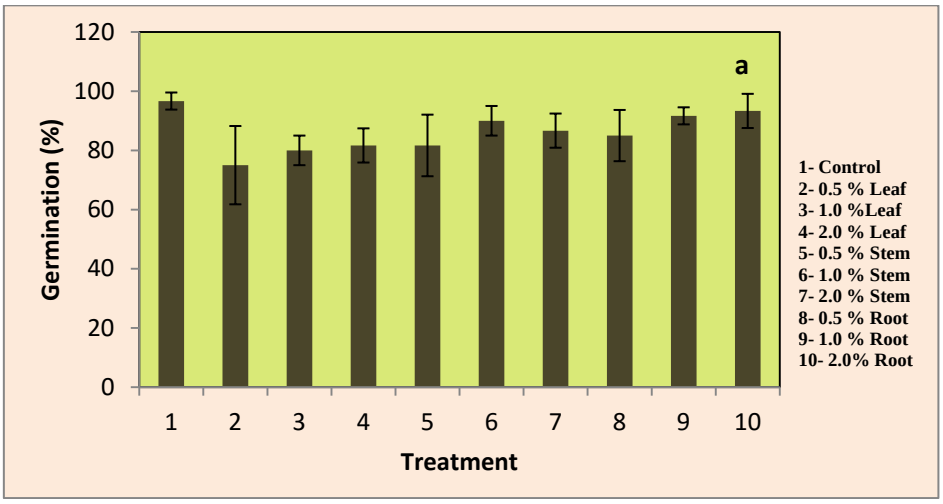
(Note: Bars represent mean ± Std)

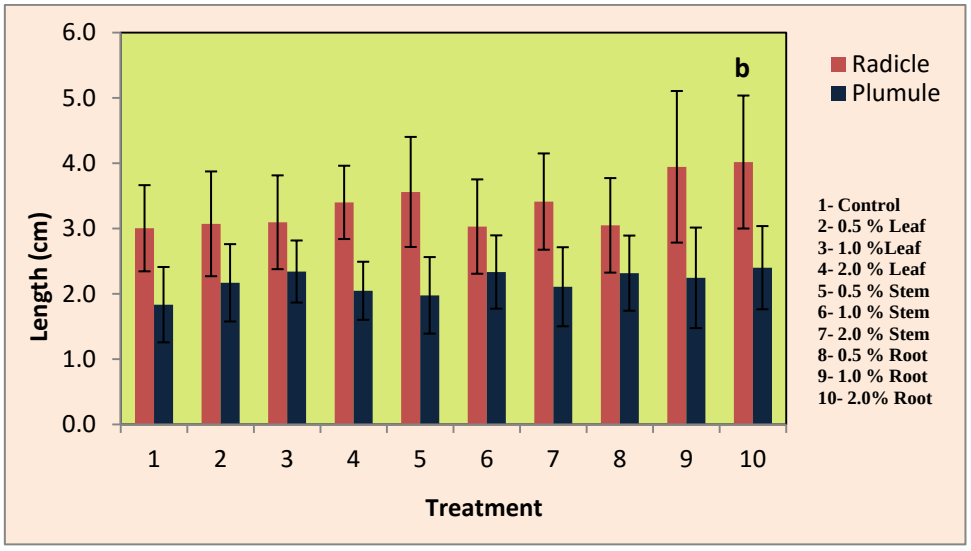
0.5 and 2.0 % also, were found to decrease the seed germination by 17 and 8 % respectively, with reference to control (Figure 3.6a). Artemisia stem extracts at 1 and 2 % concentration inhibited the germination by 8 and 5 % and the root extracts at 0.5, 1 and 2 % concentration suppressed the same by 21, 6 and 15 % as compared to the control (Figure 3.6a).

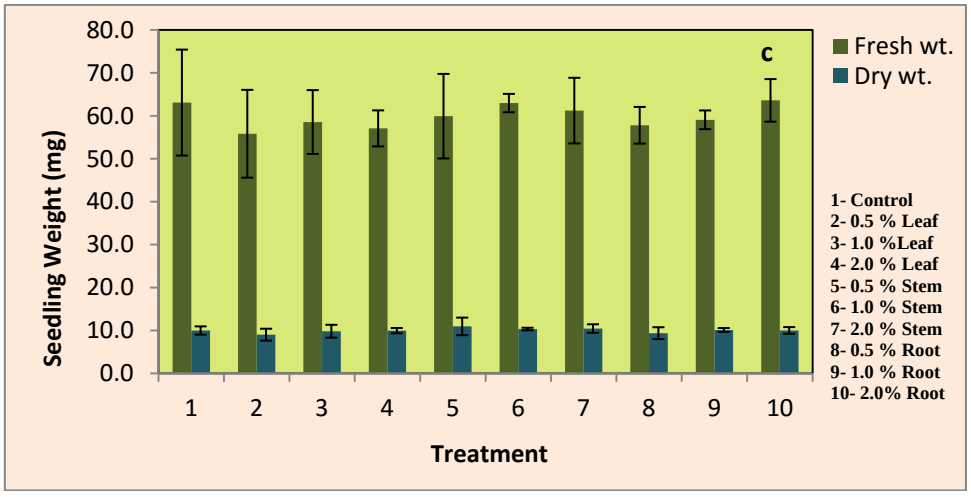
7) *Boerhaavia diffusa* L. (Bd): Radish seed germination and seedling biomass were the parameters that were affected negatively by the treatment with Bd aqueous extracts. Radicle and plumule length were not affected by any of the Bd extracts (Figure 3.7b). Leaf extracts at the rate of 0.5 %, 1 % and 2 % reduced the seed germination by 22 %, 18 % and 16 % (Figure 3.7a). Percentage reduction in radish seed germination in the treatment with stem extracts (at the rate of 0.5, 1, 2 %) was observed to be 16, 7 and 11 % respectively. Seedling fresh weight owing to the leaf extracts (at 0.5, 1, 2 %) was reduced by 12 %, 7 % and 10 % as compared to the respective controls (Figure 3.7c). Seedling dry weight was observed to decrease to 10 and 2 % in response to the leaf extracts (at 0.5 and 1 %) treatments. Plant root extracts at 0.5 % concentration inhibited the seed germination by 2 %, reduced the seedling fresh weight by 8 % and the dry weight by 6 %.

8) ***Catharanthus roseus* (L.) G. Don (Cr):** Aqueous extracts of the plant parts had no inhibition on the radish seed germination and plumule growth (Figure 3.8). Leaf extracts at the rate of 0.5 and 2 % of concentration suppressed the radicle length by 5 and 3 % as compared to the control (Figure 3.8b). Leaf extracts at the rate of 1% decreased the seedling fresh and dry weight by 9 and 17 % with respect to the respective controls (Figure 3.8c). Stem extracts at 1 % concentration reduced the fresh and dry weight of radish seedling in comparison to the controls by 12 % and 4 % (Figure 3.8c).

Figure 3.7: Effect of leaf, stem and root aqueous extracts of *Boerhaavia diffusa* L. on germination and growth parameters of Radish

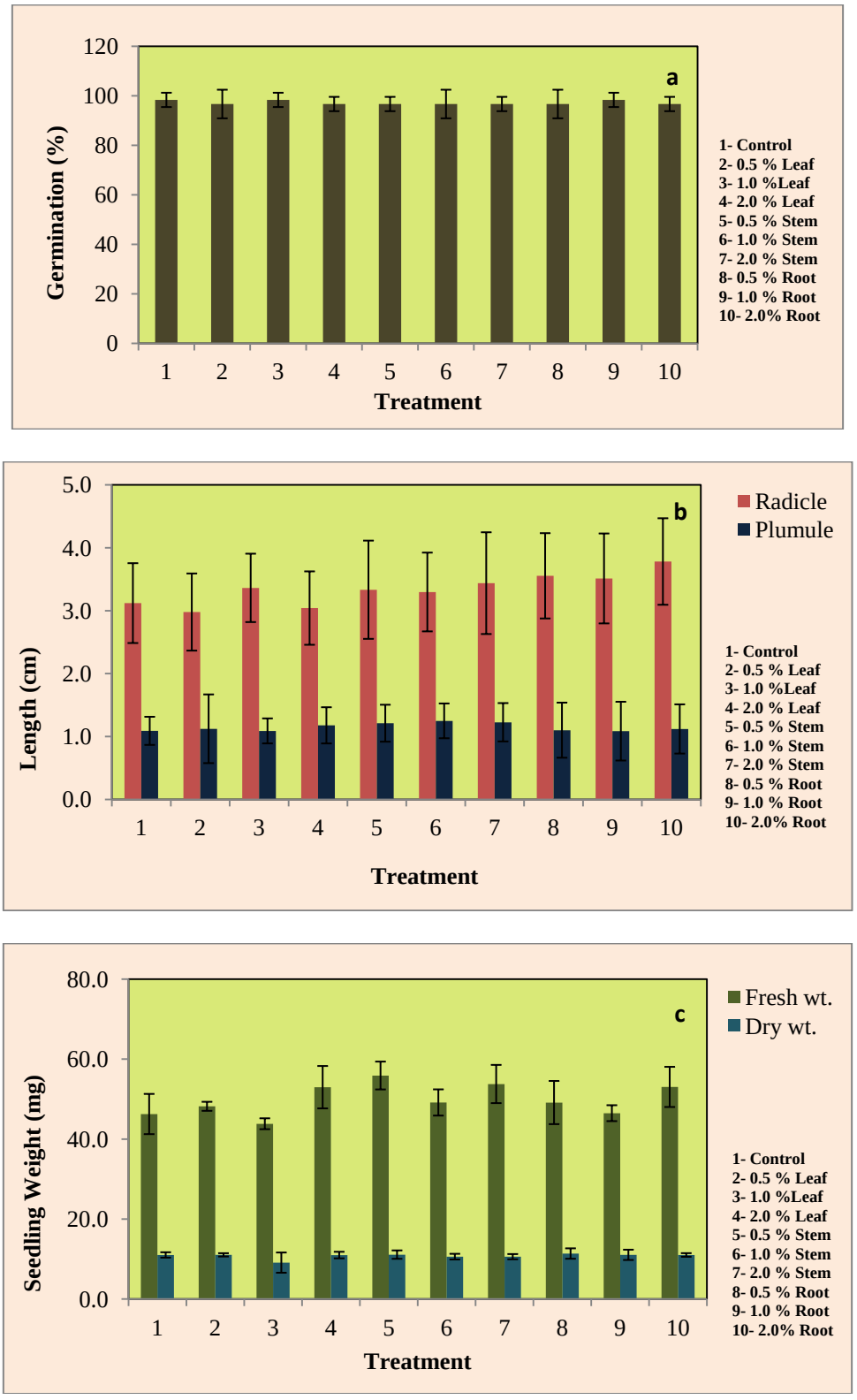






(Note: Bars represent mean ± Std)

Figure 3.8: Effect of leaf, stem and root aqueous extracts of *Catharanthus roseus* (L.) G. Don. on germination and growth parameters of Radish



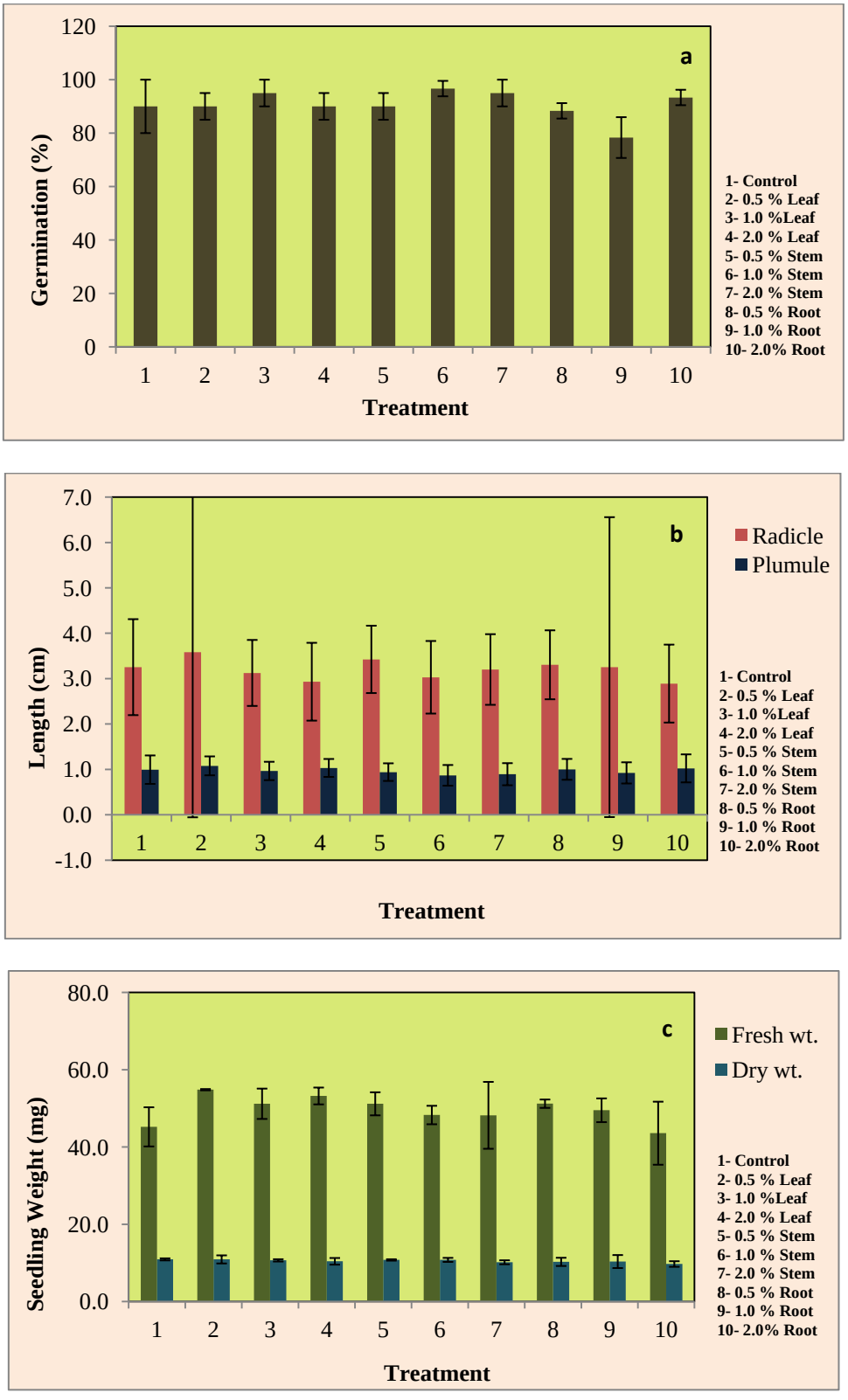
(Note: Bars represent mean ± Std)

9) *Chlorophytum borivilianum* San. and Fern. (Cb): The plant root aqueous extracts at all the studied concentrations considerably affected all the analysed parameters. The root extracts at the concentration of 0.5 and 1 % inhibited the seed germination where in the germination was reduced by 2 % and 13 % as compared to the control (Figure 3.9a). Radicle length was reduced by 11 % in the root extracts treatment applied at the rate of 2 % where as the plumule length was suppressed by 7 % in the treatment with root extracts applied at the rate of 1 % concentration (Figure 3.9b). Seedling dry weight was highly reduced in root extracts treatment (at rate of 0.5, 1 and 2 %) and the reduction was observed to be 6 %, 5% and 11 % with respect to the control (Figure 9c). Seedling fresh weight in treatment with root extracts applied at the rate of 2 % as compared to the control was reduced by 4 % (Figure 3.9c).

10) *Coleus forskohlii* Briq. (Cf): Cf leaf and stem aqueous extracts at all the studied concentrations affected the radicle length (Figure 3.10b). Of all the considered parameters only the radicle length showed the response to inhibitory effects imparted by the *Coleus* aqueous extracts (Figure 3.10 a, b, c). The leaf extracts at the studied rates of 0.5 %, 1 % and 2 % of concentration decreased the radicle length by 14, 13 and 18 % respectively in comparison to the control. Stem extracts (at rate of 0.5 %, 1 % and 2 %) inhibited the radicle length in comparison to the control by 12, 27 and 26 % respectively.

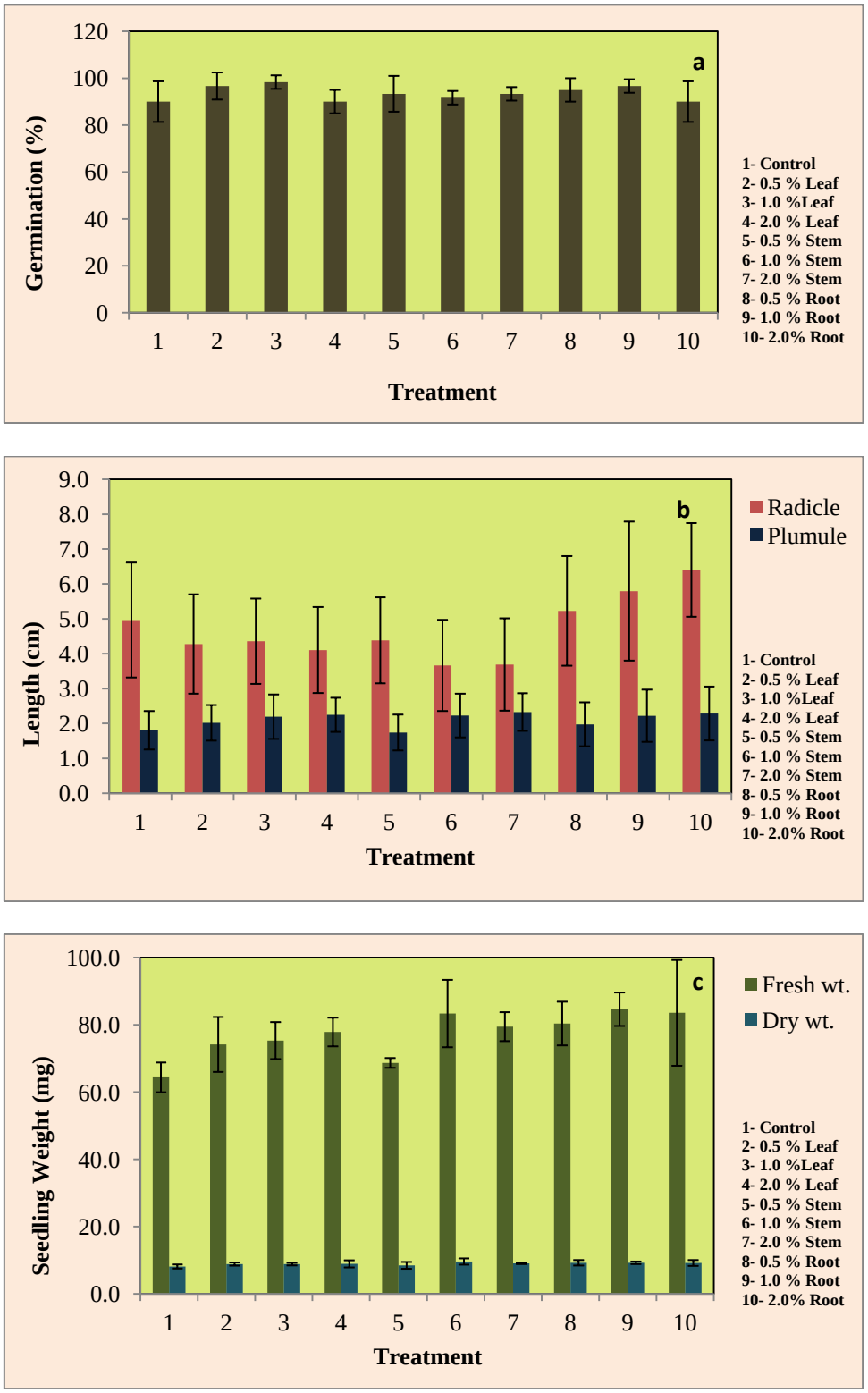
11) *Curculigo orchioides* Gaertn. (Co): Aqueous extracts from Co had varied effect on the analysed parameters of Radish (Figure 3.11 a, b, c). Trivial inhibitory effects were observed for the leaf and stem aqueous extracts. Leaf extract at the rate of 1 % of concentration suppressed the radicle length by 7 % and stem extracts reduced the plumule length by 15 % at the applied rate of 0.5 % (Figure 3.11 b). Seedling fresh weight was reduced by 8, 8 and 6 % respectively (Figure 3.11c) in the treatment with

Figure 3.9: Effect of leaf, stem and root aqueous extracts of *Chlorophytum borivilianum* San. and Fern. on germination and growth parameters of Radish



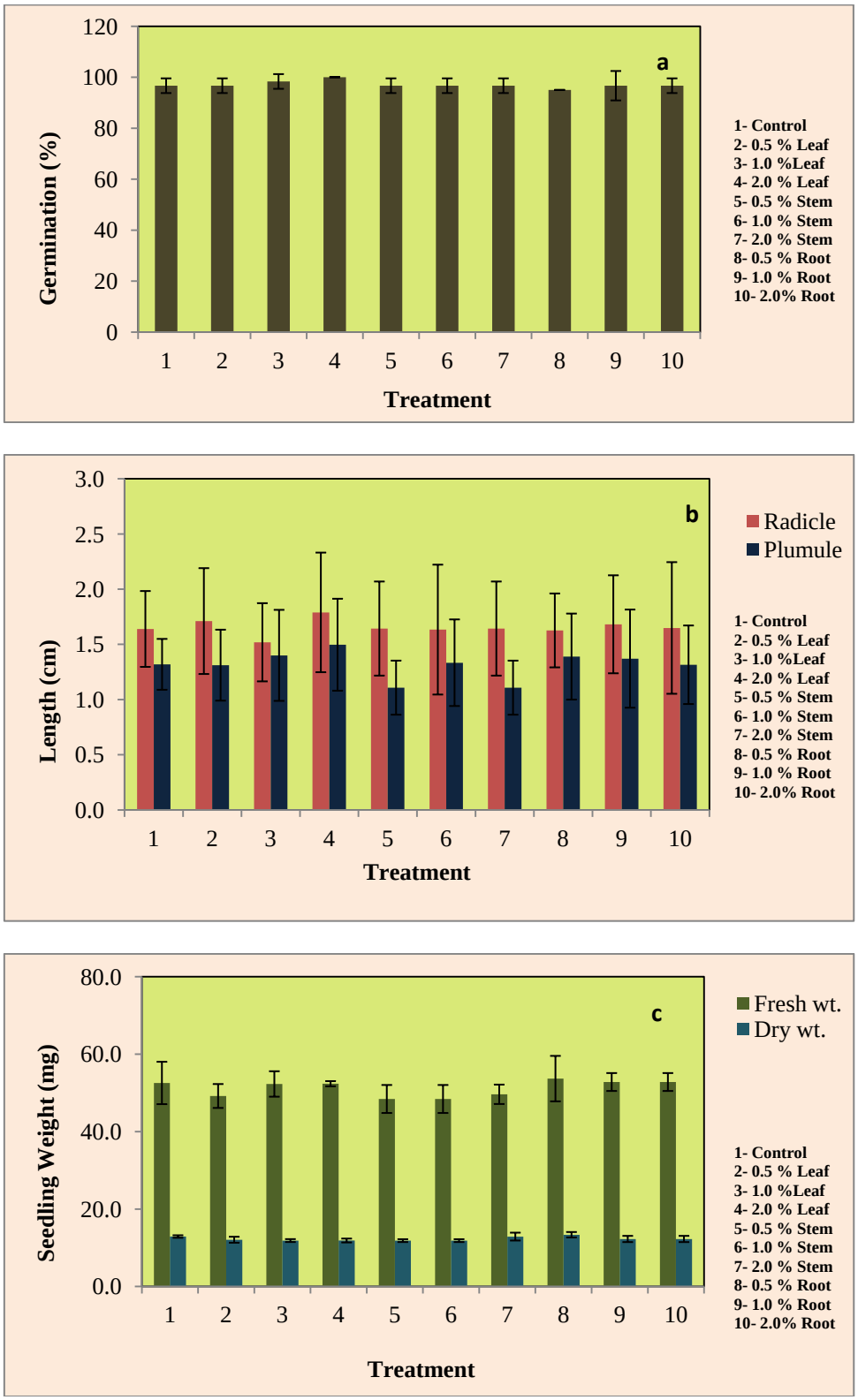
(Note: Bars represent mean ± Std)

Figure 3.10: Effect of leaf, stem and root aqueous extracts of *Coleus forskohlii* Briq. on germination and growth parameters of Radish



(Note: Bars represent mean ± Std)

Figure 3.11: Effect of leaf, stem and root aqueous extracts of *Curculigo orchioides* Gaertn. on germination and growth parameters of Radish



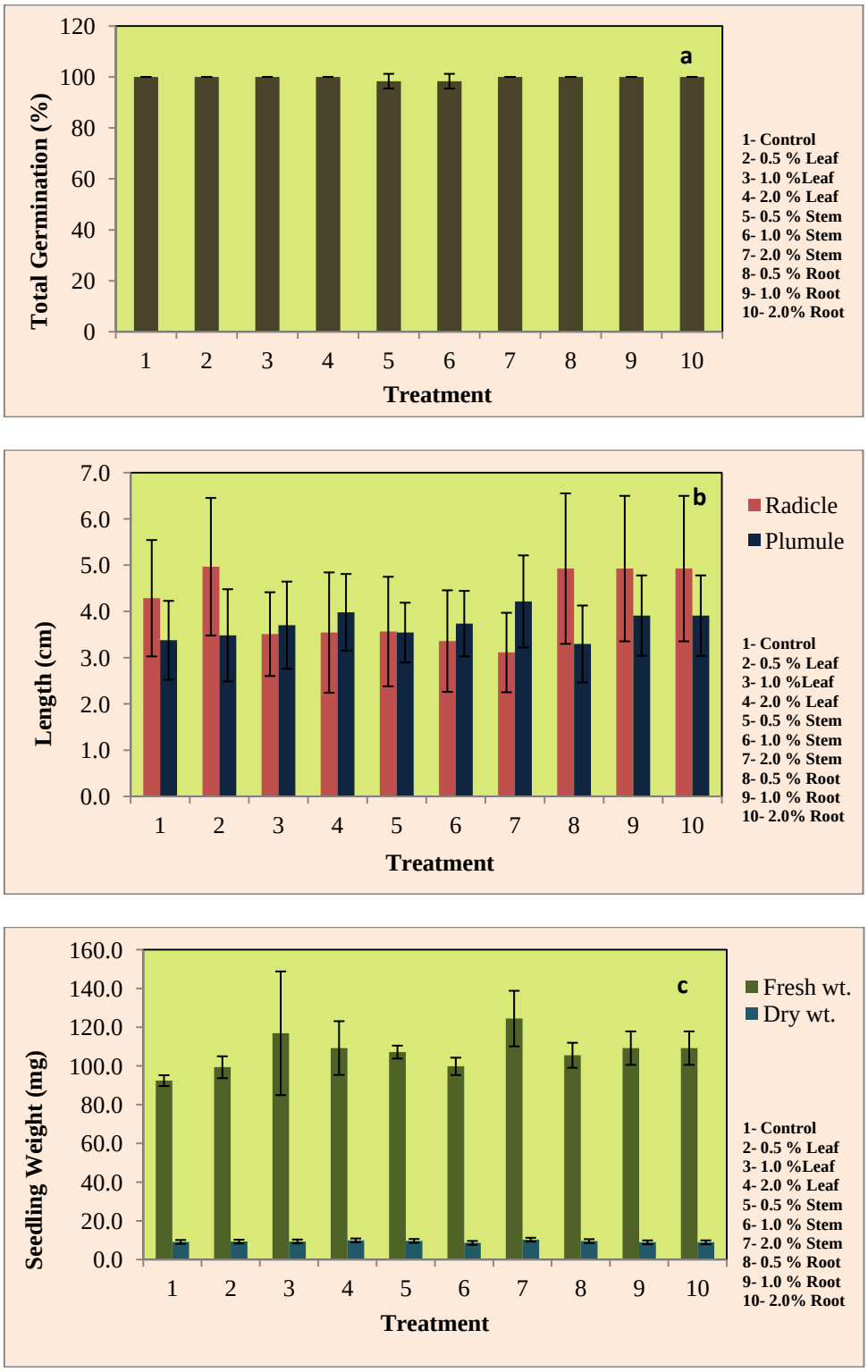
(Note: Bars represent mean ± Std)

stem aqueous extracts (0.5, 1 and 2 % concentration).

12) *Dioscorea alata* L. (Da): No aqueous extracts from Da could suppress the radish seed germination considerably (Figure 3.12a). Plumule length and seedling biomass showed no inhibitory response to any of the extract treatments (Figure 3.12 b, c). Radicle was highly affected by the leaf and tuber aqueous extracts (Figure 3.12b). Leaf extracts at both the concentrations i.e. 1 and 2 % suppressed the radicle length by 18 % (Figure 3.12b). Tuber extracts affected the radicle length in concentration dependent manner. Extracts applied at the concentration of 0.5, 1 and 2 % suppressed the radicle length by 17, 22, and 28 % respectively with reference to the control (Figure 3.12b).

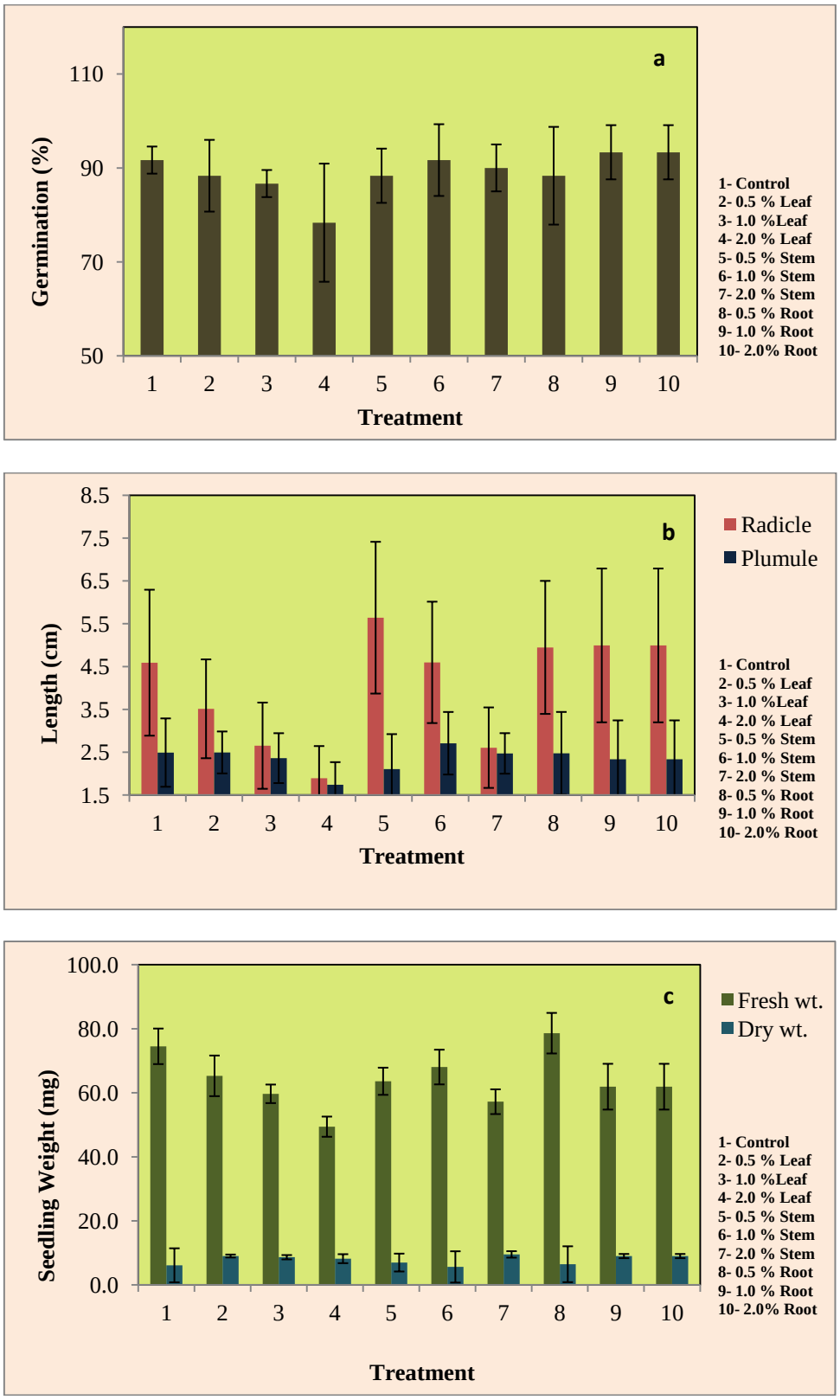
13) *Enicostemma littorale* (Blume) (El): Leaf aqueous extracts exhibited the inhibitory effect on all the studied parameters except the seedling dry weight (Figure 3.13 a, b, c). The inhibitory effect of leaf extracts was observed to be concentration dependent for all the affected parameters that is the increase in concentration of leaf extract resulted in the suppression of the radish parameters. Leaf extracts applied at the concentration of 0.5, 1 and 2 % reduced the seed germination by 4, 6 and 15 % as compared to the control (Figure 3.13a). Leaf extracts at the same concentrations inhibited the radicle by 24, 42 and 59 % (Figure 3.13b). Plumule length in comparison to the control was reduced by 6 % and 30 % respectively owing to the leaf extract treatment applied at the rate of 1 and 2 % (Figure 3.13b). Stem aqueous extracts at 2 % inhibited the radicle growth by 43 % and reduced seedling fresh weight by 23 % with reference to the respective controls (Figure 3.13c). Plant root extracts at the rate of 1 and 2 % suppressed the growth of radicle by 7 % as compared to the control. Root extracts at the same concentration also reduced the fresh weight by 17 %.

Figure 3.12: Effect of leaf, stem and root aqueous extracts of *Dioscorea alata* L. on germination and growth parameters of Radish



(Note: Bars represent mean ± Std)

Figure 3.13: Effect of leaf, stem and root aqueous extracts of *Enicostemma littorale* (Blume) on germination and growth parameters of Radish



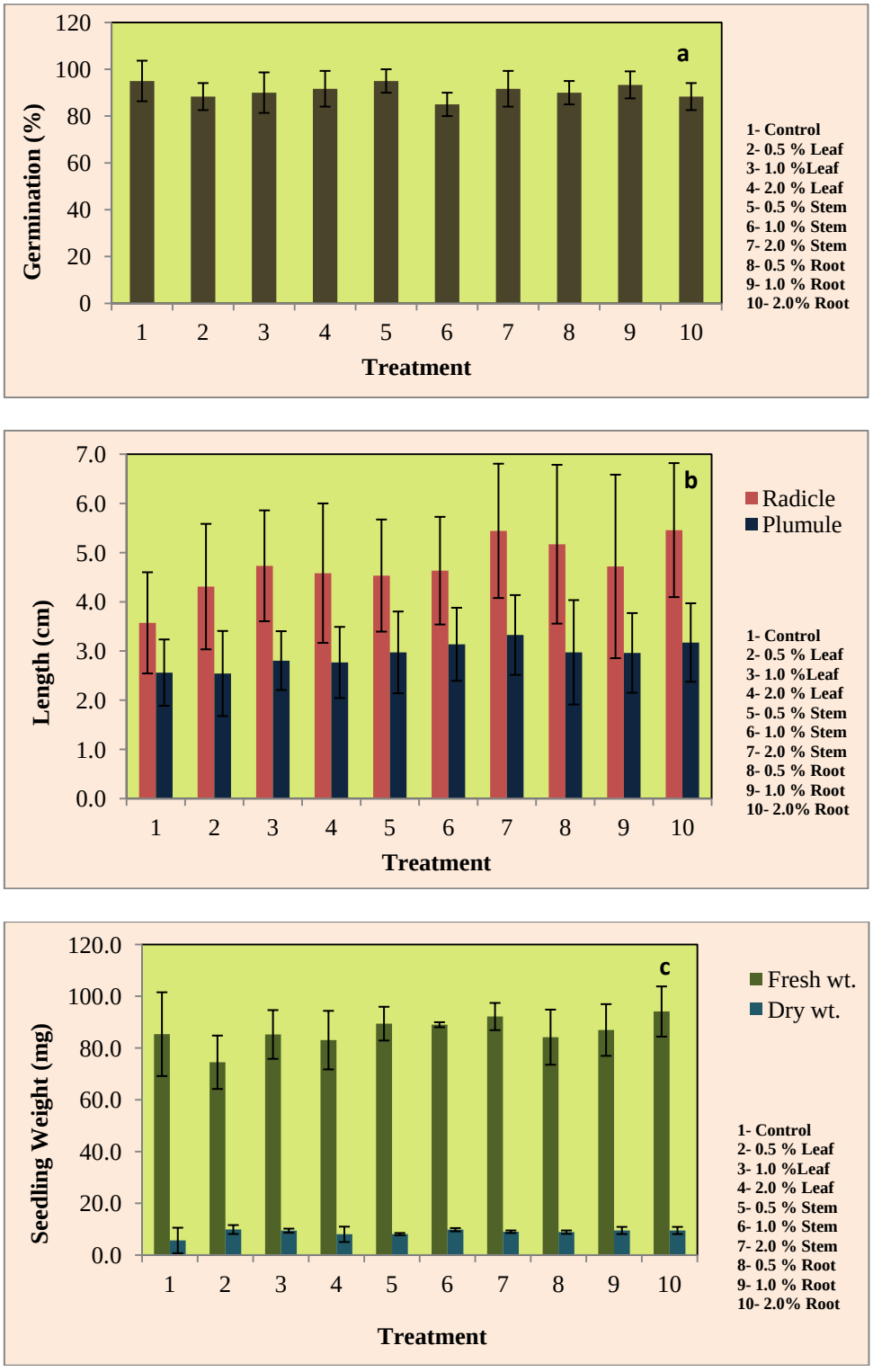
(Note: Bars represent mean ± Std)

14) *Euphorbia hirta* L. (Eh): Leaf, stem and root aqueous extracts from Eh at all the applied rates suppressed the radish seed germination (Figure 3.14). At the applied rates of 0.5, 1 and 2 % of concentration, leaf extracts reduced seed germination by 7, 5 and 4 % respectively, stem extracts reduced the germination by 0, 11 and 4 % and the root extracts reduced the germination by 5, 2 and 7 % respectively (Figure 3.14a). No significant inhibition was observed for the radicle and plumule length and fresh and dry weight (Figure 3.14 b, c). Leaf extracts at 0.5 % reduced the seedling fresh weight by 13 % as compared to control.

15) *Synedrella nodiflora* (L.) Gaertn. (Sy.n): Different aqueous extracts i.e. leaf, stem and root, had no effect on any of the radish growth parameters (Figure 3.15 a, b, c) except trivial effect on the seed germination.

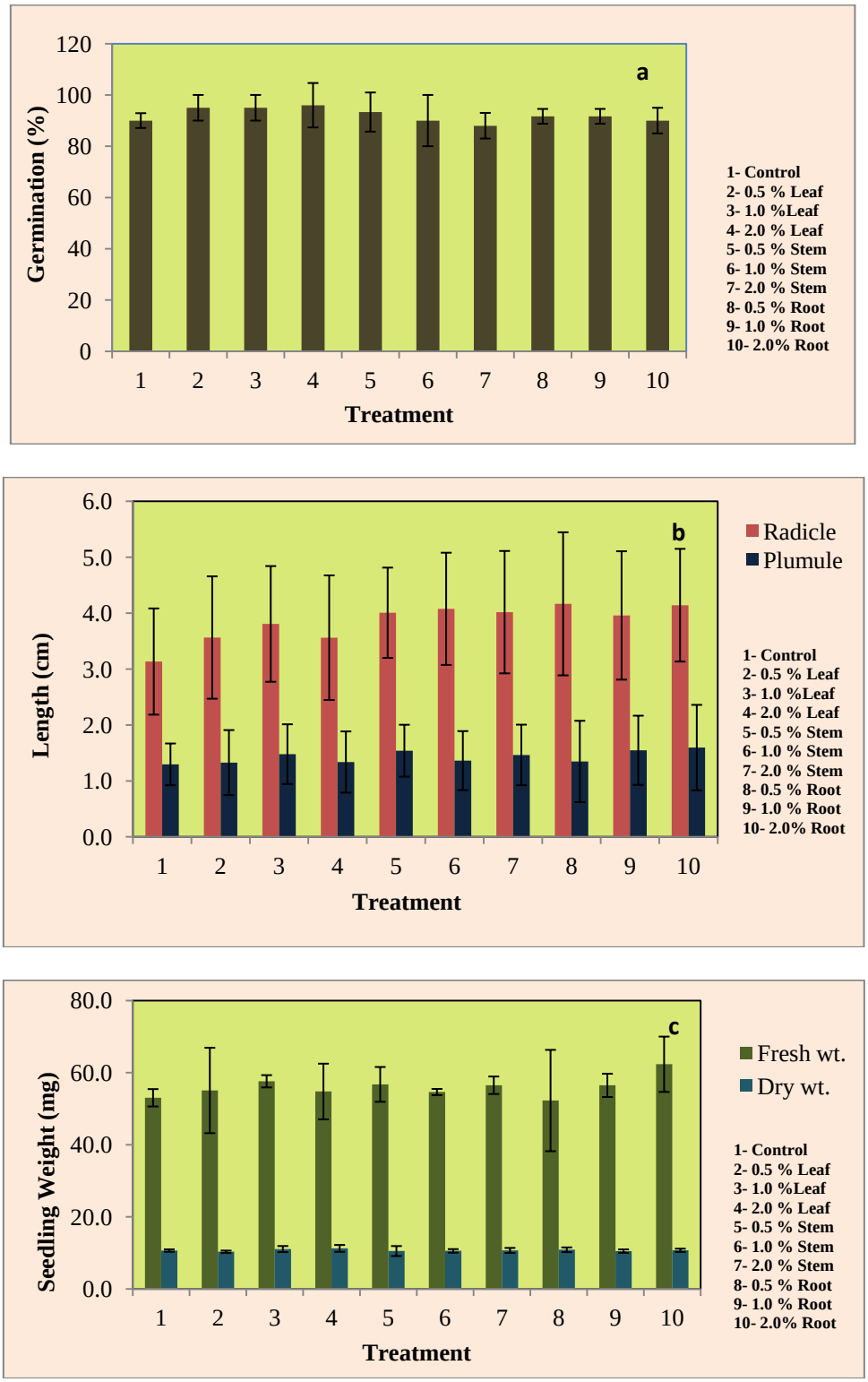
16) *Solanum nigrum* L. (So.n): So.n aqueous extracts at all the concentration suppressed the radish seed germination. Leaf extracts was suppressive at all the applied rates that is 0.5, 1 and 2 % concentration, where in seed germination in the treatments were reduced by 14, 14 and 12 % (Figure 3.16a). Stem extracts inhibited the same by 18, 9 and 7 % respectively as compared to the control. Root extracts inhibited the seed germination by 3, 7 and 0 %. Treatment with 0.5 % of leaf extracts suppressed the radicle length by 18 % as compared to control (Figure 3.16b). Plumule length was suppressed by leaf extracts at applied rates by 14, 7 and 10 % and the root extracts inhibited the same by 2, 6 and 5 % respectively. Seedling fresh weight decreased as compared to the control and the decrease was by 5 and 3 % in treatment with 0.5 and 1 % leaf extracts. No effect was observed on seedling dry weight in response to the So.n treatments.

Figure 3.14: Effect of leaf, stem and root aqueous extracts of *Euphorbia hirta* L. on germination and growth parameters of Radish



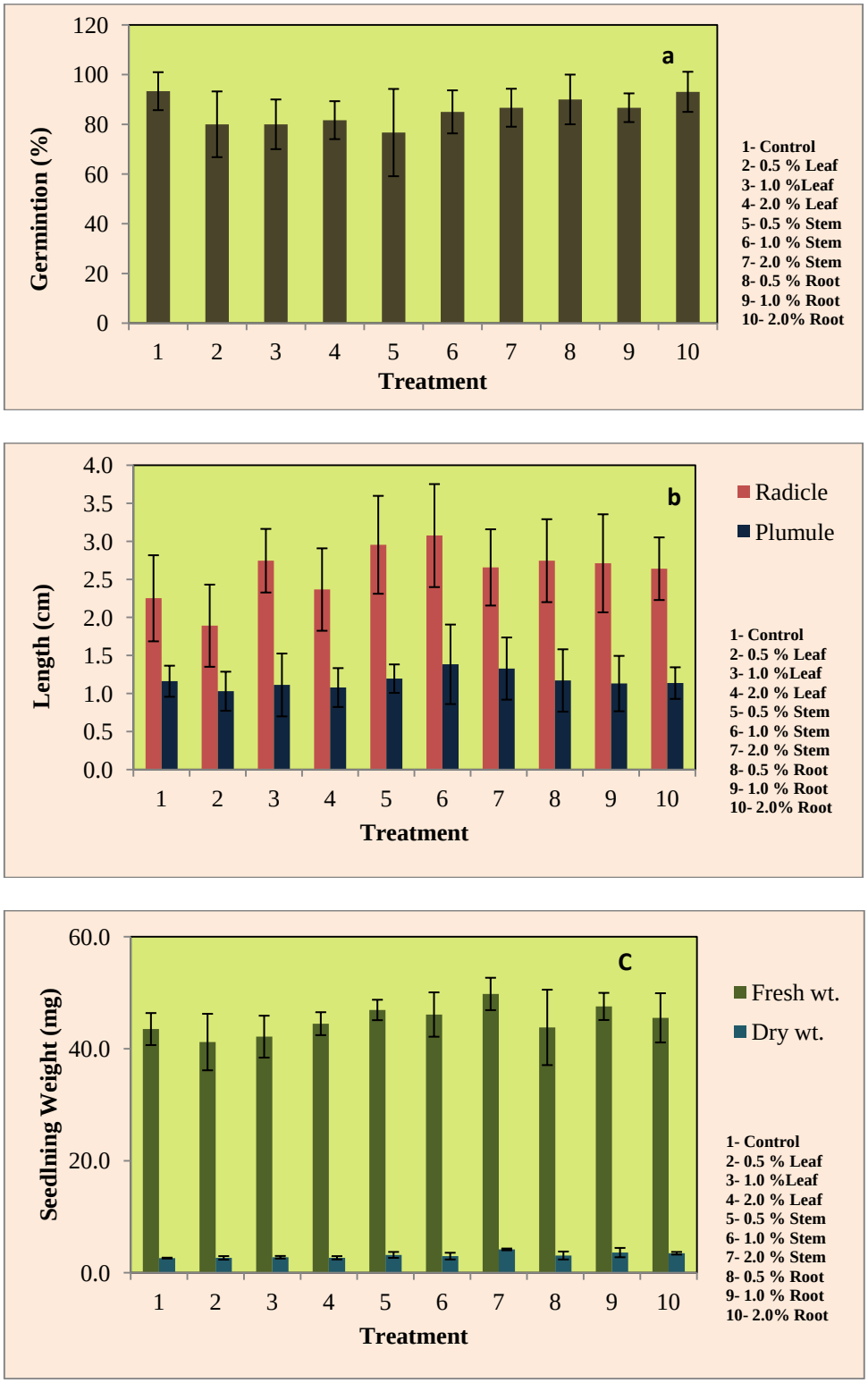
(Note: Bars represent mean ± Std)

Figure 3.15: Effect of leaf, stem and root aqueous extracts of *Synedrella nodiflora* (L.)Gaertn. on germination and growth parameters of Radish



(Note: Bars represent mean ± Std)

Figure 3.16: Effect of leaf, stem and root aqueous extracts of *Solanum nigrum* L. on germination and growth parameters of Radish

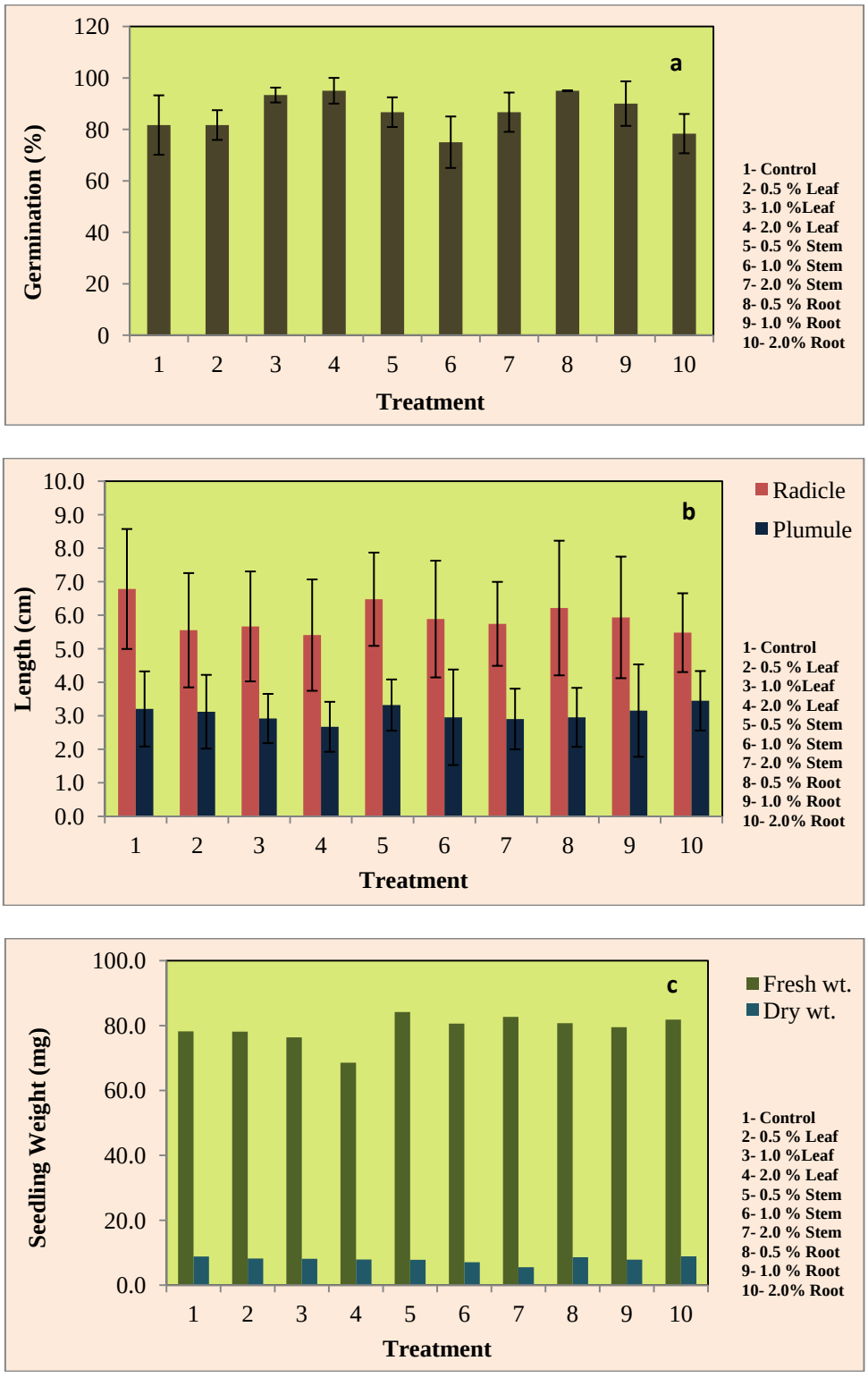


(Note: Bars represent mean ± Std)

17) *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms (Tc): Stem extracts at 1 % concentration and root extracts at 2 % concentration reduced the seed germination by 9 % and 4 % respectively (Figure 3.17a). Leaf, stem and root extracts at all the concentrations inhibited the radicle length (Figure 3.17b). At the applied rates of 0.5 %, 1 % and 2 %, leaf extracts suppressed the radicle length by 18, 17 and 20 %, stem extracts reduced the radicle length by 5, 13 and 16 % and the root extracts reduced the same by 9, 13 and 19 % with reference to the control. Plumule length was reduced by leaf extracts at the applied rates by 2, 9 and 17 % (Figure 3.17b). The same was reduced by stem extracts (at the rate of 1 and 2 %) by 8 and 9 %. Seedling fresh weight decreased by 2 and 12 % as compared to the control owing to the leaf extracts treatment applied at the rate of 1 and 2 % (Figure 3.17c). Seedling dry weight was affected by the leaf and stem extracts in and the effect was observed to be concentration dependant (Figure 3.17c). Reduction in fresh weight imposed by leaf extracts was 7, 8 and 10 % as compared to control. Stem extracts reduced the same at the applied rates by 11, 20 and 30 %. Plant root at 0.5 and 1 % rates decreased the seedling dry weight by 3 and 11 % respectively.

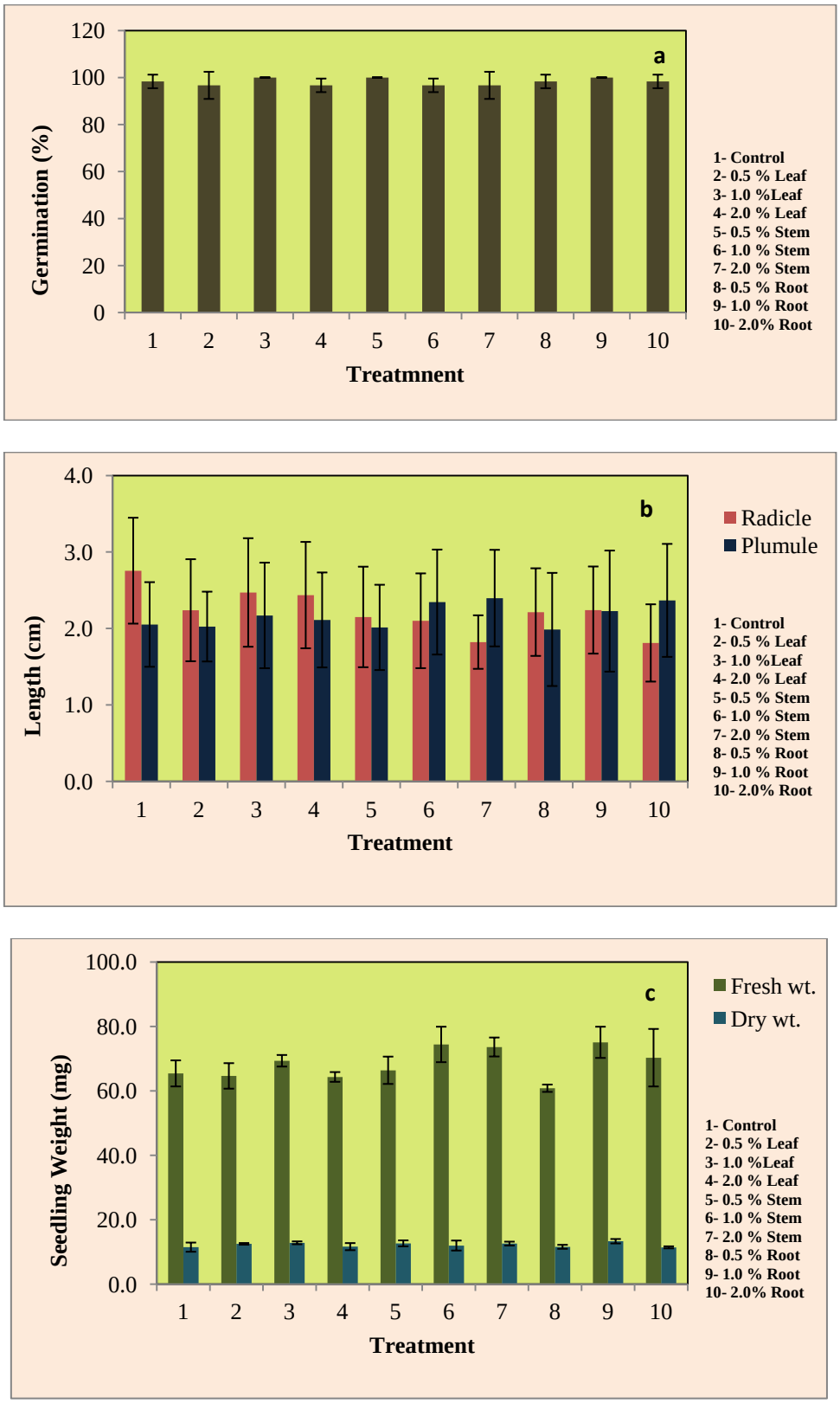
18) ***Urginea indica* (Roxb.)Kunth. (Ui):** Ui aqueous extracts had no inhibitory effect on seed germination (Figure 3.18a). Radicle length was significantly affected by all the aqueous extracts. At the applied rates of 0.5, 1 and 2 % concentration, leaf extracts inhibited the radicle length by 20, 12 and 13 %, stem extracts inhibited the radicle length by 23, 25 and 35 % and the root extracts inhibited the same by 21, 20 and 35 % respectively (Figure 3.18b). Plumule length was affected by the 0.5 % of leaf stem and root extracts by 4, 4 and 5 % respectively. Seedling fresh weight was decreased by 7 % owing to the root extracts treatment applied at the rate of 0.5 % concentration (Figure 3.18 c).

Figure 3.17: Effect of leaf, stem and root aqueous extracts of *Tinospora cordifolia* (Willd.) on germination and growth parameters of Radish



(Note: Bars represent mean ± Std)

Figure 3.18: Effect of leaf, stem and root aqueous extracts of *Urginea indica* (Roxb.) Kunth. on germination and growth parameters of Radish



(Note: Bars represent mean ± Std)

3.1.1.2. Membrane integrity: Evaluation of membrane damage was incorporated as a parameter in the preliminary analysis of medicinal plants toxicity on membrane integrity of radish seedling. Figure 3.19 (a,b,c) shows the effect of aqueous plant extracts from different medicinal plants. Extracts were observed to impart varied extent of membrane damage to the radish seedlings. Cellular membrane integrity was assessed based on the conductivity of bathing medium imparted by the ion leakage from immersed radish seedlings with reference to the control. Higher conductivity values represented higher electrolyte or ion leakage occurring due to high membrane injury to the radish seedlings. Notable membrane damage was observed for extracts treatment from Ai, Ap, Aa, Bd, Cr, Cb El, Sn, Tc and Cf. No effect was observed for the aqueous extracts from Av, Al, Ar, Co, Da, Eh, Sn and Ui on the seedling membrane integrity. Percentage increase in the conductivity values that were observed in the range of 25.0 - 75.0 %, were considered as significant values for membrane damage in radish seedlings. The Stem and root extracts of Ai at all the concentrations (0.5, 1, 2%) increased the percentage conductivity and induced the seedling membrane damage. Percentage conductivity values obtained for the same are 66.67 % in case of stem extracts applied at all the concentration and 75.0, 100.0 and 80.0 % in case of root extracts applied at the rate of 0.5, 1, 2% respectively whereas the same in control was 33.3 %. Ap stem extracts (at the applied rates of 0.5, 1.0 and 2.0 %) induced membrane damage by 50.0, 25.0 and 40.0 % respectively in comparison to the control where there was no membrane damage. Aa root extracts applied at the rate of 2 % caused 100.0 % leaching of ions in radish seedlings as compared to control where it was 66.7 %. In case of Bd all the three that is leaf, stem and root extracts applied at all the rates induced ion leakage in comparison to the control where there was no ion leakage as the membranes must not have undergone

damage. The percentage conductivity observed in leaf (0.5, 1, 2 %) extract was 50.0, 33.3, 33.3 % respectively, in stem (0.5, 1, 2 %) extracts it was 50.0, 40.0, 50.0 % and in root extracts it was 33.3, 50.0 and 75.0 % while in control there was no ion leakage at all. For Cr, leaf and stem extracts applied at the rate of 1 % induced 80.0 and 100 % ion leakage in radish seedlings with reference to the control where value was 66.7 %. Cb leaf extracts at the rate of 0.5 % and its stem extracts applied at the rate of 1% both caused 100.0 % ion leakage when the same in control was 50.0 %. Aqueous extracts from Cf imparted varied level of injury to the radish seedling where in, seedlings in the respective control had no injury at all. Leaf extracts at 1 and 2 % rate caused 25.0 and 33.3 % ion leakage, stem extracts at the rate of 0.5 and 1 % caused 25.0 and 33.3 % ion leakage and root extracts (0.5, 1, 2 %) caused 33.3, 25.5 and 33.3 % of the same. In case of Co only the leaf extracts applied at rate of 1% caused ionic leakage to 66.6 % in comparison to control where the same was 50.0 %. El leaf extracts at all the applied rates (0.5, 1, 2 %) induced ionic leakage in the radish seedlings, by 33.3, 25.0 and 25.0 % respectively as compared to the control where there was no ionic leakage at all. Stem extracts at the rates of 0.5 and 2 % both caused the ionic leakage by 33.3 %. Prominent damage in case of Eh extracts was observed for the root extracts applied at the rate of 2 % where the ionic leakage was 50.0 % in comparison to the control where the value was 25.0 %. Sy.n leaf extracts at the rate of 0.5 % and root extracts at the rate of 1.0 % induced the ion leakage in radish seedlings by 75.0 and 60.0 % however in the respective control the ionic leakage was 50.0 %. Aqueous extracts of So.n at all the applied rates caused serious membrane damage to the radish seedlings, wherein in case of the respective control there was no ion leakage observed. So.n leaf extracts (0.5, 1, 2 %) caused 50.0, 50.0 and 100.0 ionic leakage, stem extracts at the applied rates (0.5, 1, 2 %) induced 100.0, 33.3 and 67.0 % and the

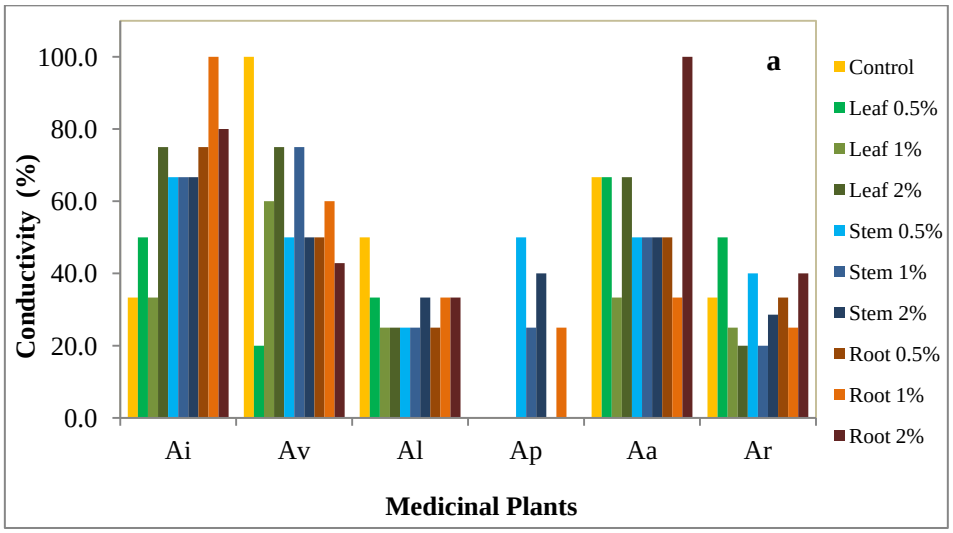
root extracts at all the rate (0.5, 1, 2 %) caused 100% ionic leakage in radish seedlings. All the Tc extracts imparted high damaged to the seedling membrane as compared to the control, where no membrane injury was observed. Tc leaf extracts caused 33.3, 25.0 and 25.0 % ionic leakage, stem extracts caused 33.3, 25.0 and 100.0 % ionic leakage and root extracts caused 50.0 75.0 and 75.0 % of the same.

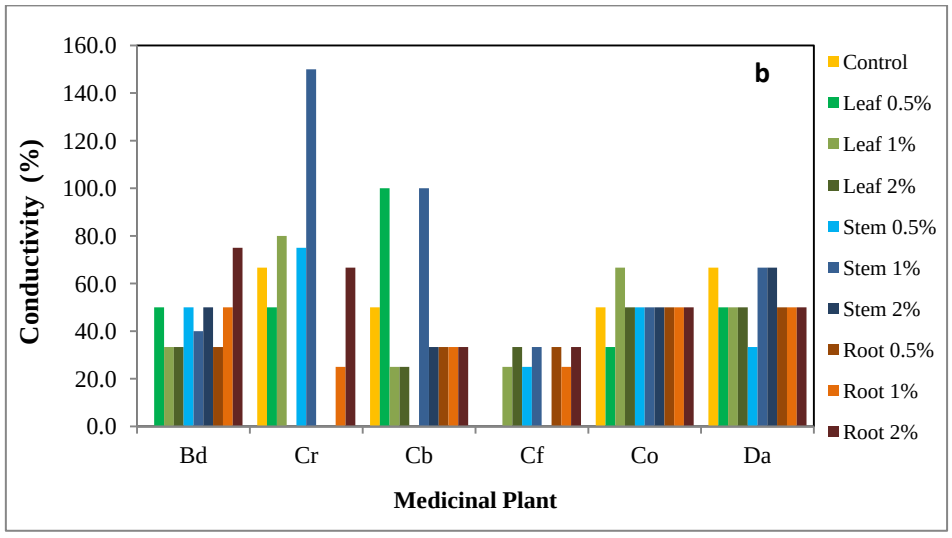
The plant extracts that were observed to be most toxic, imparting high membrane injury to the radish seedlings include root extracts from Ai, Aa and Bd, stem extracts from Cr, So.n and Tc and leaf extracts from So.n.

3.1.1.3. Lipid peroxidation: MDA content (µM/g Fw) in the radish seedlings was considered as a parameter to measure the allelopathic stress imparted by treatment with various medicinal plants aqueous extracts and increase in the content observed by the magnitude of 1 and more was considered important as a measure of stress. Aqueous extracts from few plants were observed to increase the MDA content in radish seedlings (Table 3.1 A, B, C). In the radish seedlings treated with Ai aqueous extracts no effect was observed on the MDA content in treated seedlings. For Av treatment, stem extracts applied at the rate of 0.5 and 1 %, the MDA content was 21.71 and 24.28 in comparison to this the same in control seedling was 18.61. Treating the radish seedlings (MDA conc. in the control was 23.93) with Al leaf extracts (1, 2 %), MDA content increased to 28.77 and 27.81 and treating with stem extracts (0.5, 1 %) the MDA content was increased to 33.25 and 42.22 respectively. MDA concentration in the seedling treated with Ap and Ar leaf extract applied at the rate of 1 % was 23.31 and 27.21 respectively which was higher than the same in their control (19.90 and 23.73 respectively). In the control kept along with the Aa treatments, MDA content in the radish seedlings was 68.5, treatment with the leaf extracts at the rate of 1 and 2% increased the MDA content to 104.4 and 95.5 and

treatment with the stem extracts at the rate of 0.5 and 2 %, the MDA content in radish seedling was observed to be 91.6 and 79.2 respectively. Leaf and stem extracts from Bd were also observed to affect the MDA content in radish seedlings. MDA content in the control kept along with Bd treatment was 17.04, the same was observed to increase, in the treatment with leaf extracts (1 and 2 %) by 24.98 and 21.91, in treatment with stem extracts (0.5 %) by 20.67 and in treatment with root extracts by 22.35. Cr leaf and stem extracts applied at the rate of 1 % affected the MDA content and the increase observed was 23.55 and 21.22 respectively with reference to the control where the MDA conc. was 18.57. MDA concentration in the radish seedlings treated with, leaf (at rate of 2 %), stem (1%) and root (1 and 2 %) extracts from El were 20.56, 18.30, 20.33 and 20.33 respectively which was higher than the same in the control (16.26). Eh leaf extracts (0.5 %) affected the MDA content in radish seedlings and increase it to 13.68 as compared to the same in control where the MDA conc. was 18.25. Sy.n root extracts at the rate of 0.5 % increased the MDA conc. to 29.42 as compared to the control where the MDA content was 25.47. All the aqueous extracts from the plant So.n, imparted stress and thus increased the MDA concentration in radish seedling as compared to the control where the concentration was 20.07. MDA concentration in the seedlings treated with leaf extracts applied at the rate of 0.5, 1, 2 % were 23.02, 23.78, 25.24, in the seedlings treated with stem extracts (0.5, 1, 2 %) were 22.28, 27.06, 23.62 and in the seedlings treated with root extracts applied at the rate of 0.5 % was 22.30. So.n, leaf extracts were observed to impart stress to the radish seedlings in a concentration dependent manner. Aqueous extracts of the medicinal plants such as Ai, Cb, Cf, Co, Da, Tc and Ui had no remarkable effect on the MDA content in radish seedlings.

Figure 3.19: Effect of medicinal plants aqueous extracts on membrane integrity in Radish seedling





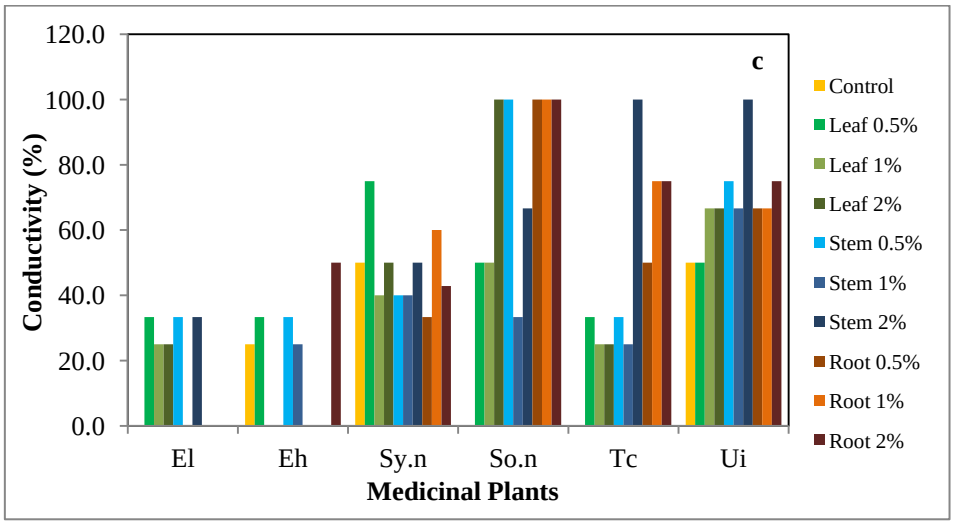


Table 3.1: Effect of different medicinal plants aqueous plant extracts on Lipid peroxidation (MDA µM/ g^{-1} FW) in Radish seedlings:

Table1A						
Treatment	**Ai**	**Av**	**Al**	**Ap**	**Ar**	**Aa**
1	36.17 ± 3.8	18.61 ± 3.3	23.93 ± 5.8	19.90 ± 1.4	23.73 ± 2.5	68.5 ± 4.9
2	28.1 ± 2.4	19.18 ± 1.7	16.82 ± 8.3	23.31 ± 2.7	27.21 ± 5.3	7.36 ± 0.5
3	31.0 ± 5.0	18.08 ± 0.4	28.77 ± 7.3	19.96 ± 2.64	23.52 ± 0.2	104.4 ± 10.1
4	27.16 ± 0.7	19.32 ± 2.4	27.81 ± 1.9	20.36 ± 3.5	21.58 ± 5.1	95.5 ± 7.8
5	29.84 ± 4.5	21.71 ± 2.1	33.25 ± 1.4	15.70 ± 13	22.31 ± 3.6	91.6 ± 10.8
6	27.12 ± 2.2	24.28 ± 2.3	42.22 ± 20.2	14.47 ± 12.3	20.99 ± 0.8	61.1 ± 6.6
7	25.91 ± 2.6	19.15 ± 2.1	21.63 ± 10.1	20.03 ± 1.4	18.47 ± 2.3	79.2 ± 4.6
8	28.90 ±5.3	18.74 ± 2.5	20.59 ± 2.6	15.29 ± 1.32	13.52 ± 14.2	56.0 ± 1.5
9	25.84 ± 6.7	18.51 ± 1.3	24.71 ± 6.0	17.58 ± 1.6	20.27 ± 2.5	59.1 ± 6.3
10	20.75 ± 1.9	19.55 ± 0.5	15.62 ± 4.9	17.96 ± 2.18	17.37 ± 1.6	38.3 ± 7.4

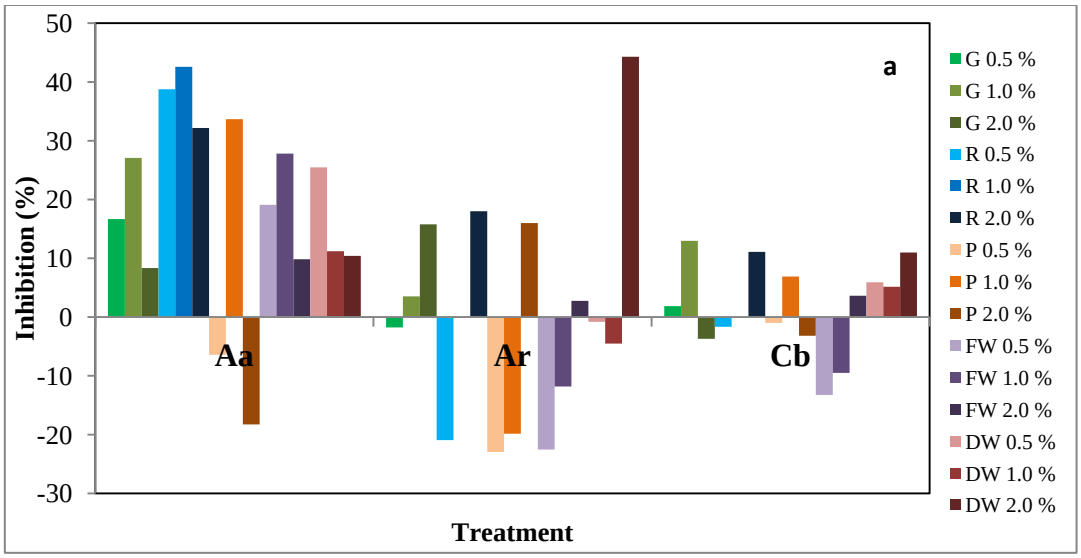
Table1B						
Treatment	**Bd**	**Cr**	**Cb**	**Cf**	**Co**	**Da**
1	17.04 ± 3.1	18.57 ± 0.8	20.21 ± 2.1	23.47 ± 1.3	1.80 ± 0.8	5.01 ± 0.5
2	16.28 ± 4.5	18.09 ± 0.5	13.62 ± 9.7	19.86 ± 1.9	1.58 ± 0.6	3.37 ± 1.3
3	24.98 ± 5.3	23.55 ± 3.9	15.79 ± 2.0	19.14 ± 1.9	1.33 ± 0.1	4.43 ± 0.5
4	21.91 ± 1.2	20.00 ± 0.7	18.23 ± 4.8	18.47 ± 1.7	1.60 ± 0.4	6.58 ± 2.8
5	20.67 ± 4.6	20.13 ± 1.3	17.73 ± 1.12	19.69 ± 2	1.60 ± 0.4	5.07 ± 0.4
6	18.25 ± 2.3	21.22 ± 3.7	17.66 ± 0.51	18.02 ± 4.2	1.99 ± 0.3	4.45 ± 0.5
7	18.58 ± 1.8	17.34 ± 1.9	20.96 ± 5.7	15.89 ± 3.9	1.24 ± 1.8	4.72 ± 1.0
8	22.35 ± 3.0	19.06 ± 2.0	18.55 ± 1.5	19.15 ± 4.1	1.15 ± 1.2	3.64 ± 0.5
9	12.07 ± 2.0	18.68 ± 1.9	13.23 ± 0.3	13.14 ± 2.5	1.15 ± 1.2	3.14 ± 1.2
10	11.60 ± 0.5	17.29 ± 1.8	12.43 ± 1.6	10.67 ± 1.2	1.53 ±0.8	3.14 ± 1.2

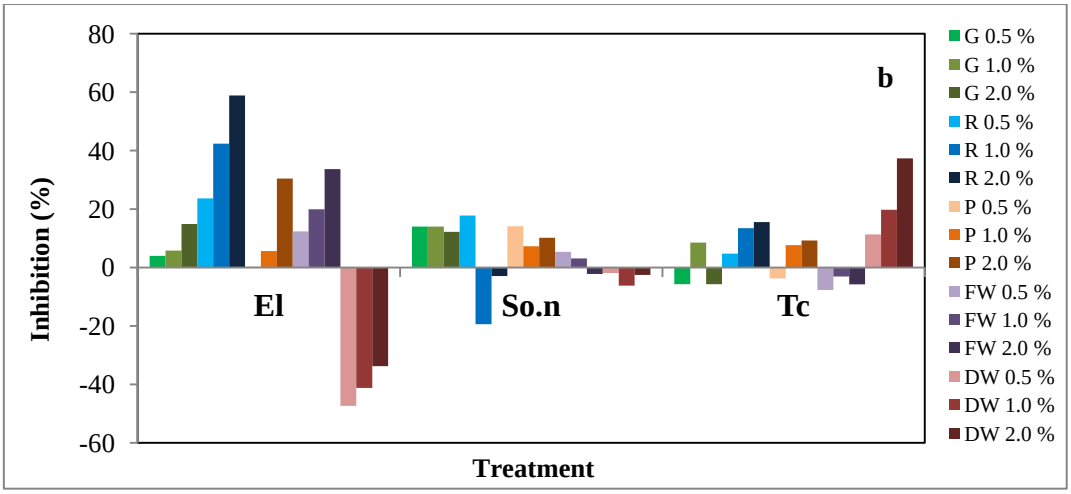
Table1C						
Treatment	**El**	**Eh**	**Sn**	**Sn**	**Tc**	**Ui**
1	16.26 ± 5.8	13.68 ± 2.9	25.47 ± 2.9	20.07 ± 3.9	17.35 ± 4.9	0.54 ± 0.3
2	17.84 ± 0.9	18.25 ± 4.1	24.62 ± 2.1	23.02 ± 2.9	14.51 ± 2.3	0.44 ± 0.4
3	17.59 ± 1.6	12.18 ± 1.4	24.52 ± 1.1	23.78 ± 5.1	14.76 ± 2.1	0.66 ± 0.4
4	20.56 ± 3.3	12.81 ± 1.7	18.73 ± 6.6	25.24 ± 0.7	17.04 ± 1.4	0.66 ± 0.3
5	17.37 ± 0.6	12.93 ± 0.4	23.60 ± 2.3	22.28 ± 1.3	15.97 ± 4.4	1.21 ± 1.3
6	18.30 ± 6.4	13.22 ± 0.9	23.72 ± 6.3	27.06 ± 3.2	12.70 ± 1.3	0.50 ± 0.4
7	17.28 ± 6.8	13.87 ± 3.2	18.75 ± 8.0	23.62 ± 4.1	15.44 ± 2.5	0.22 ± 0.1
8	15.94 ± 3.3	11.32 ± 0.3	29.42 ± 3.8	22.30 ± 3.0	12.73 ± 3.1	0.33 ± 0.3
9	20.33 ± 7.8	12.03 ± 5.4	16.25 ± 0.0	19.20 ± 1.4	10.63 ± 0.8	0.50 ± 0.6
10	20.33 ± 7.8	10.37 ± 2.3	18.46 ± 1.7	15.87 ± 3.4	7.16 ± 6.4	0.38 ± 0.2

(Note: MDA (µM/ g-1 FW) values are expressed as mean ± Std)

From the results of preliminary analysis, medicinal plants observed to impart maximum relative inhibition to the analysed parameters in toto in Radish were selected for the further allelopathic studies. Six medicinal plants exhibiting higher inhibitory effects and also having high medicinal utility were selected for further allelopathic studies. For all the six medicinal plants, the plant part that is known to confer medicinal importance and utility to the plant were also found to possess allelopathic potential. The selected plants include Aa, Ar, Cb, El, So.n and Tc. Figure 3.20 (a and b) depicts relative inhibitory effect offered by the aqueous extracts of the selected allelopathic plant part at all the applied concentration on the studied parameters of radish. Aqueous leaf extracts of Aa at all the applied concentration suppressed the growth parameters, that is seed germination, radicle and plumule length (except for leaf extracts applied at rate of 0.5 and 2 %), fresh and dry weight of radish seedlings, however the most inhibited was the seedling radicle length. Leaf extracts also increased lipid peroxidation in radish seedlings. Root aqueous extracts from Ar were toxic and inhibited all the parameters of radish except membrane integrity and lipid peroxidation. Aqueous extracts of Cb roots also affected all the parameters except the lipid peroxidation and the most affected parameter was radish seed germination. El leaf aqueous extracts was inhibitory to all the parameters except seedling dry weight. So. n leaf extracts exhibited the inhibitory effects to all the parameters however there was no effect of the same on seedling dry weight. Considering the results described above, the potential part of these six medicinal plants was subjected to chemical fractionation following the Harborne's fractionation protocol. Further allelopathic studies were performed on a commonly found monocot weed *Chloris barbata* Sw.

Figure 3.20: Relative inhibitory effect of the medicinal plants selected for the detailed study





(Note: Radish Germination and Growth parameters i.e. G-Germination, R-Radicle, P-Plumule, FW-Fresh Weight, D-Dry Weight)

3.2. Evaluation of medicinal plant part toxicity by fractionation guided bioassay

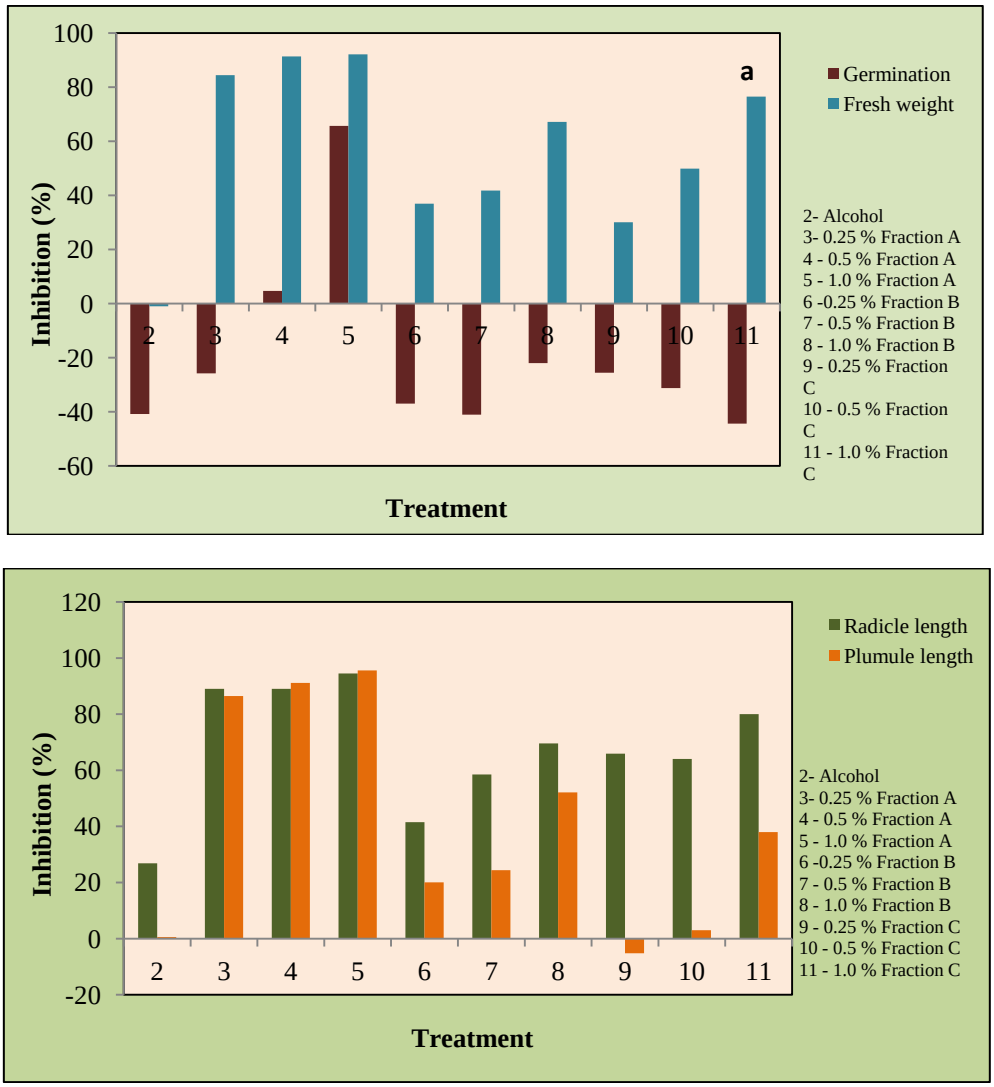
These bioassays were conducted on a weed plant, i.e. *Chloris barbata* Sw. Results of phytotoxic analysis, for all the following bioassays are presented in the form of percentage inhibition observed with reference to the respective control. Positive values represent the inhibitory and negative values represent the stimulations.

3.2.1. Allelopathic analysis of Harborne's fraction's A, B and C:

1) *Artemisia annua* L.: Figure 3.21 a and b shows the inhibition offered by plant fraction on Chloris. Treatment with fraction A inhibited the Chloris seed germination by 4.7 and 67.5 % where as fraction B and C failed to suppress seed germination in Chloris (Figure 3.21 a). Seedling fresh weight was found to reduce in response to treatment with all the three fractions however the maximum reduction was observed in treatment with fraction A (Figure 3.21 a). Seedling fresh weight was reduced by 84.4, 91.3 and 92.1 % in comparison to the control. Growth of radicle length was highly retarded owing to fraction A treatment (Figure 3.21 b) and for the applied rates of fraction the percentage inhibition observed was 89.0, 89.0 and 94.5 %. Plumule growth was also retarded by the fraction A treatment (Figure 3.21 b) and the decrease in length for the applied rates was 86.5, 91.2 and 95.6 % respectively which was also the highest suppression observed for plumule.

2) *Asparagus racemosus* Willd.: Out of the three fractions evaluated for their phytotoxicity, fraction B was found to be most inhibitory to all the studied parameters in Chloris (Figure 3.22 a, b and c). Seed germination (Figure 3.22 a and b) was highly suppressed owing to fraction B treatment. Speed of germination was retarded by 5.1, 63.6 and 88.2 % and the total germination was reduced by 3.2, 45.4 and 82.1% for the fraction B applied at the rates of 0.25, 0.5 and 1 %. Seedling fresh weight was reduced by 36.4, 55.3 and 65.8 % respectively (Figure 3.22 b). For the applied rates of fraction B, the plumule length was suppressed by 13.3, 53.5 and 35.4 % and radicle length was suppressed by 0.1 and 39.5 %, for fraction B applied at the rates of 0.5 and 1 % (Figure 3.22 c). Percentage inhibition offered by fraction B to the Chloris growth parameters ranged from 0.1 to 88.2 %.

Figure 3.21: Inhibitory effect imposed by Harborne's fractions of *Artemisia annua* L. leaf to the growth parameters of Chloris



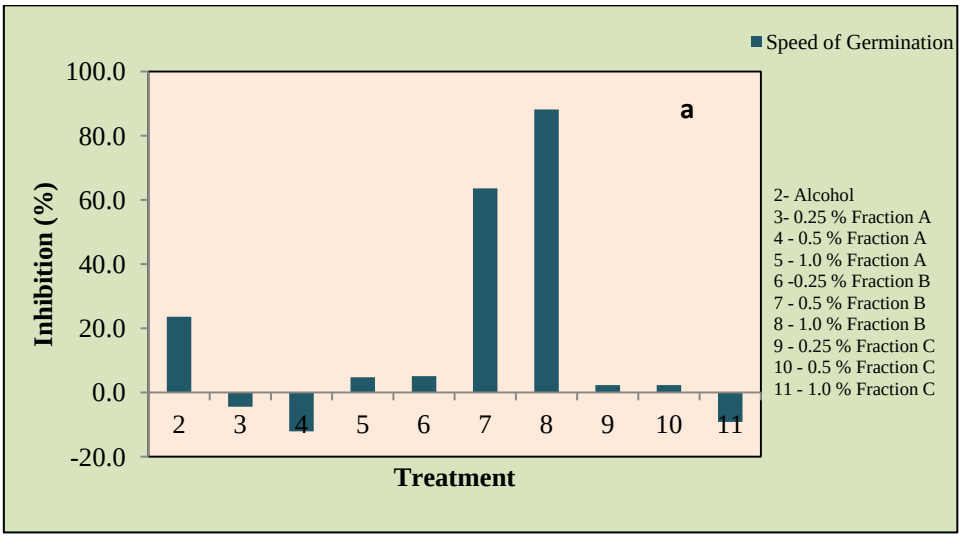
3) *Chlorophytum borivilianum* San. and Fern: Fraction A was the most allelopathic fraction and was found to inhibit all the growth parameters of Chloris (Figure 3.23 a, b and c). Speed of germination was retarded by 16.0, 25.7 and 84.3 % respectively (Figure 3.23 a) in fraction A (applied rates 0.25, 0.5, 1 %). The total germination was reduced by 1.3 and 77.0 % and seedling fresh weight was reduced by 36.0 and 38.8 % respectively in the treatment with fraction A applied at the rates of 0.5 and 1 %

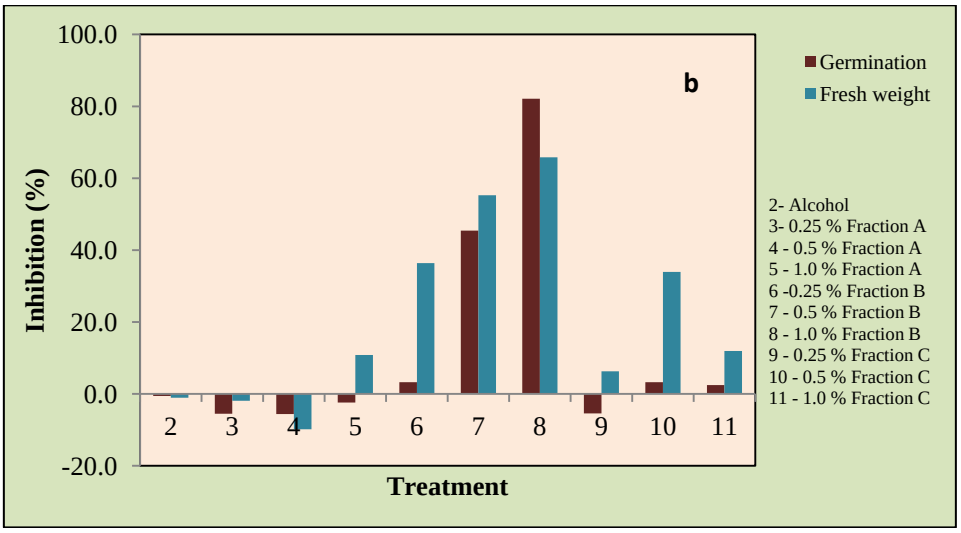
(Figure 3.23 b). Treatment with fraction A reduced the radicle length by 8.5, 86.2 and 88.1 % and the plumule length by 29.7, 69.7 and 88.4 % respectively (Figure 3.23 c). Radicle and plumule were the most affected parameters. Percentage inhibition offered by fraction A treatment ranged from 1.0 to 89.0 %.

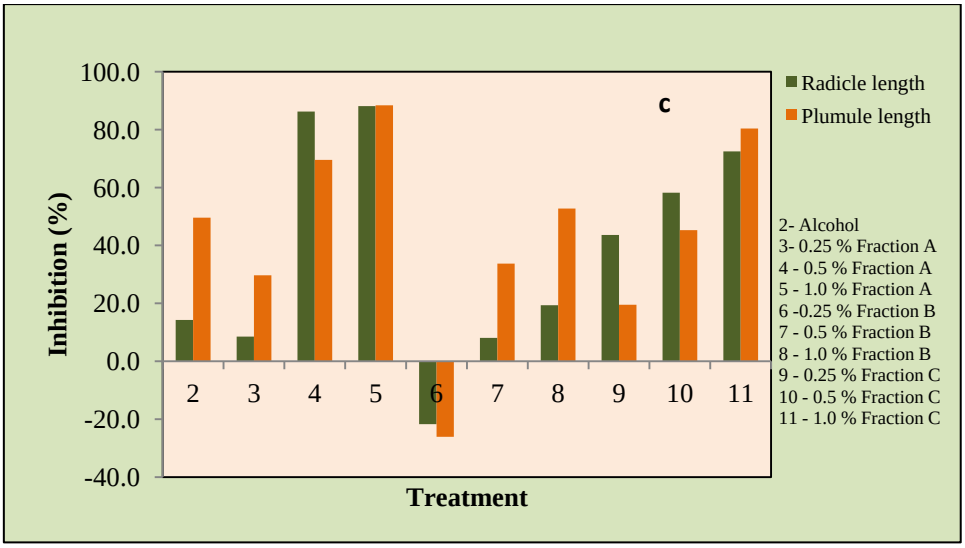
4) *Enicostemma littorale* (Blume): Of all the three fractions analysed fraction B at all the applied rates was the most allelopathic and was inhibitory to all the parameters of Chloris (Figure 3.24a, b, and c). The percentage inhibition imparted by fraction B on Chloris parameters ranged from 29.0 – 92.5 %. Growth suppression ability of fraction B was concentration dependent. Chloris germination was the most affected parameters and fraction B inhibited most at the applied rates (0.25, 0.5, 1 %). The germination speed was retarded by 44.4, 89.9 and 92.5 % (Figure 3.24 a) and the total germination was retarded by 37.2, 86.4 and 92.5 % (Figure 3.24b) respectively. Seedling fresh weight was observed to decrease by 13.2 and 36.2 % owing to treatment with fraction B at the applied rates of 0.5 and 1 % respectively (Figure 3.24 b). Radicle length was suppressed by 60.7, 70.0 and 80.0 % and plumule length was suppressed by 25.9, 74.9 and 84.0 % respectively owing to treatment B (application rates 0.25, 0.5, 1 %) (Figure 3.24c).

5) *Solanum nigrum* L.: The highest allelopathic activity was observed for Fraction A at all the application rates. Percentage inhibition observed due to fraction A treatment ranged from 22.0 – 84 %. The inhibitory effects were found to be concentration dependent. Highest affected was the seed germination. Fraction A treatment resulted in to decrease in the speed of seed germination by 43.8, 69.1 and 83.9 % and decrease in total germination by 22.2, 55.6 and 75.9 % respectively (Figure 3.25 a and b).

Figure 3.22: Inhibitory effect imposed by Harborne's fractions of *Asparagus racemosus* root Willd. to the growth parameters of Chloris.



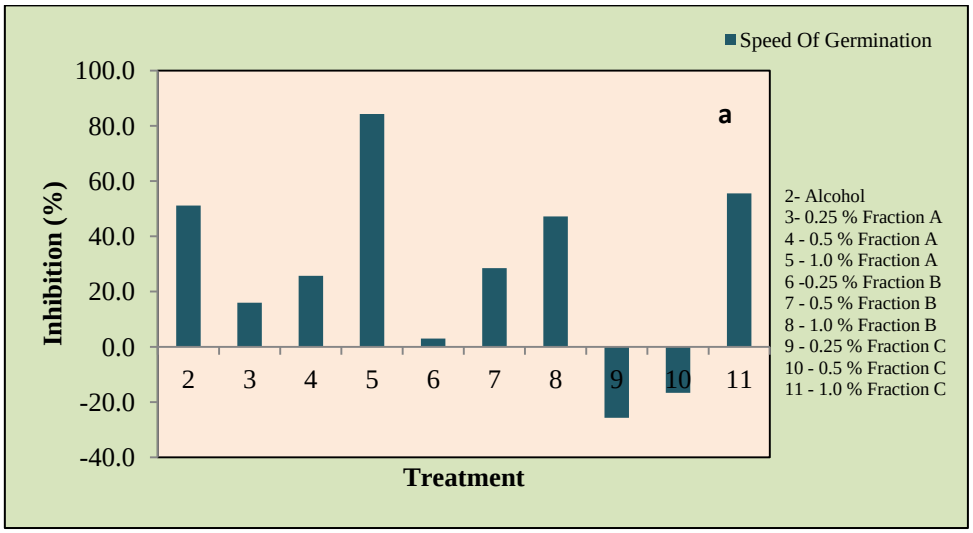


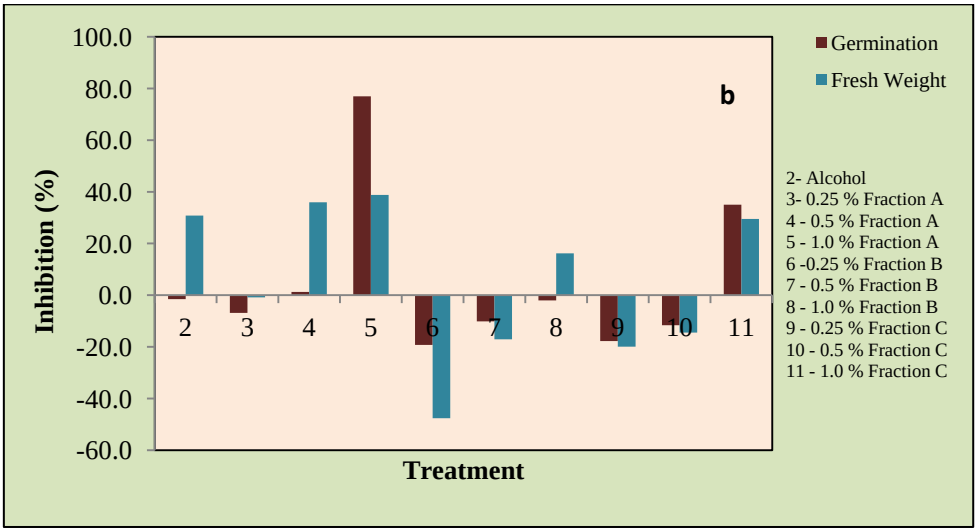


Seedling fresh weight was reduced by 47.3, 52.4 and 82.3 % respectively (Figure 3.25 b) owing to treatment with the fraction A (for the applied rates: 0.5, 1, 2 %). Fraction A treatment also affected radicle and the plumule length at the applied rates of 0.5, 1, 2 %. Retardation observed for radicle length was 75.9 and 77.8 % and for plumule length was 67.2 and 69.7 % (Figure 3.25 c).

6) *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms: The percentage inhibition exhibited by the fraction B ranged from 0 – 85.0 %. Speed of germination was suppressed by 0.2, 14.8 and 36.9 % respectively (Figure 3.26 a) in treatment with fraction B (0.5, 1 and 2%). The total germination was reduced only in the 1 % fraction B and was decreased by 70.4 % (Figure 3.26 b). However the highest effect on fresh weight was imparted by fraction A where the decrease in weight was by 55.4 and that in fraction B was 38.7 % both produced the effect at the highest application rate that is 1%. Radicle length was suppressed only by the 0.5 and 1 % fraction B and the suppression was by 19.3 and 79.4 % (Figure 3.26 c). Plumule length was affected in a concentration dependent manner (Figure 3.26 c) and the decrease in growth was by 26.5, 38.6 and 84.9 % respectively for the fraction B (applied rates: 0.25, 0.5, 1%).

Figure 3.23: Inhibitory effect imposed by Harborne's fractions of *Chlorophytum borivilianum* San. and Fern root to the growth parameters of *Chloris*





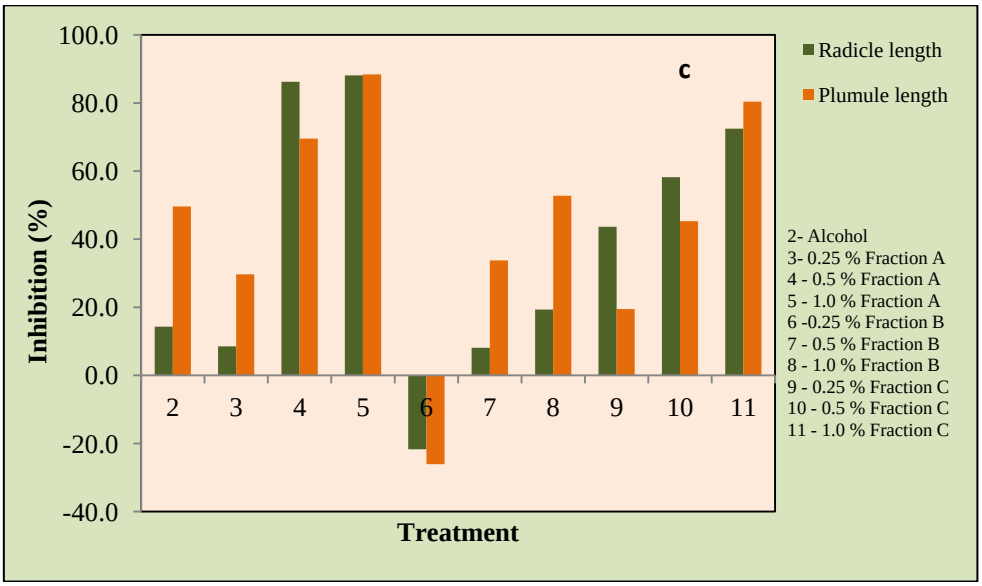


Figure 3.24: Inhibitory effect imposed by Harborne's fractions of *Enicostemma littorale* (Blume) leaf to the growth parameters of *Chloris*

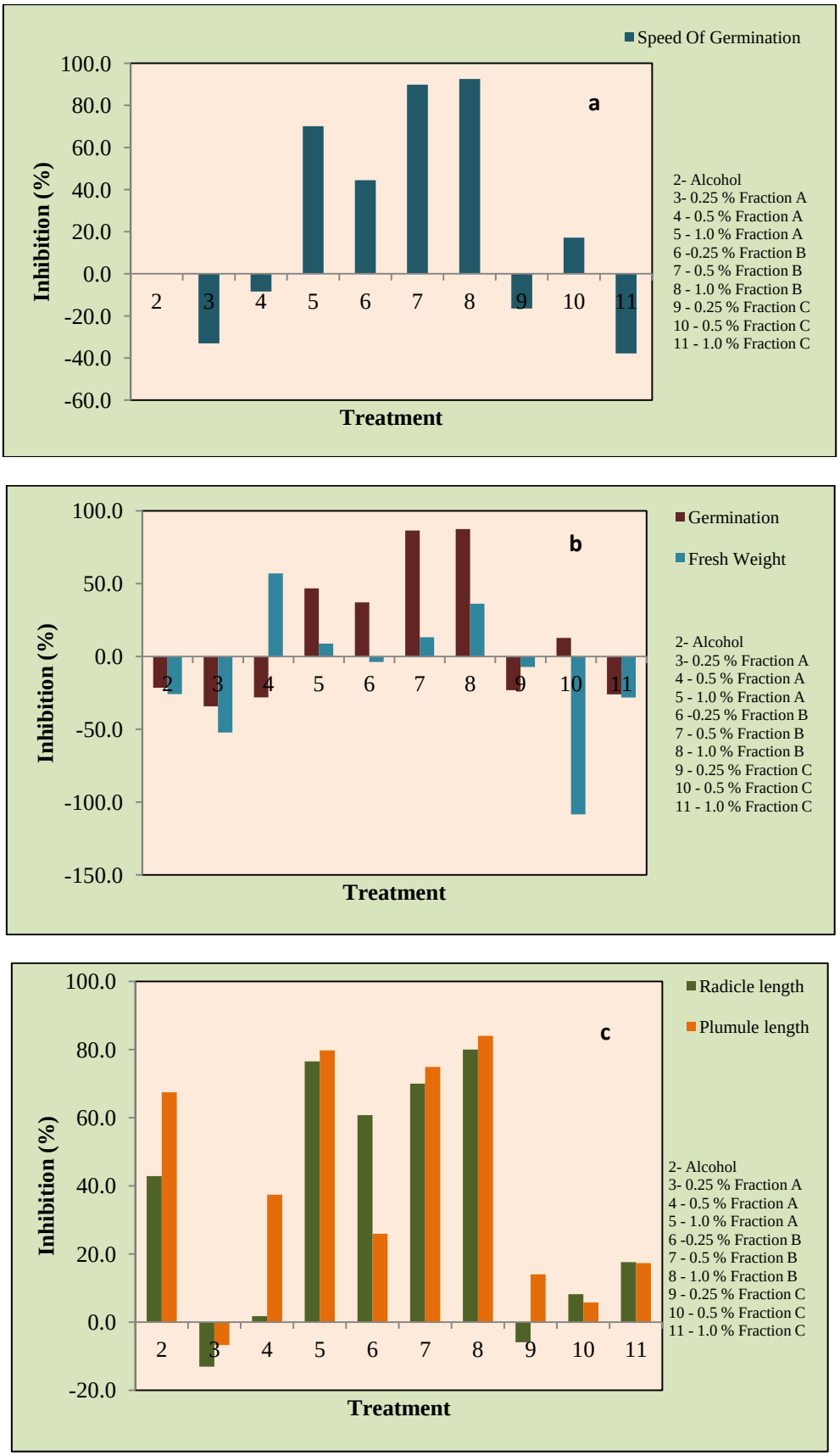
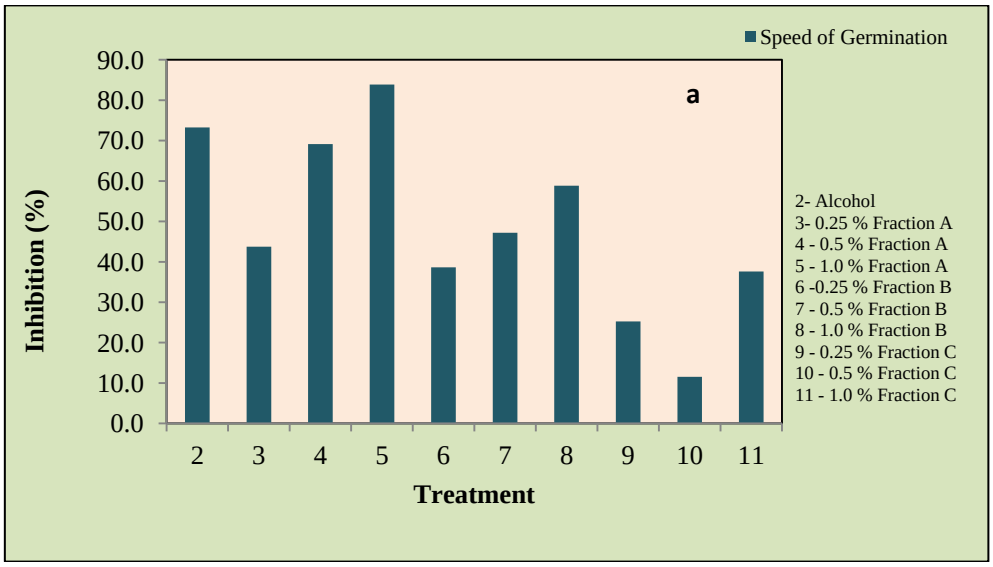
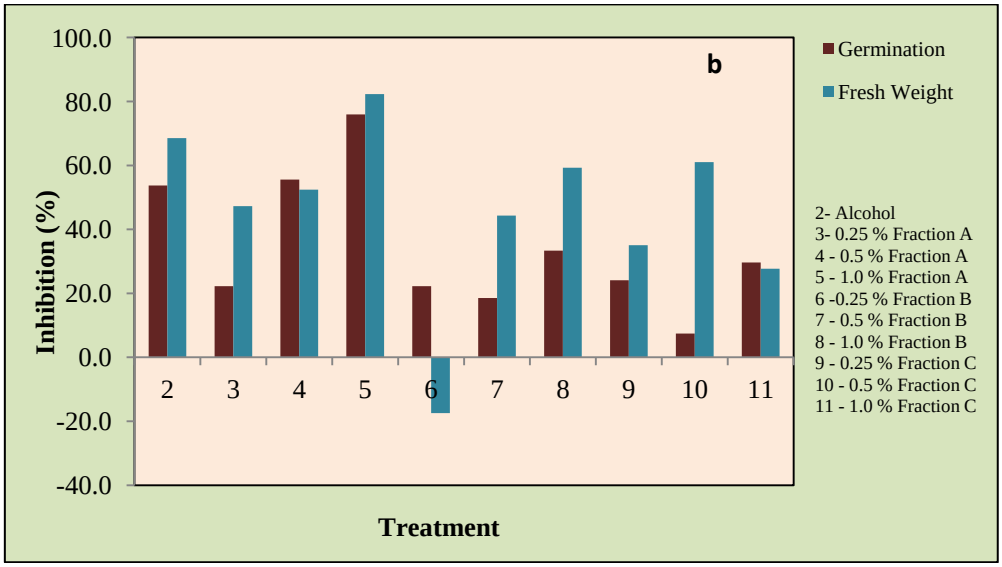


Figure 3.25: Inhibitory effect imposed by Harborne's fractions of *Solanum nigrum* L. leaf **to the growth parameters of *Chloris***





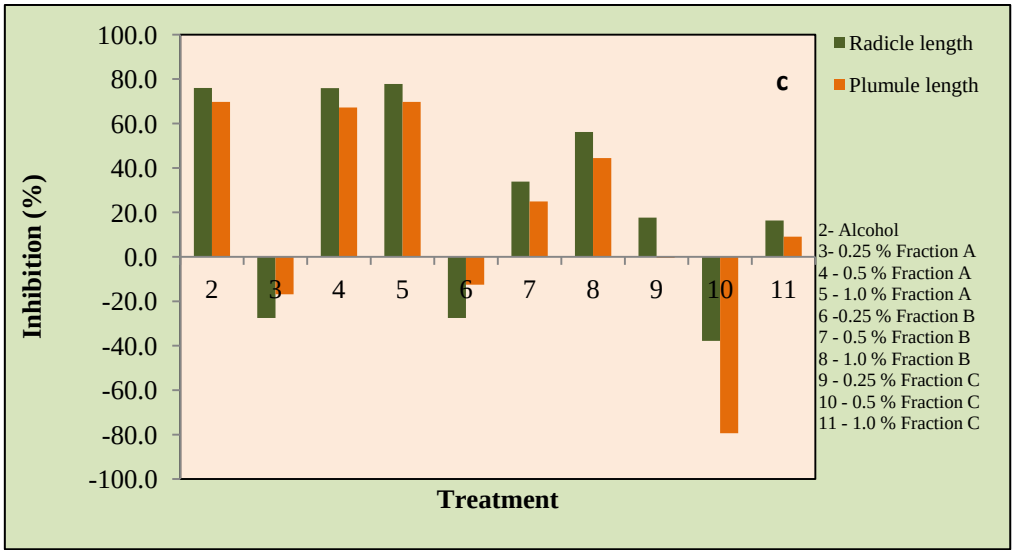
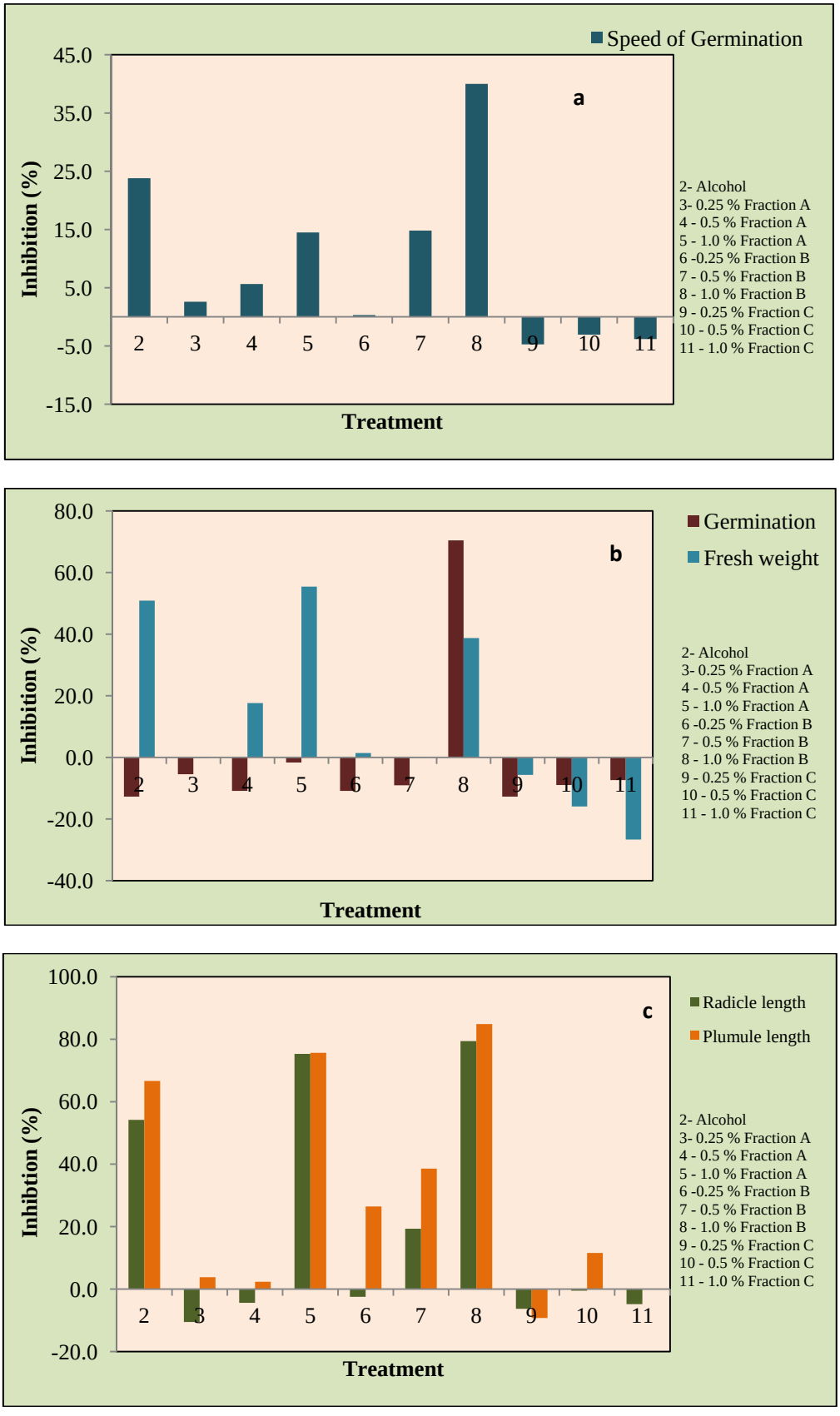


Figure 3.26: Inhibitory effect imposed by Harborne's fractions of *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms stem to the growth parameters of *Chloris*



3.2.2. Phytochemical analysis of the allelopathic fraction: For the planar chromatographic analysis, the phenolics were studied using 2D paper chromatography (Daniel 1991) while the terpenoids and the alkaloids were analysed using thin layer chromatography. However all the three i.e. phenolics, terpenoids and alkaloids were also analysed using HPLC. For the HPLC analysis of phenolics, the chromatograms were analysed referring the given chromatograms by Tumbas et al. (2004); Anjum and Bajwa 2010; Gursoy 2012; Nour et al. (2012); Saleem et al. (2013). HPLC chromatograms for terpenoids were analysed by referring the chromatograms given by Majaz et al. (2011), Kalyani and Laddha (2009), Chua et al. (2015) and that of alkaloids were analysed using the HPLC chromatograms given by Sheludko et al. (1999), Paltinean et al. (2013) and Hisiger and Jolicoeur (2007).

1) *Artemisia annua* L.: Fraction A from Aa was analysed for presence of terpenoids and phenolics using planar chromatographic techniques and HPLC analysis. Harbone's fraction A showed presence of three simple phenols identified as protocatechuic acid, vanillic acid and trans-ferulic acid (Figure 3.27 A1 and A2). Protocatechuic acid gave violet colour in diazotized p- nitra aniline and no color at all in the diazotized sulphanilic acid. vanillic acid gave purple colour in diazotized p-nitra aniline and orange colour in the diazotized sulphanilic acid. trans- ferulic acid gave blue colour in diazotized p- nitra aniline and greenish purple colour in the diazotized sulphanilic acid. For the TLC analysis of Aa, along with fraction A some other extracts from the plants were also analyzed and compared. Usually terpenoid compounds develop pink to purple coloration with the particular spray reagent used. Standard artemisinin developed orange pink color on heating (Figure 3.28 C- lane 1 'e') the Rf of which was 0.55 (Table 3.3). Extract in lane 2, 5, 6 and 7 as can be seen in figure 28 showed similar spot ('e') at the same Rf (Table 3.3), indicating presence

of artemisinin. However in extract lane 2, 5, 6, and 7, additional eight spots at different Rf (Table 3.3) gave pink/purple/ blue color. Extracts 3 and 4 had no pink or purple colored spots.

HPLC chromatogram for phenolics gave eleven peaks with six peaks being the relatively major peaks (Figure 3.30A). Retention time of the peaks, are given in Table 3.5 (A). Depending upon the eluting sequence and considering the relative elution time, the compounds in a sequence starting from 1, may be identified as gallic acid; protocatechuic acid, hydroxybenzoic acid, vanillic acid; chlorogenic acid; caffeic acid; syringic acid; epicatechin; p-coumaric acid; ferulic acid and sinapic acid. The compounds need to be re-identified by running along with the internal standards.

HPLC chromatogram of Harborne's fraction A (Figure 3.31 A) analysed for terpenoids gave three major peaks (1, 3 and 4) appearing at 1.90, 2.94 and 3.23 min and five minor peaks (2, 5, 6, 7, 8) respectively (retention time given in Table 3.6). Major peaks appeared within initial five minutes with additional minor peaks in the later duration of the spectrum. Peak number 8 (Figure 3.31A) having Rt 10.3 minutes, resembles with that of standard artemisinin (Rt: 9.59, Figure 3.47 a) and ethanolic leaf extract from Aa (Figure 3.47 b). Other peaks in Harborne's fraction A resembled to the peak number and pattern in the ethanolic leaf extract chromatograms (Figure 3.47 b). Thus the fraction A from *Artemisia annua* L. shows presence of eleven phenolic compounds atleast two of which are simple phenols and also shows presence of eight metabolites that are terpenoid in nature with one of the metabolite being artemisinin.

2) *Chlorophytum borivilianum* San. & Fern.: Fraction A was analysed for presence of phenolics and terpenoids. The 2D paper chromatogram (Figure 3.27 B) showed presence of only one compound which depending upon the color and relative location

was presumed to be either caffeic acid or cis- sinapic acid. The TLC analysis of fraction A (Figure 3.28 A) resulted into separation of four terpenoid metabolites other than chlorophyll and having different Rf values (Table 3.2).

HPLC analysis of fraction A for phenolics allowed separation of ten metabolites (Figure 3.30 B) with different retention times (Table 3.5), similar to that found in fraction A from Artemisia with the exclusion of the eleven peak seen in the later. The separated phenolics may be gallic acid; protocatechuic acid, hydroxybenzoic acid, vanillic acid; chlorogenic acid; caffeic acid; syringic acid; epicatechin; p-coumaric acid; ferulic acid and sinapic acid. HPLC analysis of fraction A executed with the selected mobile phase system, enabled separation of seven saponin compounds (Figure 3.31 B) having different retention time values (Table 3.6). These may be different borivilianoside ranging from borivilianoside-a to borivilianoside as identified by Joshi et al. (2013). Thus the fraction A from *Chlorophytum borivilianum* San. & Fern. was found to possess ten phenolics and seven terpenoid metabolites that are saponin in nature.

3) *Solanum nigrum* L.: Fraction A was observed to confer the highest phytotoxicity hence was anlaysed for presence of phenolic and terpenoid metabolites. Two simple phenols (Figure 3.27 C) were detected using 2D chromatogram. Based on their colour and relative position the metabolites were identified to be vanillic acid and syringic acid appearing purple and blue (Figure 3.27 C) in the p-nitraniline reagent. The TLC separation of terpenoids present in fraction A, apart from chlorophyll, resulted in to segregation of four Terpenoid metabolites (Figure 3.28 B) having different Rf values (Table 3.2).

HPLC chromatogram of phenolic compound from fraction A showed ten peaks (Figure 3.30C) at different retention time (Table 3.5). The compounds may be gallic acid; protocatechuic acid, hydroxybenzoic acid, vanillic acid; chlorogenic acid; caffeic acid; syringic acid; epicatechin; p-coumaric acid; ferulic acid and sinapic acid. Figure 3.31 C, shows HPLC chromatogram of Terpenoid compound present in fraction A. Twelve metabolites were detected having different retention time (Table 3.6).

Thus the fraction A showed presence of ten phenolics and twelve terpenoid compound.

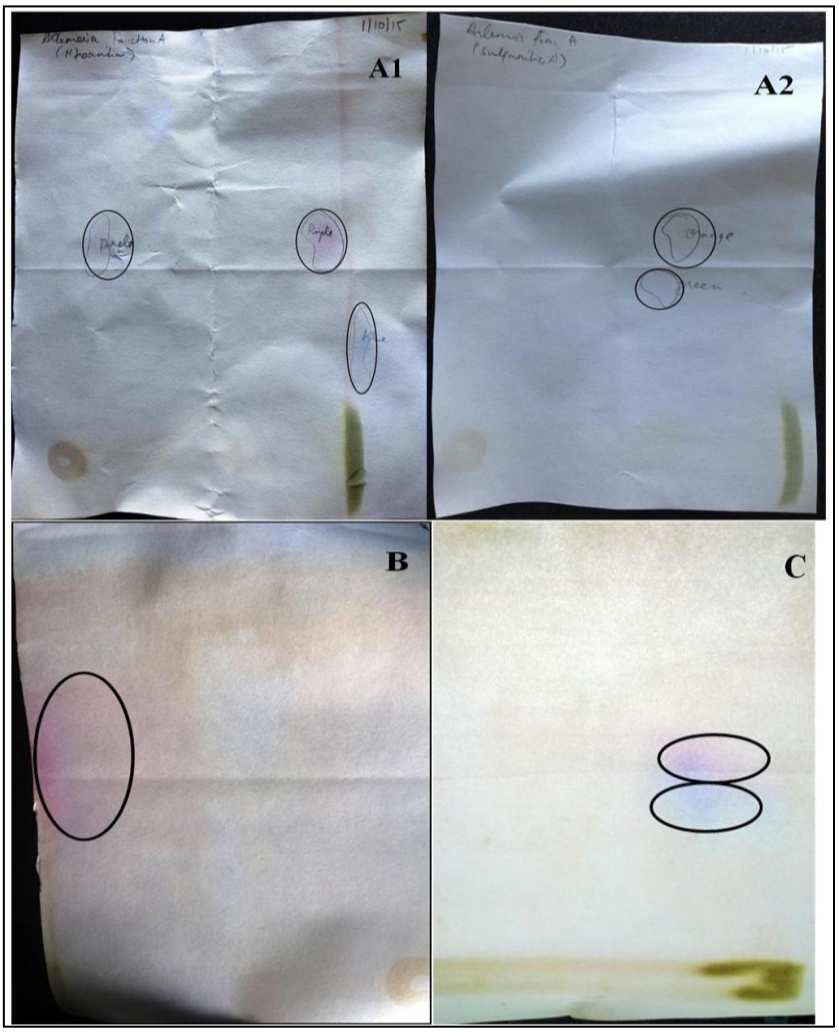
4) *Asparagus racemosus* Willd.: Fraction B from Ar was observed to confer the phytotoxic potential to the plant. Thus the fraction B was subjected to phytochemical analysis and it was analysed for presence of alkaloids. The alkaloidal nature of compound was confirmed by TLC analysis and further extended to the HPLC analysis. Figure 3.29 - (A1 and A2) shows TLC analysis of fraction B. Fraction gave coloration when developed in both the alkaloid specific solvents i.e. primary (Figure 3.29 A1, lane 1 and 2, spot a) and quaternary alkaloids (Figure 3.29 A2, lane 1 and 2, spot a), Rf values for which are given in Table 3.4. HPLC separation of the fraction B gave eleven peaks (Figure 3.32 A) having different retention times (Table 3.7), thus representing presence of atleast eleven metabolites that may be alkaloid in nature.

5) *Enicostemma littorale* (Blume): Fraction B exhibited a very high phytotoxicity and hence the fraction was analysed for alkaloid compound. Figure 3.29 B1 shows fraction B giving orange coloured spots at almost the same Rf (Table 3.4) for the fraction spotted at three different concentrations (Figure 3.29, B1 lane 1, 2, 3-a). This color development was obtained for the TLC plates developed in solvent system

specific to the separation of primary alkaloids. None of the metabolite could be separated out for the TLC plates developed in quaternary alkaloid specific solvent system (Figure 3.29 B2 lane 1 and 2). HPLC analysis of fraction B resulted in to separation of fourteen alkaloid metabolites (Figure 3.32 B) at different retention time (Table 3.7). Fraction B from El may have one to fourteen alkaloids.

6) *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms: Out of the three fractions analysed for their phytotoxicity, fraction B was the most inhibitory fraction. It was analysed for the presence of alkaloids. TLC analysis of the fraction B allowed separation of two metabolites (Table 4), for low (Figure 29 C1, 1) as well as high (Figure 29 C1, 2) concentration spots. The results were same with both solvent systems specific to the primary (Figure 3.29 C1) and to the quaternary alkaloids (Figure 3.29 C2). HPLC analysis of fraction B (Figure 3.32 C) allowed detection of eleven metabolites (retention values given in Table 3.7).Thus fraction B must have atleast two to eleven metabolites that were recognized to have alkaloidal nature.

Figure 3.27: The 2D chromatogram of Harborne's fraction A from medicinal plants



Note: Alphabets represents Harborne's fraction A from Aa (A1, A2), Chlorophytum (B), Solanum (C)

Table 3.2: The Rf values of compounds separated by TLC analysis of Terpenoids

Lane → Spot ↓	**1** (Harborne's Fraction A from Cb, Figure 28 A to be referred)	**1** (Harborne's Fraction A from So.n, Figure 28 B to be referred)
a	0.65	0.7
b	0.5	0.56
c	0.25	0.29
d	0.084	0.19

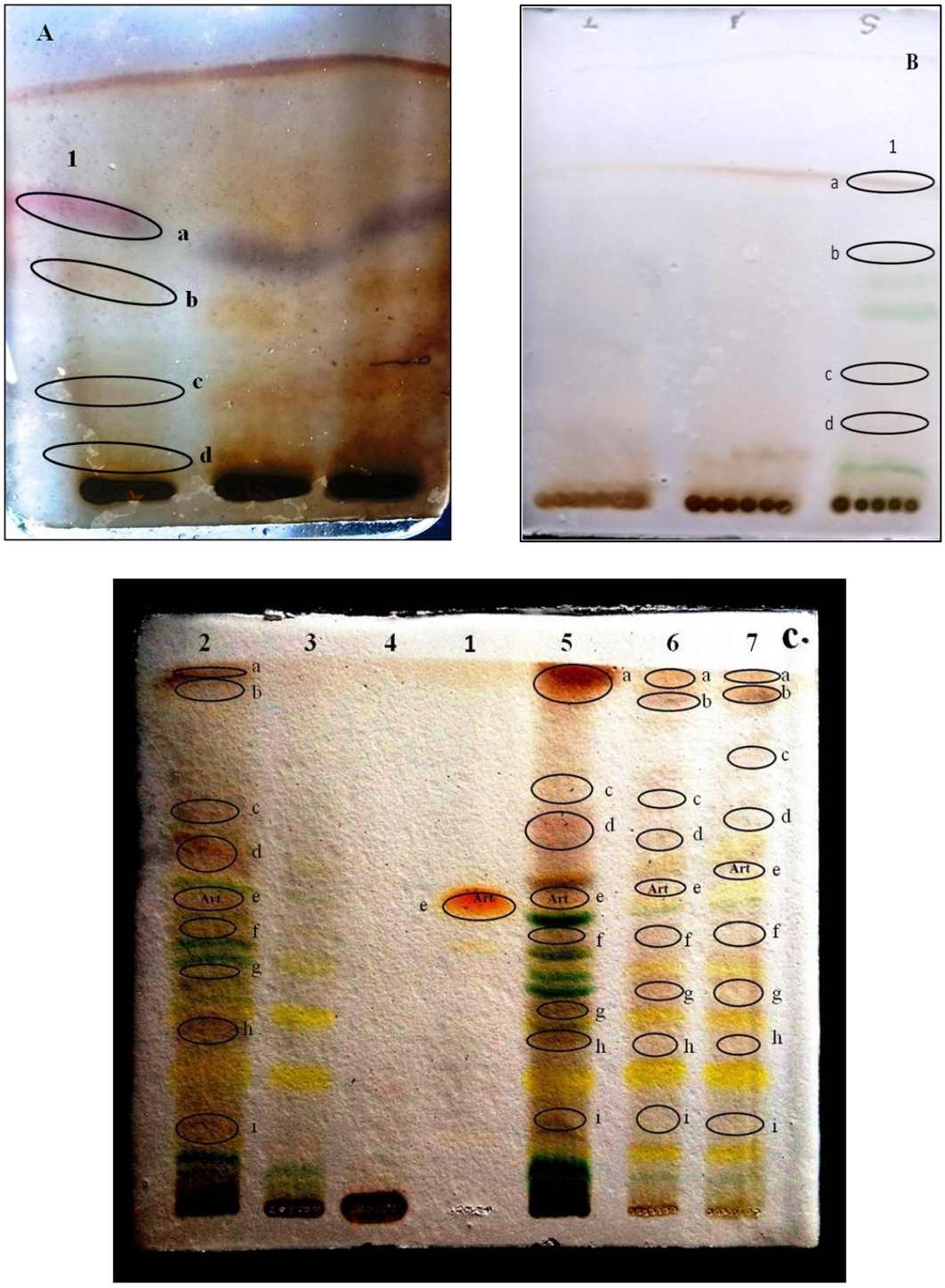
Table 3.3: The Rf values of compounds separated by TLC analysis of Terpenoids

(Figure 28 C to be referred)							
Lane → Spot ↓	**1 (Artemisinin)**	**2 (Fraction A Old extracts)**	**3 (Fraction B)**	**4 (Fraction C)**	**5 (Fraction A new and high conc.)**	**6 (Fraction A new and low conc.)**	**7 (Ethanolic leaf extract)**
a	-	0.98	-	-	0.96	0.98	0.98
b	-	0.95	-	-	-	0.94	0.96
c	-	0.73	-	-	0.77	0.76	0.83
d	-	0.65	-	-	0.68	0.68	0.72
e	0.55	0.56	-	-	0.58	0.59	0.63
f	-	0.52	-	-	0.5	0.50	0.51
g	-	0.44	-	-	0.37	0.41	0.40
h	-	0.33	-	-	0.32	0.31	0.31
i	-	0.15	-	-	0.18	0.17	0.16

Table 3.4: The Rf values of compounds separated by TLC analysis of Alkaloids

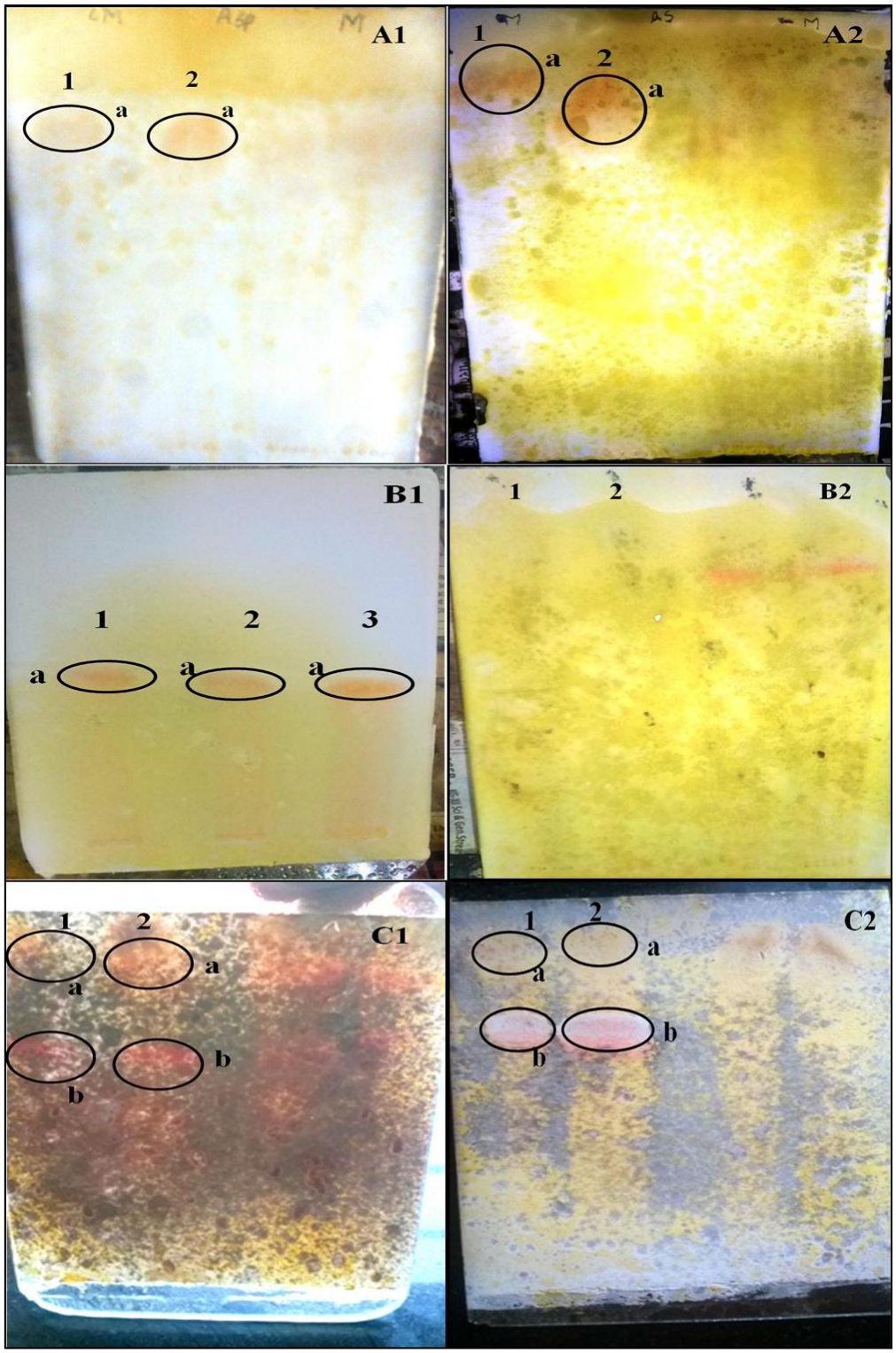
Figure 29 to be referred													
	A1		**A2**		**B1**			**B2**		**C1**		**C2**	
Lane → Spot ↓	**1**	**2**	**1**	**2**	**1**	**2**	**3**	**1**	**2**	**1**	**2**	**1**	**2**
a	0.89	0.87	0.88	0.80	0.47	0.44	0.43	-	-	0.90	0.87	0.96	0.97
b	-	-	-	-	-	-	-	-	-	0.59	0.57	0.73	0.72

Figure 3.28: TLC chromatograms of terpenoid metabolites



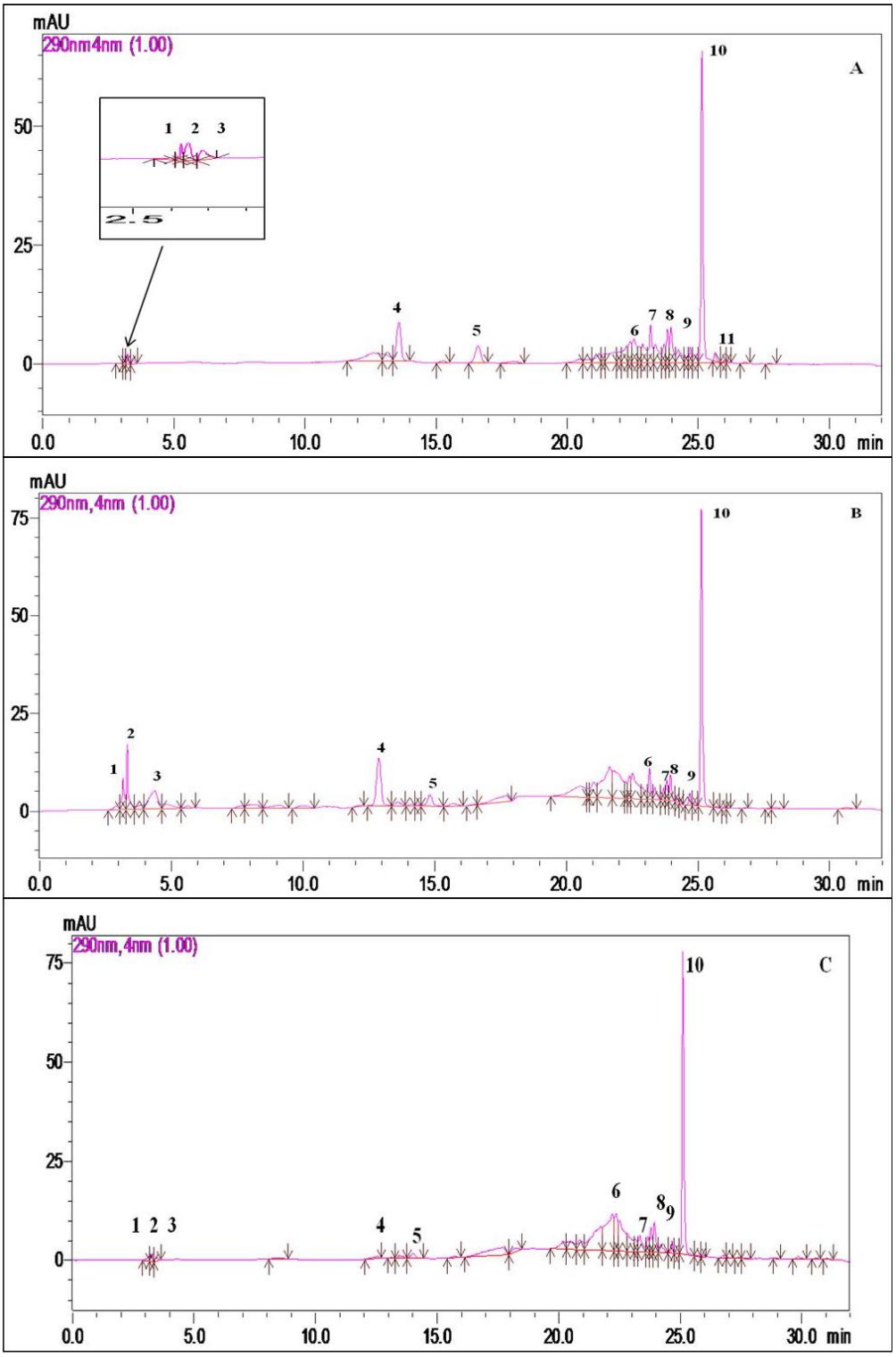
Note: Alphabets represents Harborne's fraction A from Cb (A), So.n (B), Aa (C)

Figure 3.29: TLC chromatograms of alkaloid metabolites



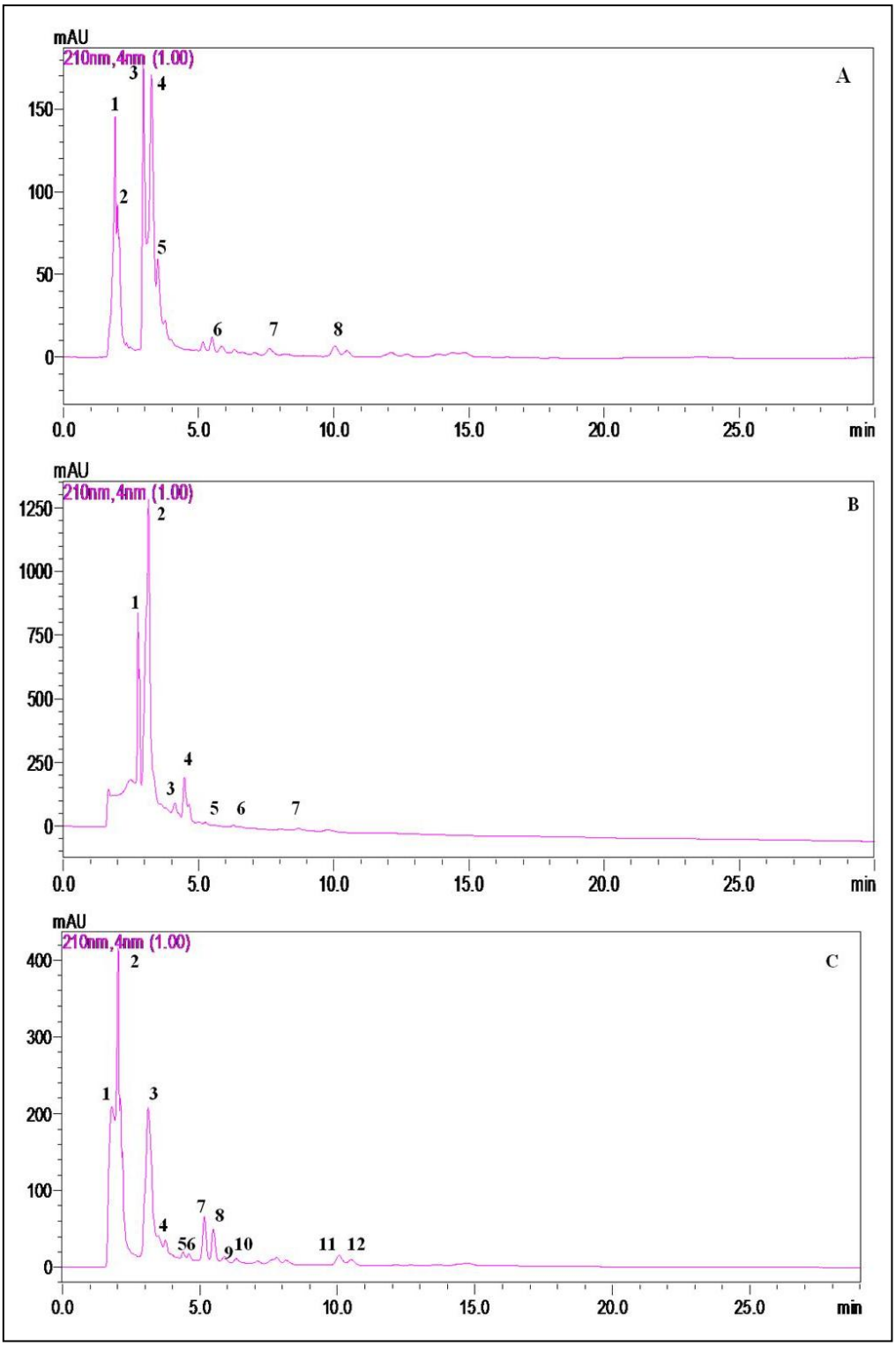
Note: Alphabets represents Harborne's fraction A from Ar (A), El (B), Tc (C)

Figure 3.30: HPLC chromatograms of phenolic compounds



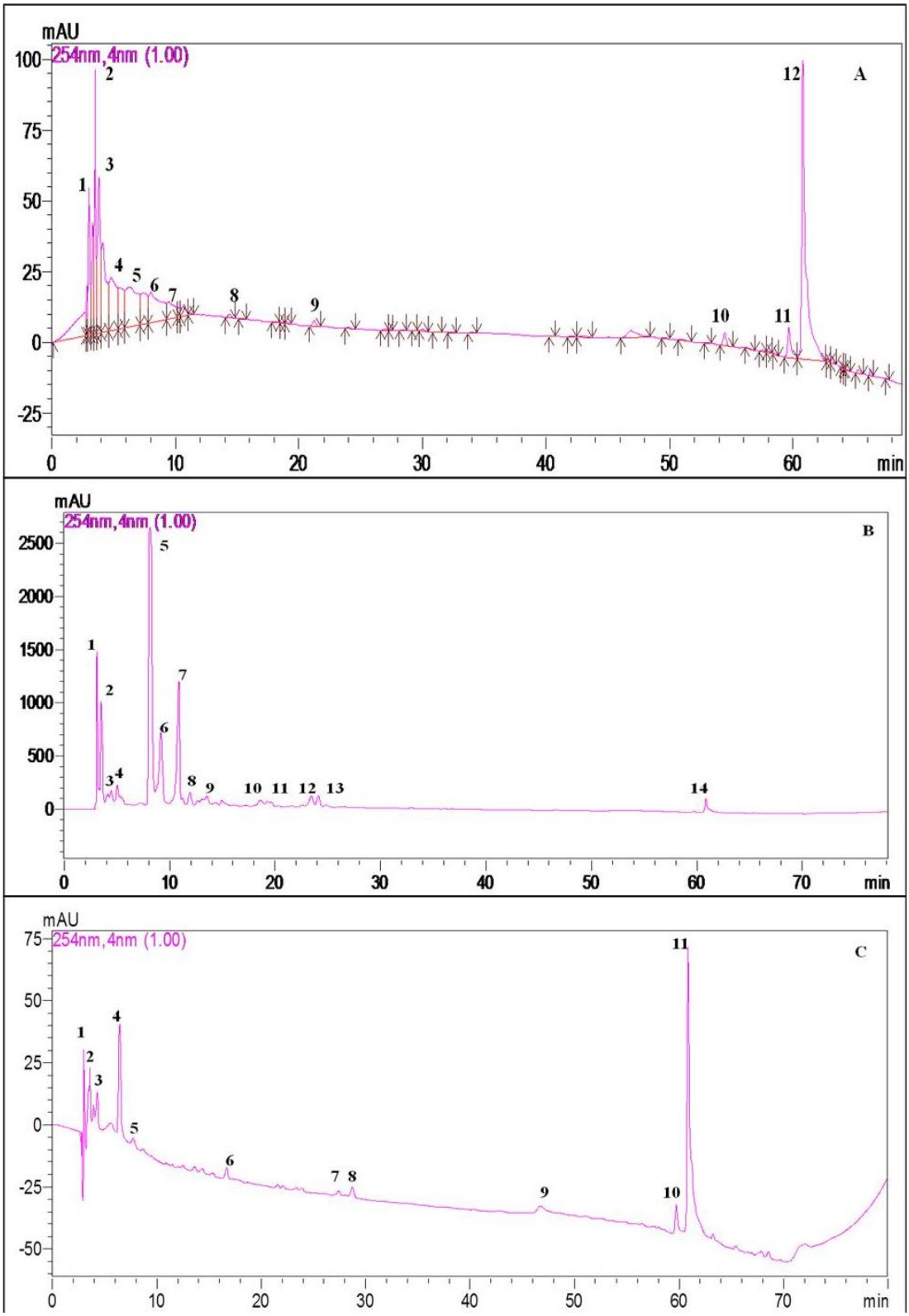
Note: Alphabets represents Harborne's fraction A, from Aa (A), Cb (B), So.n (C)

Figure 3.31: HPLC chromatograms of terpenoid compounds



Note: Alphabets represents Harborne's fraction A, from Aa (A), Cb (B), So.n (C)

Figure 3.32: HPLC chromatograms of alkaloid compounds



Note: Alphabets represents Harborne's fraction B, from Ar (A), El (B), Tc (C)

Table 3.5: Phenolic metabolites observed in the HPLC chromatograms

	Retention time (Minutes)		
Chromatogram → / **Metabolite ↓**	**A** (Aa)	**B** (Cb)	**C** (So.n)
1	2.84	3.15	3.11
2	3.22	3.32	3.21
3	3.41	4.36	3.43
4	13.58	12.87	14.00
5	16.59	14.81	22.19
6	22.86	23.14	22.50
7	23.17	23.80	23.66
8	23.35	24.29	23.93
9	23.81	24.29	24.65
10	25.12	25.11	25.10
11	25.83	-	-

Table 3.6: Terpenoid metabolites observed in the HPLC chromatograms

	Retention time (Minutes)		
Chromatogram → / **Metabolite ↓**	**A** (Aa)	**B** (Cb)	**C** (So.n)
1	1.90	2.48	1.7
2	1.99	3.14	2.02
3	2.94	4.11	3.11
4	3.23	4.47	3.74
5	3.47	5.25	4.38
6	5.47	6.27	4.59
7	7.60	8.68	5.15
8	10.3	-	5.48
9	-	-	5.88
10	-	-	6.31
11	-	-	10.049
12	-	-	10.059

Table 3.7: Alkaloid metabolites observed in the HPLC chromatograms

	Retention time (Minutes)		
Chromatogram → / **Metabolite ↓**	**A** (Ar)	**B** (El)	**C** (Tc)
1	3.28	3.086	2.82
2	3.49	3.47	3.45
3	4.09	4.42	4.27
4	4.75	5.01	6.44
5	6.35	8.13	7.71
6	7.42	9.15	16.67
7	9.50	10.85	27.39
8	14.48	11.91	28.71
9	21.29	13.52	46.72
10	55.17	18.57	59.73
11	59.64	19.62	60.86
12	60.77	23.43	-
13	-	24.09	-
14	-	60.85	-

3.3. Analysis of biochemical, physiological and cytotoxic effects*:* *Artemisia annua* L. (Aa) and *Enicostemma littorale* (Blume) (El) were the most allelopathic medicinal plants with fraction A and fraction B being the potential allelopathic chemical fractions from the plants respectively, these plant fractions were analysed for their possible mechanism of action on Chloris. Cytotoxic potential of these chemical fraction were analysed on *Allium cepa* L.

3.3.1. Lipid peroxidation: Lipid peroxidation was evaluated by measuring MDA content (µM/ g^{-1} FW) in the treated and non treated Chloris seedlings (Table 3.8). In the control seedlings, MDA content was 9.97 and in the seedlings treated with alcohol it was 17.98 which was two times higher than the control. The El treatment imparted to Chloris seedlings resulted in increase of MDA content when compared to the control wherein it was lower than the alcohol treatment. The Lipid peroxidation in Chloris was affected by El treatments in a concentration dependent manner where in the MDA content of Chloris seedling was 12.0, 12.12 and 14.88 respectively. Treatment with fraction A from Aa had no effect on the lipid peroxidation in Chloris.

Table 3.8: Effect of Harborne's fraction treatment on MDA level in *Chloris* seedling

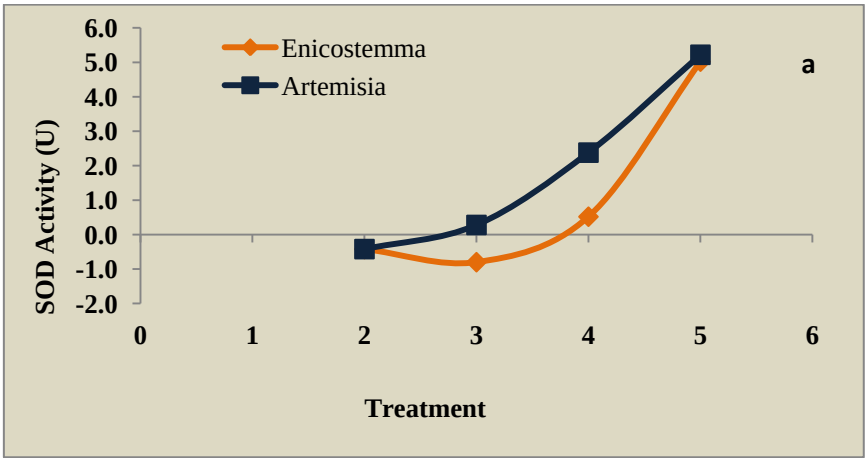
Treatment Code	Treatment	MDA (µM/ g^{-1} FW)
1	Control	9.97 ± 8.3
2	Alcohol	17.98 ± 2.16
3	El FB (0.125 %)	12.0 ± 2.06
4	El FB (0.25 %)	12.12 ± 0.11
5	El FB (0.5 %)	14.88 ± 2.70
6	Aa FA (0.125%)	2.0 ± 0.0
7	Aa FA (0.25 %)	2.03 ± 0.0
8	Aa FA (0.5 %)	2.016 ± 0.0

3.3.2. Antioxidant enzymes assays:

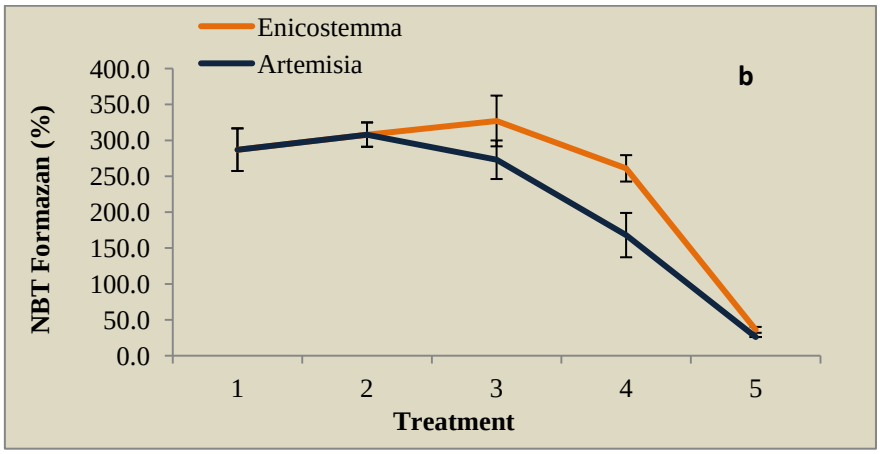
3.3.2.1. Super oxide dismutase: SOD activity showed distinct response to the chemical fractions from both the phytotoxic medicinal plant (El and Aa). The NBT

Formazan concentration and SOD activity with reference to the NBT- SOD assay are inversely related that is the NBT concentration decreases when the SOD activity increases and NBT concentration increases when the SOD activity decrease (Figure 3.33 a and b). The effect of fractions from both the plants was concentration dependant. Treatment imparted by Fraction B from El increased the SOD activity gradually with the highest value observed in treatment 5 (applied rate: 0.5 %) and the increase in SOD activity was by 5 units as compared to the control and alcohol (Figure 3.33a). SOD activity was found to increase in Chloris seedlings owing to treatment with fraction A from Aa. The SOD activity was 0.3, 2.4 and 5.2 U in the seedlings treated with 0.125, 0.25, 0.5 % of fraction A from Aa.

Figure 3.33: Effect of the Harborne's fraction treatments on Super Oxide Dismutase activity in *Chloris* seedling



Note: 2- Alcohol, 3, 4, 5- Harborne's fraction (0.125, 0.25, 0.5 %)



Note: 1- Control, 2- Alcohol, 3, 4, 5 - Harborne's fraction (0.125, 0.25, 0.5 %)

3.3.2.2. Catalase: Chloris seedlings treated with the fraction A from Aa showed higher Catalase activity as compared to the seedlings in control (Table 3.9). The CAT activity in control was 6.58 and in the treatment with Aa fraction A for the applied rates (0.125, 0.25, 0.5 %) were 7.50, 7.90 and 8.13 respectively. Thus the increase in Catalase activity was concentration dependant for Aa treatments. Catalase activity was not found to increase owing to treatment with fraction B from El (Table 3.9).

3.3.2.3. Peroxidase: Treatment with fractions from El and Aa had no considerable effect on the peroxidise activity in Chloris seedlings and the values were not found to increase owing to fraction treatments (Table 3.9).

Table 3.9: Effect of Harborne's fraction treatments on Catalase and Peroxidase activities in *Chloris* seedling

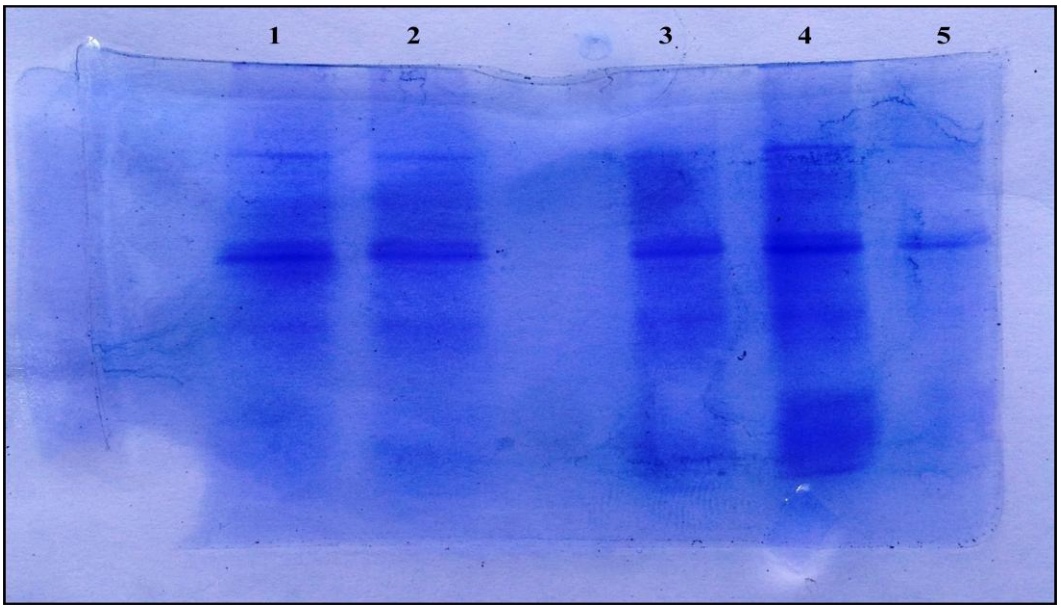
Sr. No.		**Treatment**	**CAT** U/ mg Protein	**POD** U/ mg Protein
1	**Control**	Water	6.58 ± 0.3	0.197 ± 0.08
2	**Solvent ethanol**	Ethanol	7.93 ± 0.6	0.097 ± 0.04
3	***El***	0.125 % Fraction B	5.01 ± 0.1	0.190 ± 0.2
4		0.25 % Fraction B	5.25 ± 0.4	0.070 ± 0.0
5		0.5 % Fraction B	5.20 ± 0.8	0.140 ± 0.0
6	***Aa***	0.125 % Fraction A	7.50 ± 0.2	0.077 ± 0.0
7		0.25 % Fraction A	7.90 ± 0.1	0.000 ± 0.0
8		0.5 % Fraction A	8.13 ± 1.0	0.000 ± 0.0

3.3.3. Protein analysis: Protein isolated from non-treated (control and solvent ethanol) and treated (Harborne's fraction from El and Aa) seedlings were analysed using SDS PAGE. Chloris seedlings showed varied response to medicinal plant fraction treatments. Same amount of isolated protein was loaded for each treatment. Band pattern in protein isolated from control and alcohol treated seedlings was similar. Equal or more number of protein bands were observed for Aa fraction treatment at the applied rates when compared to the control and alcohol treated seedlings (Figure 3.34). Number of bands, were more in treatment with 0.25% where as number of bands were less in treatment with 0.5 % of fraction A from Aa. In

the Chloris seedlings treated with fraction B from El, the number of the bands were highest for the protein isolated from fraction B applied at the rate of 0.125 %. Band pattern appeared similar for the protein isolated from seedlings in control and alcohol and those treated with 0.25 and 0.5 % fraction B from El.

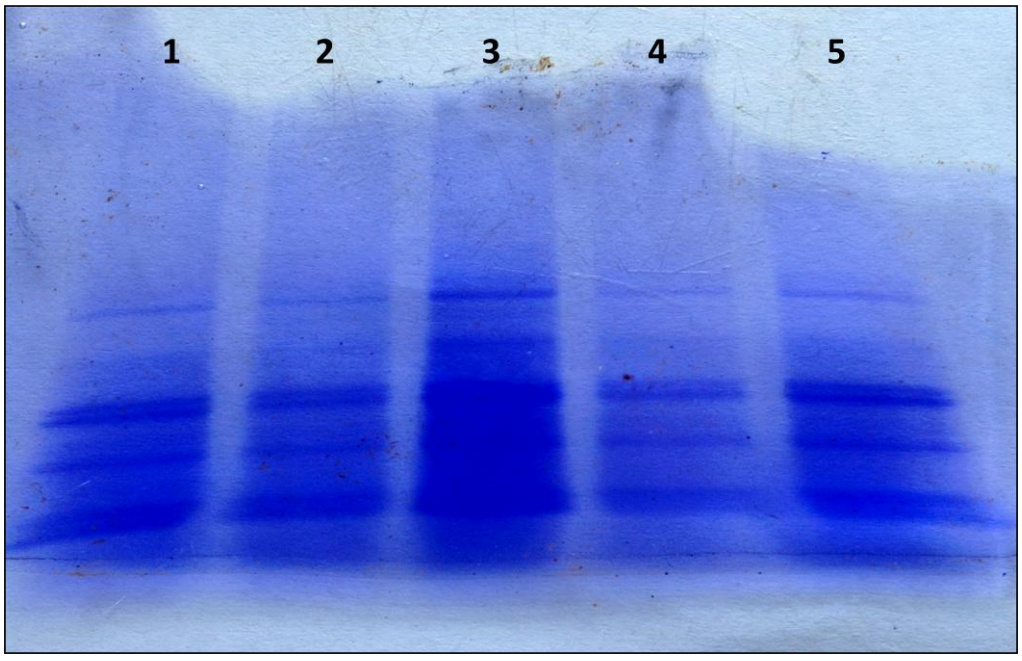
3.3.4. Analysis of RNA: Upon Gel electrophoresis of Chloris RNA samples**,** two bands: one band for 28 S RNA and the other of 18 S RNA were observed (Figure 3.36). The absorbance ratio of the RNA samples at 260 nm and 280 nm (wavelength) and concentration of RNA in the respective sample preparation are given in Table 3.10. As can be seen from Figure 3.36, in lane 1 a very low and equal intensity bands are visible for both 28 S and 18 S RNA. The absorbance ratio A260/280 obtained for the same was 2.05, which indicates no contamination by polysaccharides. RNA yield for the control sample was 3520 (µg/ml). Lane 2 shows RNA sample isolated from seedlings of the alcohol treatment (Figure 3.36). As can be seen, sharp bands of almost equal intensities are visible for the same with the A260/280 ratio of 1.72 and RNA yield of 4680 (µg/ml). For the RNA samples isolated from El treatments applied at the rates of 0.125, 0.25 and 0.5 % (Figure 3.36 lane 3, 4, 5) the two distinct bands were observed only for lowest and highest concentration treatments. The A260/280 ratio for the same, were 2.42, 0.24 and 1.82 while their RNA yield was 3200, 880 and 8440 (µg/ml) respectively. Similar to the EL treatments, for the RNA samples pooled form Aa treatment applied at the rate of 0.125, 0.25 and 0.5 % (Figure 3.36 lane 6, 7, 8) two distinct bands were observed for lowest and highest concentration treatments. The A260/280 ratio for the same were 1.92, 1.61, 1.66 and the RNA yield was 5760, 10880 and 7880 (µg/ml) respectively.

Figure 3.34: Comparison of protein in non treated and *Artemisia annua* L. treated samples using PAGE



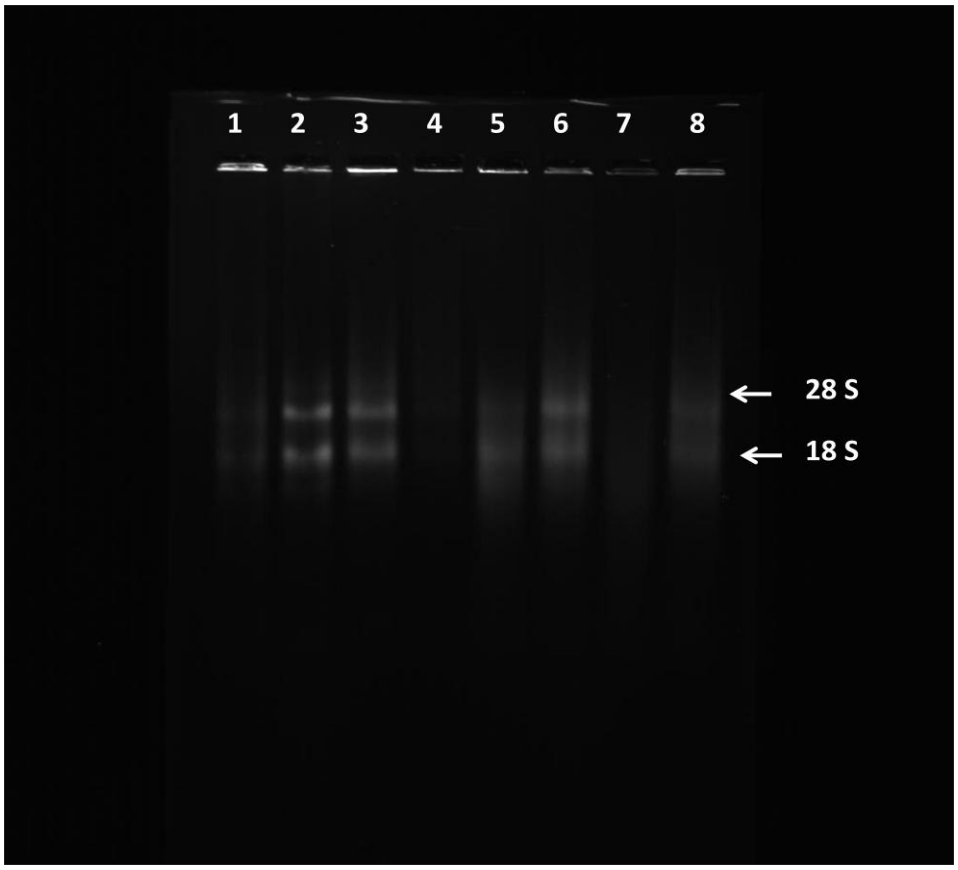
Note: Lane numbers represents protein sample viz. 1-control; 2-solvent ethanol; 3, 4, 5 - 0.125, 0.25, 0.5 % of Harborne's fraction A respectively)

Figure 3.35: Comparison of protein in non treated and *Enicostemma littorale* (Blume) treated samples using PAGE



Note: Lane numbers represents protein sample viz.: 1-control; 2-solvent ethanol; 3, 4, 5 - 0.125, 0.25, 0.5 % of Harborne's fraction B respectively

Figure 3.36: Effect of Harborne's fraction treatments from *Enicostemma littorale* (Blume) and *Artemisia annua* L. on the RNA content in *Chloris* seedling



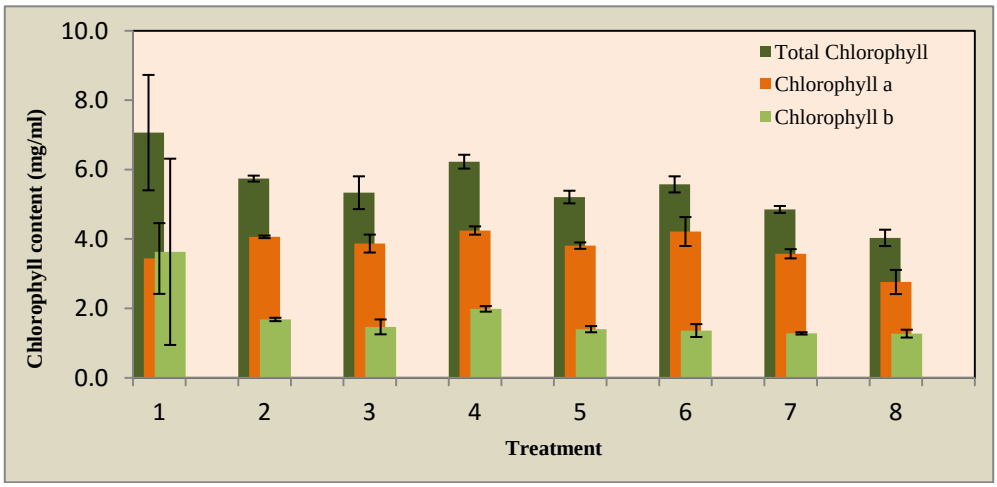
Note: Lane numbers represent RNA sample from treated and not treated *Chloris* seedlings, 1- control, 2-solvent ethanol; 3, 4, 5 - 0.125, 0.25, 0.5 % of Harborne's fraction B from *Enicostemma*, 6,7,8 - 0.125, 0.25, 0.5 % of Harborne's fraction A from *Artemisia*

Table 3.10: RNA yield and quality in the samples isolated from Chloris seedlings treated with Harborne's fraction from *Enicostemma littorale* (Blume) and *Artemisia annua* L.

Treatment Code	RNA Samples	RNA quality (A 260/ A280)	RNA Yield (µg/ml)
1	**Control**	**2.05**	3520
2	Alcohol	1.72	4680
3	El FB (0.125 %)	2.42	3200
4	El FB (0.25 %)	0.24	880
5	**El FB (0.5 %)**	**1.82**	8440
6	**Aa FA (0.125%)**	**1.92**	5760
7	Aa FA (0.25 %)	1.61	10880
8	Aa FA (0.5 %)	1.66	7880

3.3.5. Analysis of Chlorophyll content: Chlorophyll content in the Chloris leaf blades was analysed as a parameter representing the seedling health. The amount of total Chlorophyll, Chlorophyll a and b were highest in the seedlings in the control and the values for the same were 7.1, 3.4 and 3.6 mg/ml respectively. Chlorophyll content was observed to decrease in response to the treatments provided in form of fractions from both the medicinal plants i.e. El and Aa (Figure 3.37). In the Chloris seedlings treated with El fraction B (applied at the rates of 0.125, 0.25 and 0.5 %) the total chlorophyll content was 5.3, 6.2 and 5.2 mg/ml, amount of Chlorophyll a was 3.9, 4.2 and 3.8 mg/ml and Chlorophyll b was 1.5, 2.0 and 1.4 mg/ml respectively. Chlorophyll content in the seedlings treated with fraction A from Aa applied at the rates of 0.125, 0.25 and 0.5 %, was highly reduced as compared to that in the control seedlings. The total Chlorophyll content of Aa treated seedlings was 5.6, 4.9 and 4.0, amount of Chlorophyll a and Chlorophyll b in the same were 4.2, 3.6, 2.8 mg/ml and 1.4, 1.3 and 1.3 mg/ml respectively.

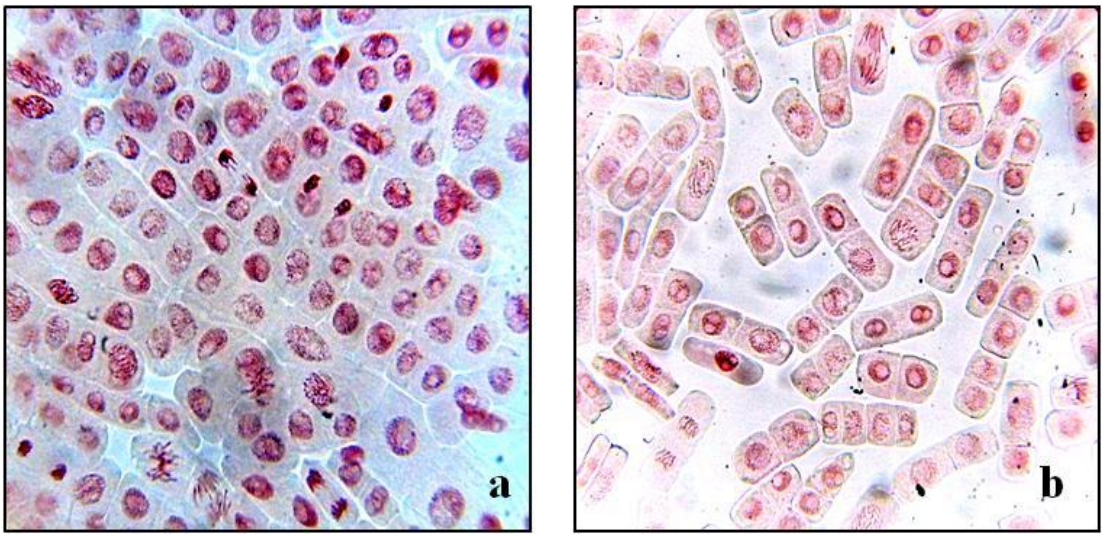
Figure 3.37: Effect of Harborne's fraction from *Enicostemma littorale* (Blume) and *Artemisia annua* L. on Chlorophyll content in *Chloris*

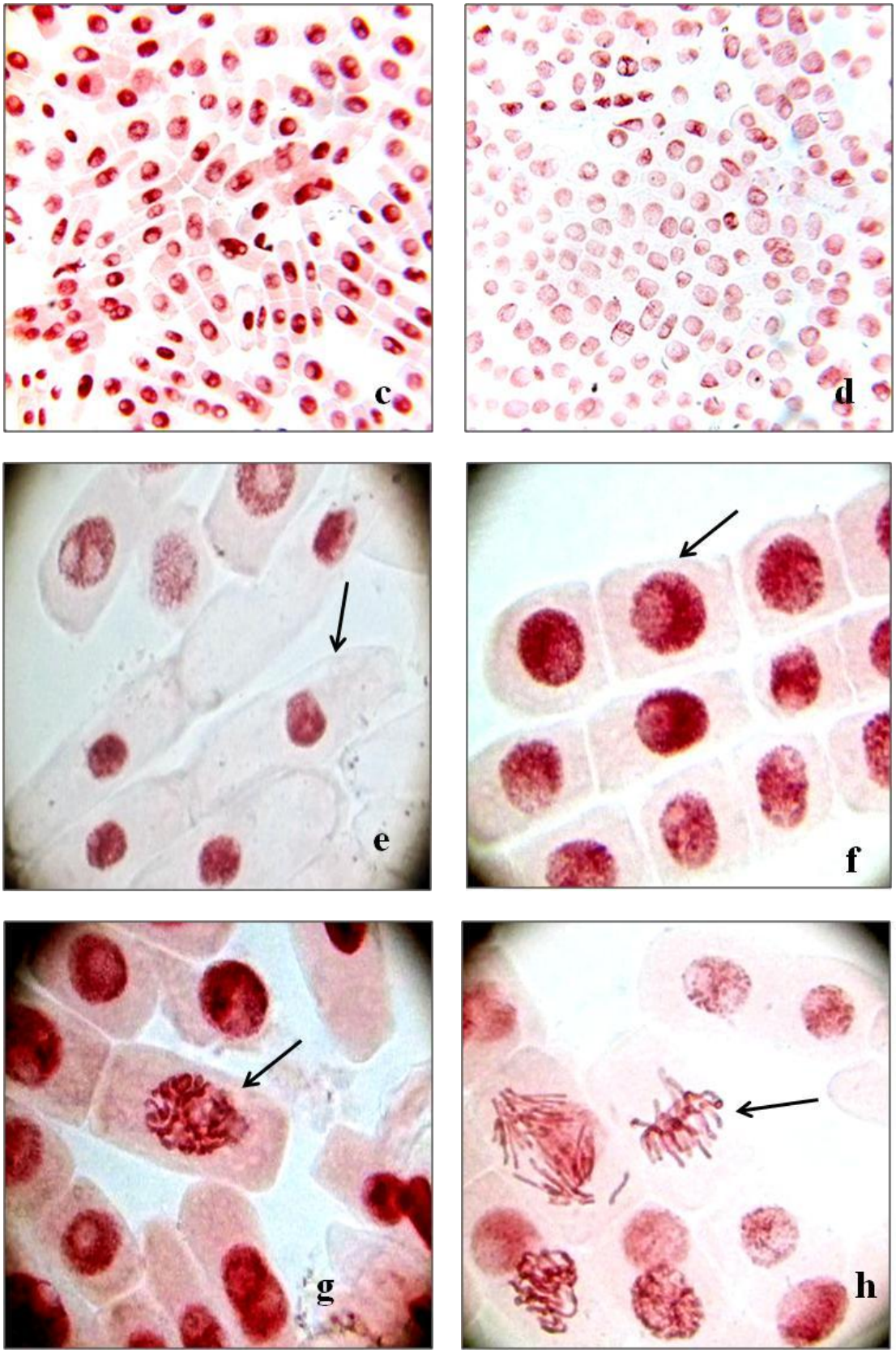


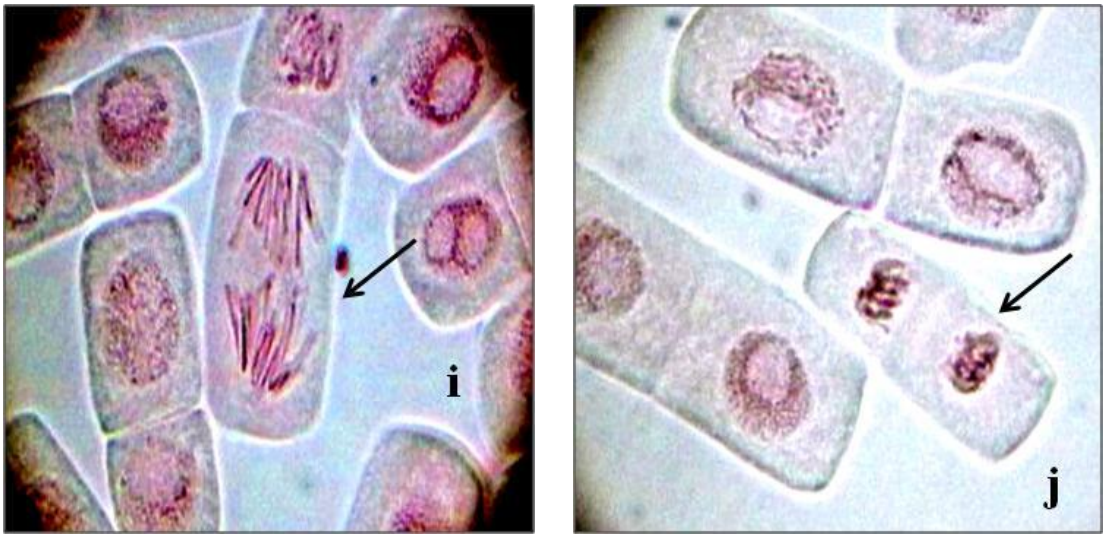
Note: 1- Control, 2- Alcohol, 3, 4, 5 - El FB (0.125, 0.25, 0.5 %), 6, 7, 8 - Aa FA (0.125, 0.25, 0.5 %)

3.3.6. Cytotoxicity analysis: Cytotoxicity of the Harborne's fractions were analysed using *Allium cepa* test. Figure 3.38 shows the onion root cell plates. Figure 3.38 a and b, shows the cell plates from control and alcohol treated cell. Figure 3.38 c and d shows the onion cells treated with El fraction B and Aa fraction A respectively. Figure 3.38 e, f, g, h, i and j shows non dividing and dividing cells that were found in different stages of cell division viz. nterphase, Prophase, Metaphase, Anaphase and Telophase. Treatment with medicinal plant fractions had high inhibitory effect on the Onion root cell division and the mitotic index was found to reduce owing to the treatment toxicity.

Figure 3.38: Mitotic cell plates illustrating non-treated and plant extract treated onion root cells



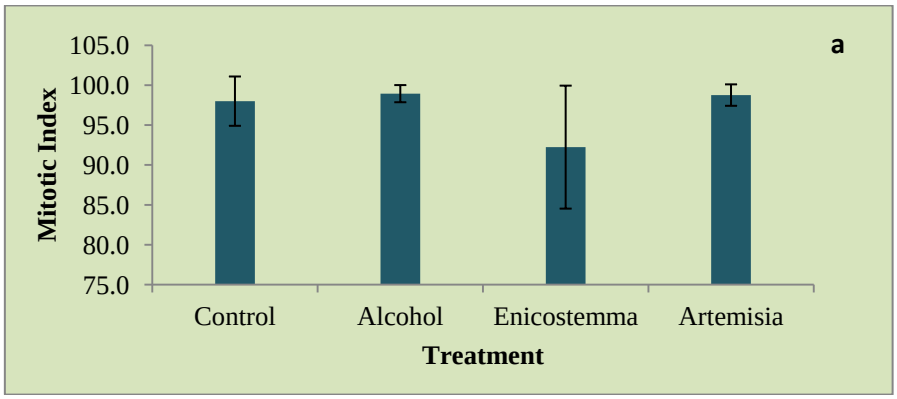

c
d
e
f
g
h



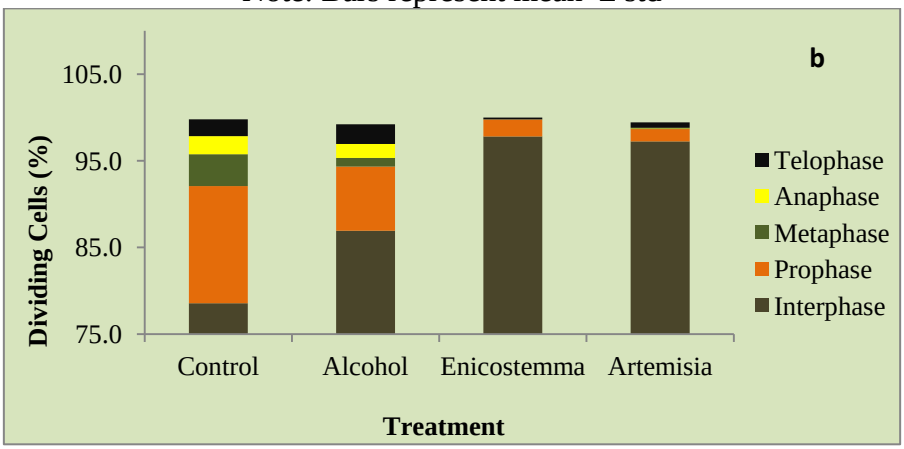
Note: Onion root cells in: a) Control, b) Alcohol treatment, c) El treatment, d) Aa treatment, e) non dividing cell,
f) Interphase, g) Prophase, h) Metaphase, i) Anaphase, J) Telophase

Mitotic index (MI) in the control was 98.0 %, in the alcohol treatment was 98.9 %, in treatment with El the MI was 92.2 % (Figure 3.39 a) and in the Aa treatment MI was 98.8 % (Figure 3.39 a). The cell in all phases of cell divisions were observed for onion roots in the control, wherein out of the total dividing cells, 78.6 % cell were in Interphase, 13.5 % in Prophase, 3.7 % in Metaphase, 2.1 % in Anaphase and 1.9 % in Telophase (Figure 3.39 b). Similarly in the alcohol treatment, out of the dividing cells 86.9 % cells were in Interphase, 7.4 % in Prophase, 1 % in Metaphase, 1.6 % in Telophase and 2.3 % of cells were in Anaphase (Figure 3.39 b). In the onion root cells treated with the El fraction B, 97.8 % of the dividing cells were in Interphase, 2 % cells were in Prophase and 0.2 % cells were in Telophase, however no cells were observed in Metaphase and Anaphase (Figure 3.39 b). In case of onion root cells treated with Aa fraction A, out of the dividing cells, 97.2 % cells were in Interphase, 1.4 % cells were in Prophase, 0.1 % cells in each Metaphase and Anaphase and 0.6 % cells were in the Telophase (Figure 3.39 b).

Figure 3.39: Mitotic index in Onion root tip cells treated with Harborne's fraction from *Enicostemma littorale* (Blume) and *Artemisia annua* L.



Note: Bars represent mean ± std



Note: Bar height represents total dividing cells and sections in each bar shows percentage of cells in different phases of cell divisions

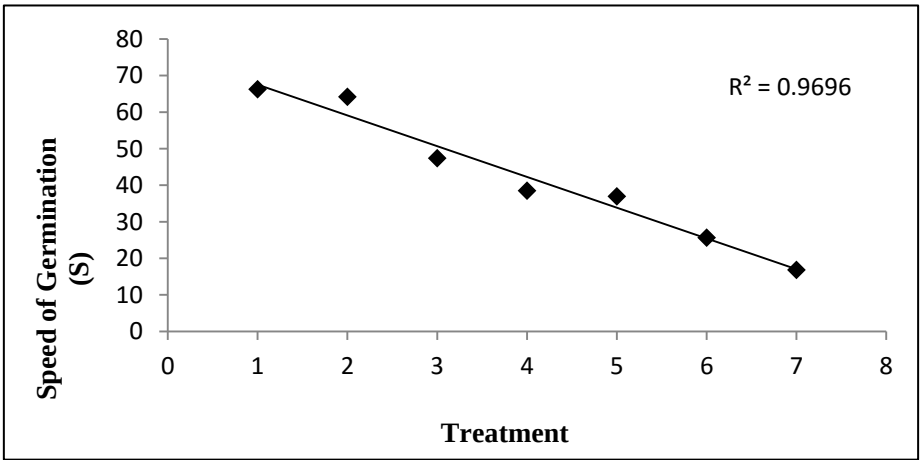
3.4. *Artemisia annua* L., artemisinin & plant metabolite absorbance by *Chloris*:

3.4.1. Agar based bioassays for *Artemisia annua* L. related treatments:

3.4.1.1. Allelopathic effect of ethanolic extract from *Artemisia annua* L. leaf: Regression analysis, with allelopathic treatments in addition to control taken as independent variable and the speed of germination taken as the dependent variable gave R^2 value of 0.969 and showed best fit relationship between the two to be linear (Figure 3.40). Germination (%) in control was observed to be 93.0 % and in solvent ethanol was 100.0 %. Hence comparative phytotoxicity of the ethanolic leaf extract treatments were performed with both, the control and the solvent ethanol separately. Seed germination in leaf extract applied at the rate of 10 mg/ml was 54.9 %. Figure

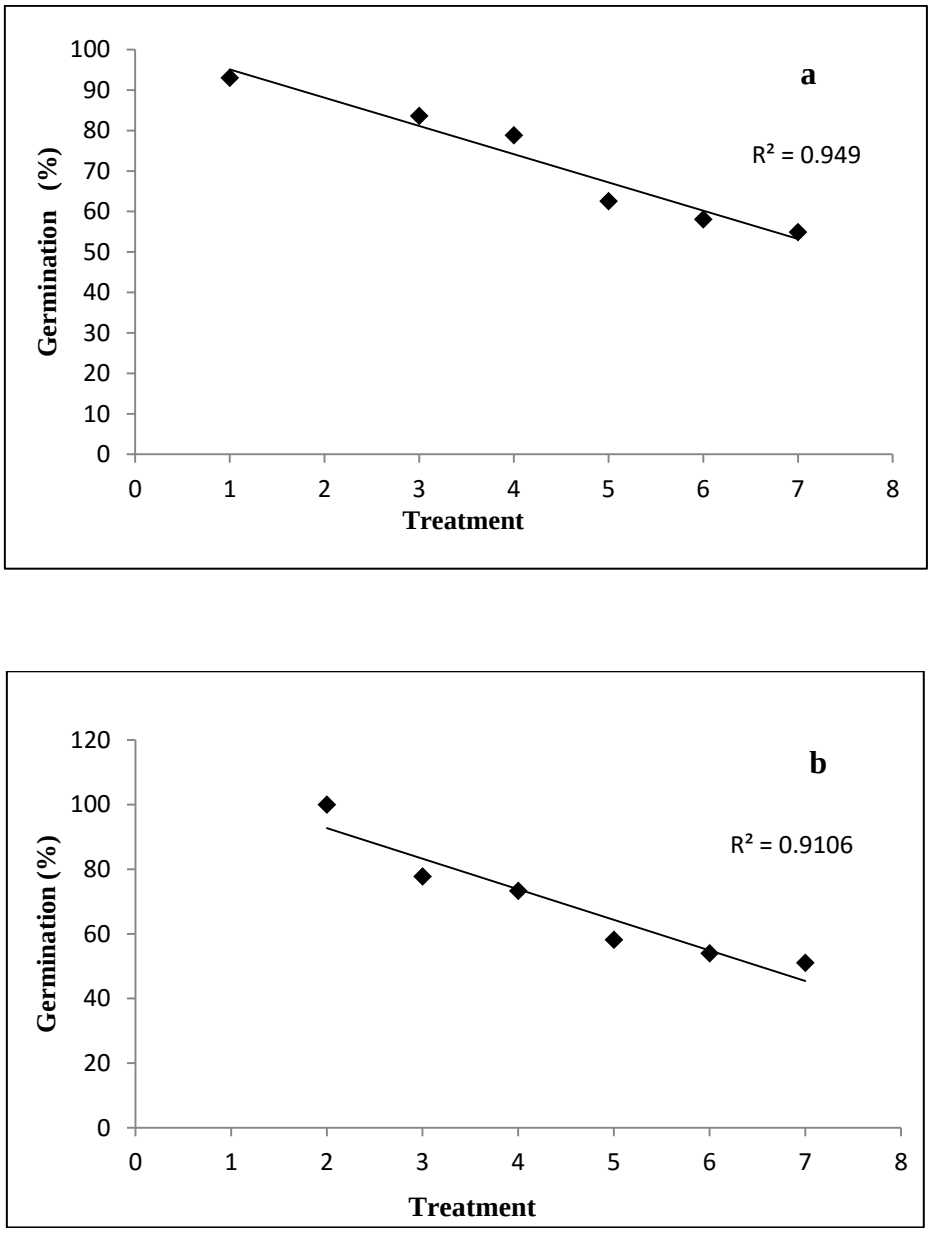
3.43 shows comparison of percentage germination, where in Figure 3.41 (a) show, control compared with treatments 3, 4, 5, 6, 7 and Figure 3.41 (b) shows solvent ethanol treatment compared with treatment 3, 4, 5, 6, 7. Regression analysis projects relation between the treatment and total germination to be linear with R^2 values to be 0.949 and 0.910 for a) and b) comparisons respectively. Results of ANOVA and post hoc test show means of treatments 3, 4, 5, 6, 7 (ethanolic leaf extract) to significantly differ ($P < 0.05$) from the treatment 1 (control) and 2 (solvent ethanol). Inhibition of radicle length was seen in treatment 3, 4, 5, 6 and 7 (Figure 3.42) as compared to that in treatments 1 and 2. As radicle length was more in treatment 2 as compared to treatment 1, the results of the performed comparisons are presented separately with each of the treatment 1 and 2. Figure 3.42 (a) show, treatment 1 compared with treatments 3, 4, 5, 6, 7 and Figure 3.42 (b) shows treatment 2 compared with treatment 3, 4, 5, 6, 7. Regression analysis was performed with treatments and the radicle length. The best-fit relation was found to be exponential with R2 value as 0.971 and 0.918. Least growth was observed in treatment 7 (10 mg/ml).

Figure 3.40: Effect of ethanolic leaf extracts on germination speed in agar grown *Chloris*



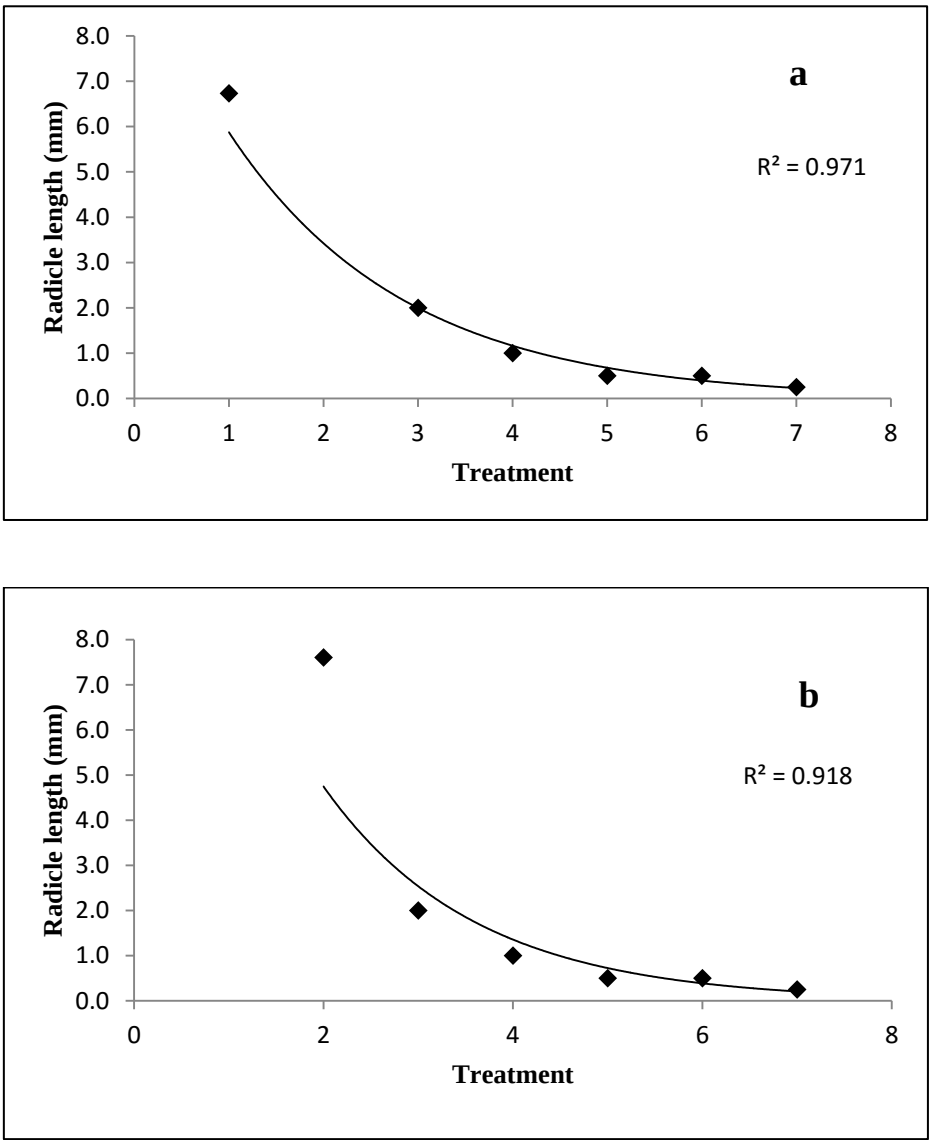
Note: Numrals stands for, 1-**contro**l, 2-**ethanol**, 3- **1.25**, 4) **2.5**, 5) **5.0** 6) **7.5,** 7)**10.0** – leaf extracts (mg/ml)

Figure 3.41: Effect of ethanolic leaf extracts on total germination in *Chloris*



Note: a- ethanolic leaf extract treatments compared with control, b- ethanolic leaf extract treatments compared with solvent ethanol), Numerals stands for, 1-control, 2-ethanol, leaf extracts (mg/ml): 3- 1.25, 4) 2.5, 5) 5.0 6) 7.5 and 7)10.0

Figure 3.42: Effect of ethanolic leaf extracts on radicle length in *Chloris* seedling



Note: a- ethanolic leaf extract treatments compared with control, b- ethanolic leaf extract treatments compared with solvent ethanol),

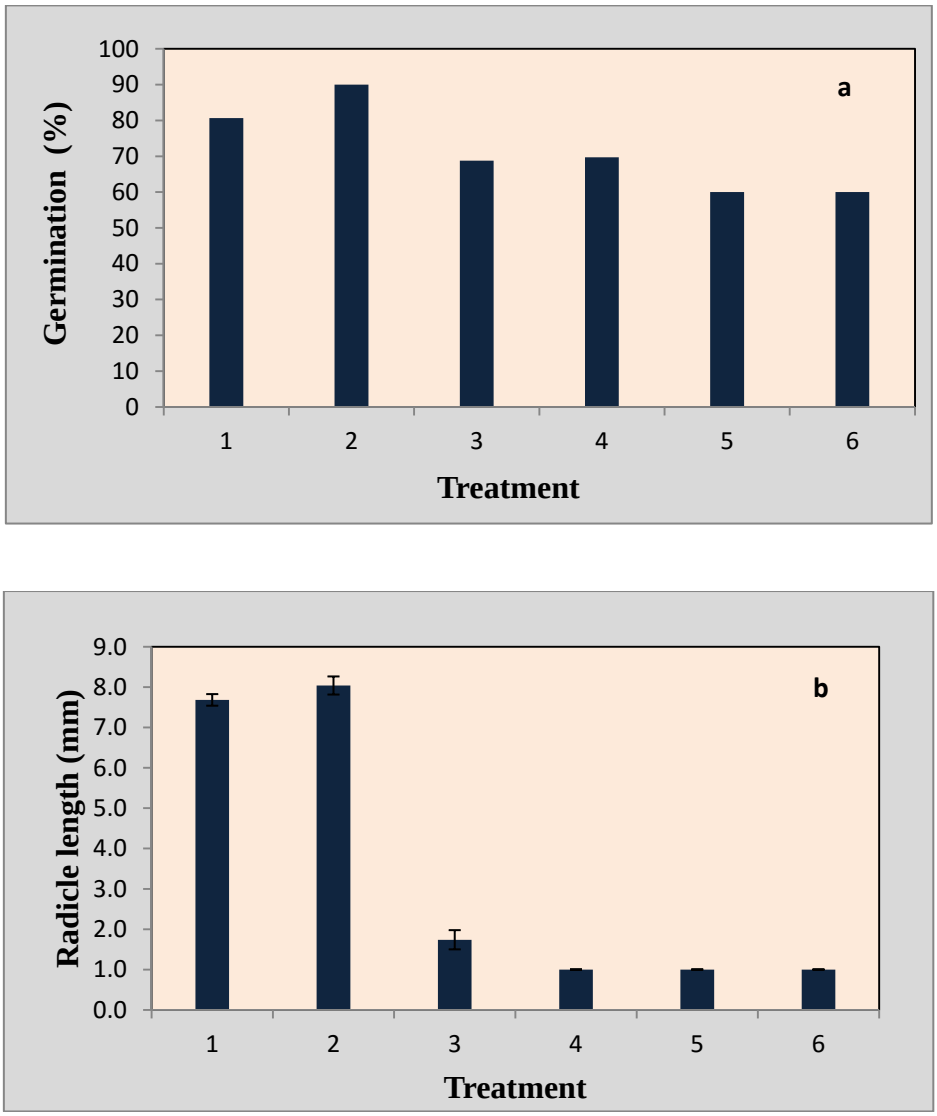
Numerals stands for, 1-control, 2- ethanol, leaf extracts (mg/ml): 3- 1.25, 4) 2.5, 5) 5.0 6) 7.5 and 7)10.0

3.4.1.2. Allelopathic effect of artemisinin: Treatment with pure artemisinin did affect the growth parameters of Chloris. A decrease in total germination was observed (Figure 3.43 a) owing to treatments 3, 4, 5, 6 as compared to treatment 1 and 2. Least

germination was found in treatment 5 and 6 where in the concentration of artemisinin were 33µM and 44 µM respectively. Total germination values in treatment 1 and 2 were 80.6 and 90.0, while in treatment 6 it was 60.0 %.

Artemisinin treatment greatly reduced (Figure 3.43 b) the radicle length. Magnitude of reduction increased with increasing concentration of artemisinin. Least radicle length was observed in treatment 4, 5 and 6, which was 1.0 mm while radicle length in treatment 1 and 2 were 7.7 and 8.0 respectively.

Figure 3.43: Effect of artemisinin on seed germination and radicle length in *Chloris*



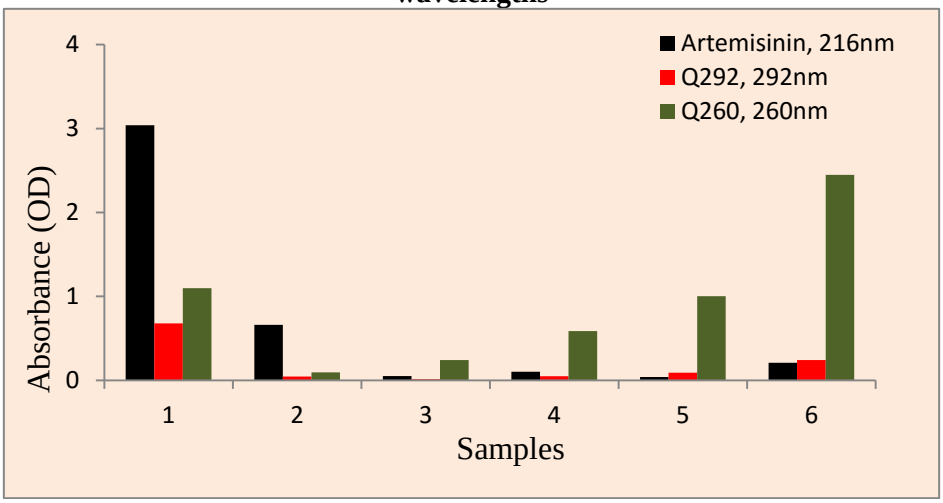
Note : Bars represent Mean ± Std

3.4.2 Qualitative analysis:

3.4.2.1. Spectrophotometric evaluation of standard artemisinin:

Pure artemisinin (underivatized) shows (Figure 3.44) maximum absorption at 216 nm, with little absorption at higher wavelengths like 292 nm and 260 nm. Ethanolic leaf extracts (underivatized) showed absorption at all the three wavelengths with maximum absorption at 216 nm. As compared to pure artemisinin (1mg/ml) the ethanolic leaf extract (1000 mg/ ml) showed less absorbance at 216 nm. The derivatized samples showed consistent increase in absorbance at 260 nm corresponding to ensured increase in concentration of Q260. The derivatized sample also showed some absorbance at other two wavelengths indicating some amount of pure artemisinin remaining underivatized at either NaOH or Acetic acid stage.

Figure 3.44: Absorbance of different samples at three different artemisinin specific wavelengths



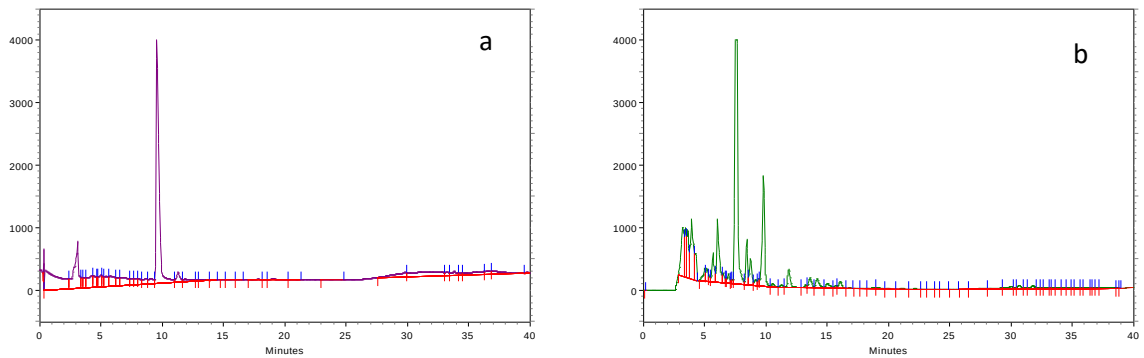
Note: Numerals stand for: 1:**artemisinin**, 2: **ethanolic leaf extract**, 3: **DC0.5**, 4: **DC1.0**, 5: **DC2.0**, 6: **DC5.0,** DC: Derivetized artemisinin and accompanied numerals indicate concentrations (mg/ml)]

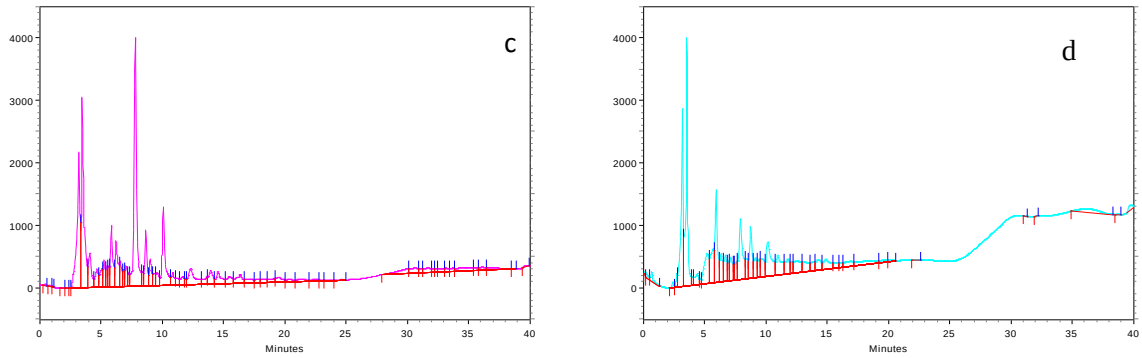
3.4.2.2. HPLC analysis of artemisinin, ethanolic leaf extract and Harborne's fraction A: Figure 3.45 a and b shows the HPLC chromatogram of pure artemisinin and ethanol leaf extract. The lambda max for the spectrum was obtained at 216 nm

wavelength. No absorbance was observed at 292 nm and 260 nm indicating absence of Q298 and Q260 metabolites in the analyzed samples. Figure 3.45 shows comparison of HPLC chromatograms. A sharp major peak was obtained for pure artemisinin with retention time at 9.59 minutes (Figure 3.45a). HPLC chromatograms of ethanolic leaf extract gave three major peaks with one peak having retention time of 9.59 minutes similar to artemisinin (Figure 3.45b). HPLC chromatogram for Harborne's fraction is given in Figure 3.31 A and explained in section 3.3.2.

3.4.3 Metabolite absorbance: *Chloris barbata* (SW.) seedling extracts pooled from different treatments were analysed for metabolites absorbed. Figure 3.45 shows comparision of chromatograms vis, a) artemisinin, b) ethanol leaf extract, c) extract of *Chloris* seedling pooled from control and d) extract of *Chloris* seedling pooled from artemisinin treated seeds. As the chromatogram pooled from the control and solvent ethanol seedling were same, only chromatogram for untreated seedling (Figure 3.45c) is presented here. Chromatogram of artemisinin treated seedlings (Figure 3.45d) showed no peak at 9.59 min. Retention time of the major peaks in both control seedlings and artemisinin treated seedling were same while differing in their intensities.

Figure 3.45: Comparison of chromatograms for presence of artemisinin

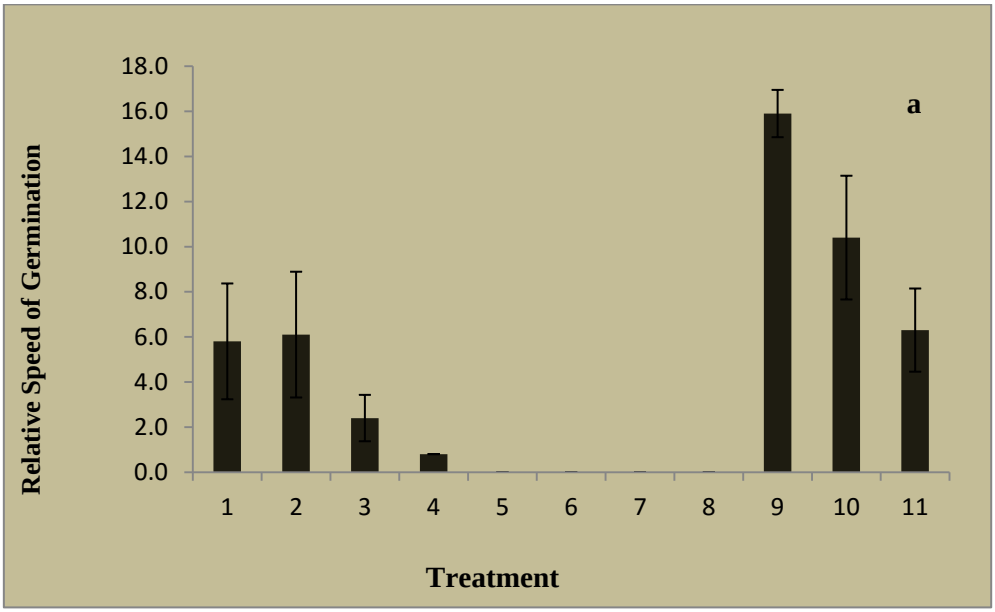


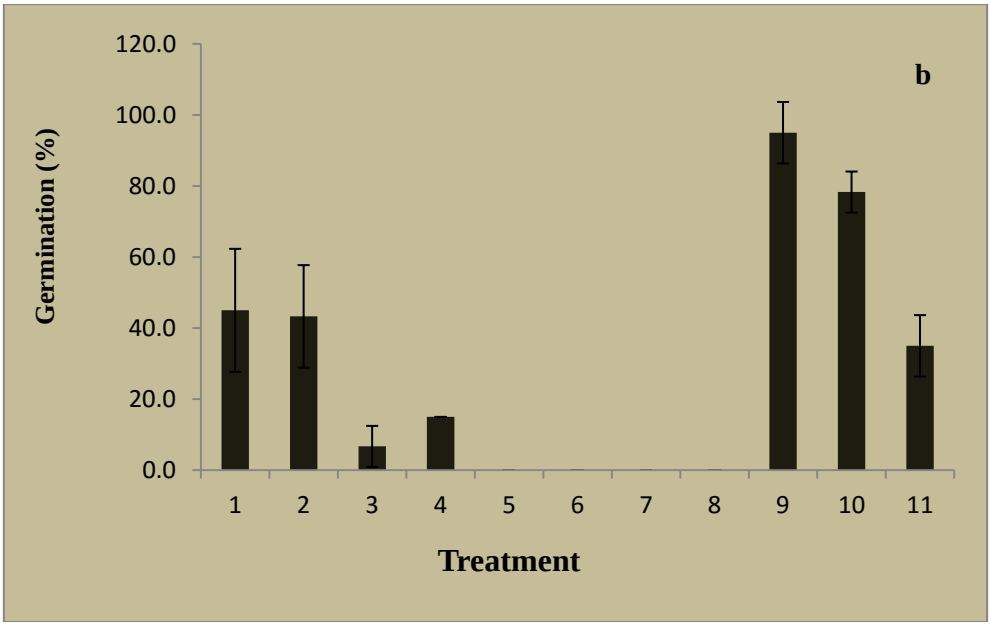


Note: Chromatograms: a) artemisinin, b) ethanol leaf extract, c) extract of *Chloris* seedling pooled from control and d) extract of *Chloris* seedling pooled from artemisinin treated seeds.

3.4.4. Soil bioassay for *Artemisia annua* L.: Figure 3.46c shows the allelopathic treatments provided to Chloris. Both, fraction A and leaf mulch were applied at the same rate that is 0.5, 1.0 and 2.0 % respectively. Artemisinin was applied at the rate of 16.5, 33 and 66 mM concentrations. In the Control, speed of germination (Figure 3.46a) and total germination (Figure 3.46b) was 5.8 and 45.0 % respectively. The same in alcohol treated Chloris was 6.1 and 43.3 %. For the Chloris seeds treated with Aa leaf mulch applied at the rate of 0.5, 1.0 and 2.0 % the speed of germination was 2.4, 0.8 and 0 respectively and the total germination was 6.7, 15.0 and 0.0 % respectively (Figure 3.46 a and b). For the seeds treated with Harborne's fraction A (applied at the rate of 0.5, 1.0 and 2.0 %), there was no seed germination, hence the values for speed of germination and total germination were 0.0. For the artemisinin treatment applied at the rates, the speed of germination was 15.9, 10.4, 6.3 and total germination was 95.0, 78.3 and 35.0 % respectively. Soil pH and Water holding capacity of the soil were analyzed from each of the pot kept for Aa soil bioassay before and after the experiment. No considerable variation was seen for both soil parameters, between the control and treatment measured before and after the experiment.

Figure 3.46: Effect of *Artemisia annua* L. treatments on germination in soil grown *Chloris*





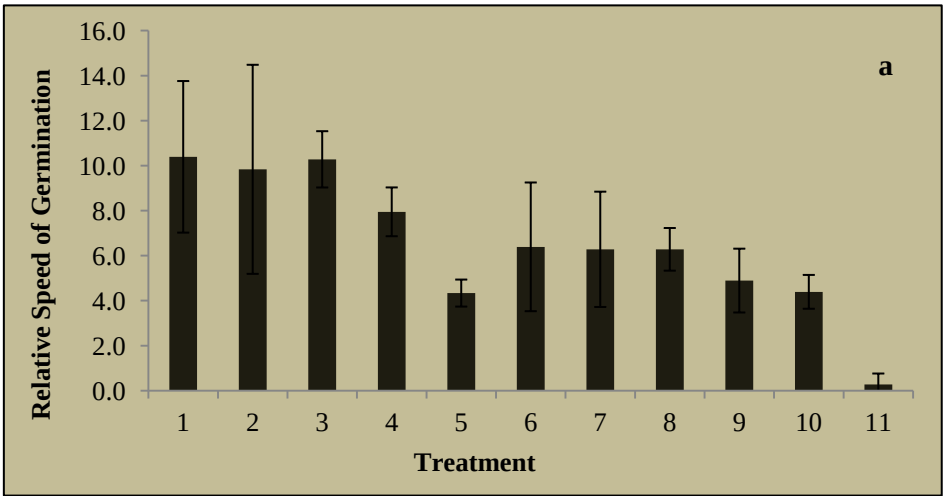
Note: Bars in Figure 3.46 a and b represents: mean ± std, Numerals represents: **1**- control, **2**- alcohol, **3**- 0.5 % Aa leaf mulch, **4** - 1.0% Aa leaf mulch, **5** - 2.0 % Aa leaf mulch,**6** - 0.5 % Aa FA, **7** - 1.0% Aa FA, **8** - 2% Aa FA, **9** – 16.5 mM artemisinin, **10** – 33 mM artemisinin, **11**- 66 mM artemisinin

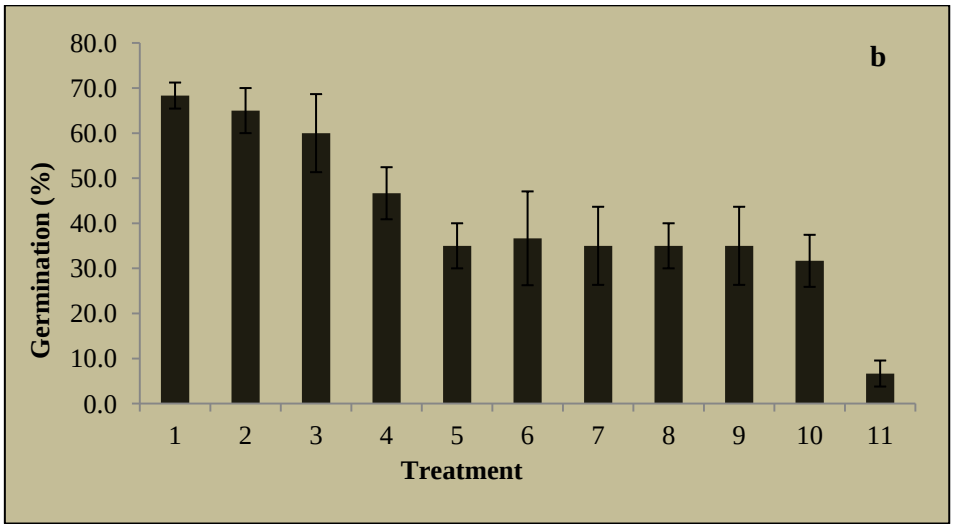
Note: Numerals in Figure 3.46c represents: **1**- control, **2**- alcohol, **3**- 0.5 % Aa leaf mulch, **4** - 1.0% Aa leaf mulch, **5** - 2.0 % Aa leaf mulch,**6** - 0.5 % Aa FA, **7** - 1.0% Aa FA, **8** - 2% Aa FA, **9** – 16.5 mM artemisinin, **10** – 33 mM artemisinin, **11**- 66 mM artemisinin

3.5. Soil bioassay for *Enicostemma littorale* Blume: Figure 3.47c shows the soil bioassay for El. All the treatments which include two chemical fractions and the leaf mulch, were applied at three rates viz. 0.5, 1 and 2 %. The speed of germination and total germination in the control was 10.4 and 68.3 %. The same in alcohol treated Chloris was 9.8 and 65.0 % respectively (Figure 3.47 a and b). Speed of Chloris seed germination in the Fraction A at the applied rates was, 10.3, 7.9 and 4.3 while the total germination was 60.0, 46.7 and 35.0 % respectively. For the fraction B treatment, the germination speed of Chloris was 6.4, 6.3, 6.3 and total germination was 36.7, 35.0 and 35.0 % respectively. The leaf mulch was most inhibitory, affecting both speed and total germination in Chloris. Speed of germination for the leaf mulch treated seeds was 4.9, 4.4 and 0.3 while the total germination was 35.0, 31.7 and 6.7 % respectively. Thus El leaf mulch was most inhibitory followed by the fraction B and than fraction A with reference to the control.

Soil pH and Water holding capacity of the soil were analyzed from each of the pot kept for El soil bioassay before and after the experiment. No considerable variation was seen for both soil parameters, between the control and treatment measured before and after the experiment.

Figure 3.47: Effect of *Enicostemma littorale* Blume treatment on germination of soil grown *Chloris*





Note Bars in Figure 3.47 a and b represents mean ± std, Numerals in Figure 3.47 a, b, c represents: **1**- control, **2**- alcohol, **3**- 0.5 % El FA, **4** - 1.0% El FA, **5** - 2% El FA, **6** - 0.5 % El FB, **7** - 1.0% El FB, **8** - 2.0 % El FB, **9** - 0.5 % El leaf mulch, **10** - 1.0 % El leaf mulch, **11**- 2.0 % El leaf mulch

3.6. Rhizosphere analysis: Rhizosphere analysis was performed for two medicinal plants viz. *Asparagus racemosus* Willd. and *Chlorophytum borivilianum* San. and Fern, which were found to exhibit allelopathic potential through their roots. The experiment was designed by placing PDMS probes in the soil, at a finite distance from the plant axis. It was hypothesised that the plant will leach compounds that will travel to a particular distance from the plant. The leaching of metabolites if any was monitored over a particular duration of time. Thus the experiment was designed for study of the spatial and temporal dynamics of compound released from allelopathic plants. Simultaneously the data was also analysed for the flux of number of metabolites. The PDMS probes have affinity towards non polar compounds, thus for the polar compounds, soil samples from the respective probe insertion sites were also collected and extracted in similar way as the probe. Subsequently, soil extracts containing polar metabolites were combined with the respective probe extracts to form a single methanol extract directly used for HPLC analysis to detect both polar (soil extracts) and non polar (PDMS extracts) metabolites. The HPLC chromatograms of all the extracts including those of root washing and SWP (Soil Without Plant) were compared with HPLC chromatograms of plant root extract, to compare the observed peaks for viewing the presence or absence of particular metabolites. Each chromatogram was studied with respect to number of peaks and their retention time (Figure 3.48 & 3.49, Table 3.11 & 3.12).

3.6.1. *Asparagus racemosus* Willd. (Ar): Figure 3.48 shows HPLC chromatograms for all the samples related to Ar rhizosphere analysis (Chromatograms in Figure 3.48 henceforth will be referred by respective extract names). Number of peaks for all the chromatograms were considered to represent number of metabolites absorbing at the wavelength of 254 nm. Plant root extracts showed six peaks (Figure 3.48 ArRE)

representing six metabolites named as 1 (Green), 2 (Red), 3 (Pink),4 (Blue), 5(Yellow) and 6 (Grey) respectively (Table 3.12). These metabolites were considered as reference coded with a particular colour and all the other extracts were evaluated for the presence of one or more of the root metabolites. Table 3.11 shows, compilation of retention time of metabolites separated for all the Ar extracts. Ar RW (Figure 3.48) showed only three peaks coinciding with compound 3, 5 and 6 present in root extracts.

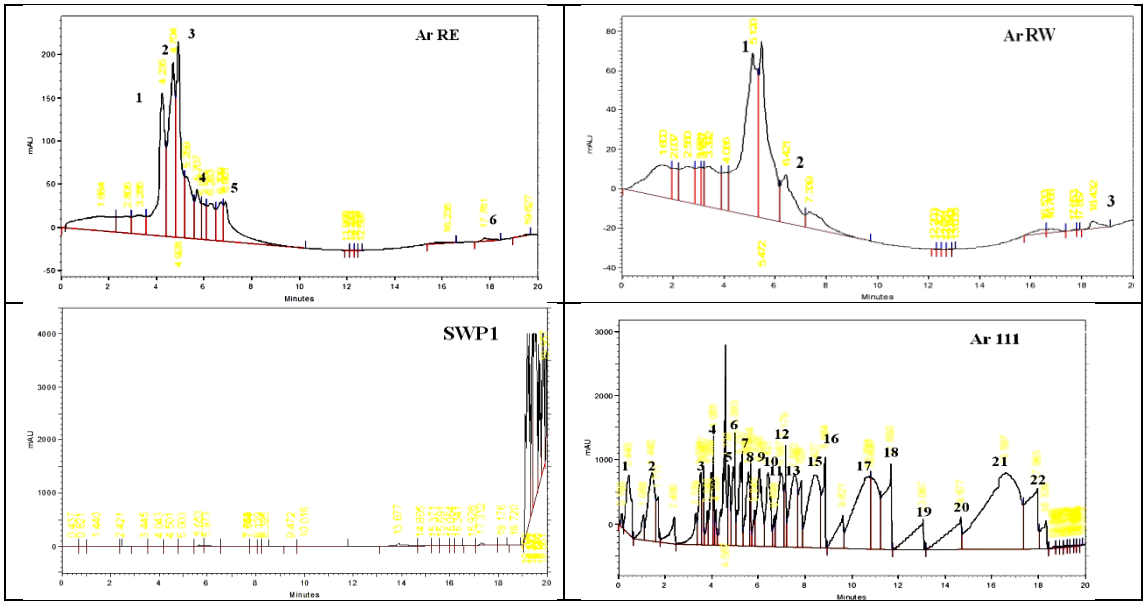
Reading one: For readings collected after ten days, Extract representing soil without plant showed no peaks (Figure 3.48 SWP1). Extract Ar111 showed 22 peaks and Ar112 showed presence of 20 peaks representing that many compounds. Table 3.12, shows out of the six root metabolites, all the six were found to be present in Ar 111extracts (probe kept at 2.5 cm) and metabolites 1,3,4,5 and 6 detected in Ar 112 (probes kept at distance of 5 cm from plant axis). Extract Ar121 showed 5 peaks one of which was similar to compound 6 found in root extract and Ar 122 showed presence of 4 peaks two of which were coinciding with compound 5 and 6 from root. Six peaks were observed in Ar131 extract with two of them appearing similar to compound 1 and 4 from root and five peaks were seen in Ar132 with one of the metabolite eluting similar to compound 4 of root extract.

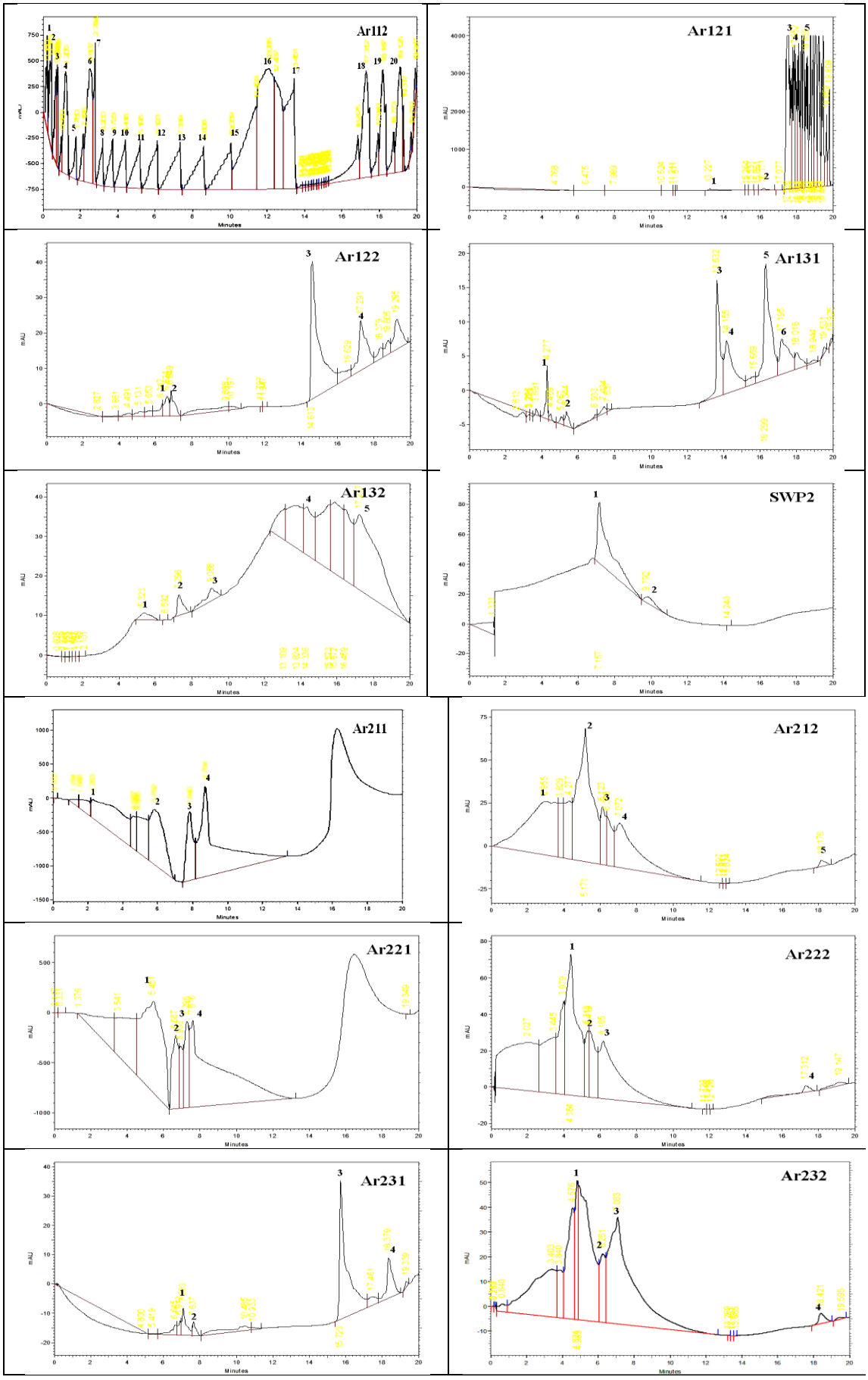
Reading two: As can be seen in Figure 3.48, SWP2 shows two peaks of a very low intensity and none of them coincide with the root metabolites. Chromatogram for Ar211 showed four peaks of which two coincide with compound 4 and 5 from root and that for Ar212 showed five peaks with three being similar to compound 3, 5 and 6 from root extract. Extracts from Ar221 and Ar222 both showed four peaks each, with former having two metabolite eluting similar to compound 5 and 6 from root and later coinciding with compound 1,3,4 and 5 from root. Both, extract from Ar231 and

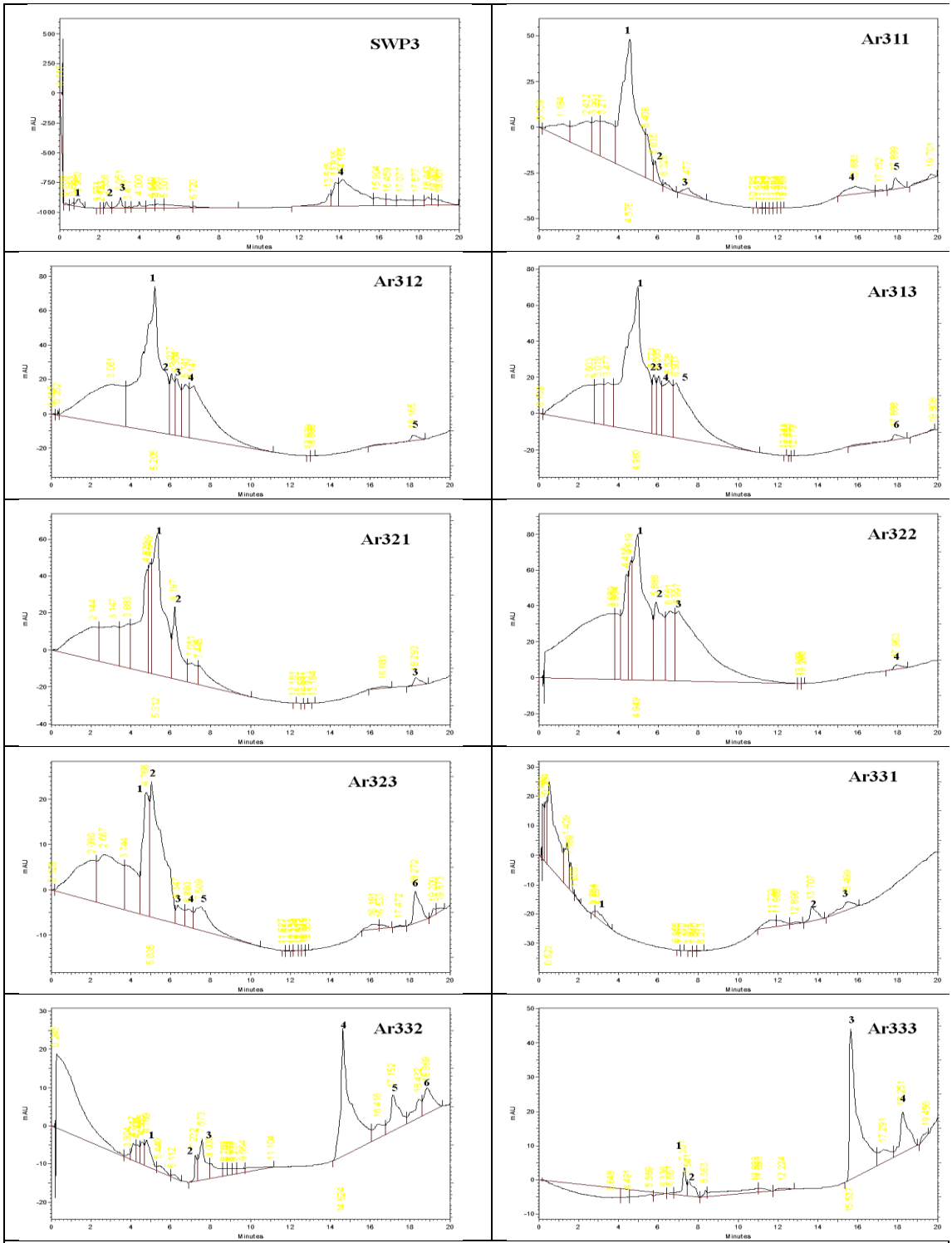
Ar232 also showed four peaks, with former having root metabolite 1 and later having root metabolite 3, 5 6.

Reading three: Figure 3.48 shows reading pertaining to third reading.SWP showed four peaks of which three initial peaks were before the root metabolite retention time and only peak number 4 appeared to be in considerable metabolite retention time. Extracts Ar311 and Ar312 showed five peaks while that of Ar313 showed six peaks. For Ar311, three of its peaks were similar to compound 1, 4 and 6 from root. For both Ar312 and Ar313, three peaks coincided with compound 3, 5 and 6 from root extract. Extracts Ar321 showed three peaks two of which were similar to compound 4 and 6 from root, Ar322 showed four peaks similar to root metabolite 3, 4, 5 and 6 and extract Ar323 showed six peaks, four of which coincided with root metabolite 2, 3, 5, and 6 respectively. Extracts Ar331, Ar332 and Ar333 showed three peaks (no peaks similar to root), six peaks (two of which were similar to metabolite 2 and 6 from root) and four peaks (one peak similar to that of compound 6 from root) respectively.

Figure 3.48: HPLC Chromatograms from rhizosphere analysis of *Asparagus racemosus* Willd.




Ar112
Ar121
Ar122
Ar131
Ar132
SWP2
Ar211
Ar212
Ar221
Ar222
Ar231
Ar232
Minutes
mAU



Note: **SWP** -Soil Without Plant (from Reading1,2,3), **ArRE**-Ar root extracts, **ArRW**-Ar root washing, Numerals in sequence **(e.g. Ar111)** starting from left, first numeral- Reading number, second numeral- plant number, third numeral – distance of probe from plant axis

Table 3.11: Retention time of the metabolites separated in HPLC analysis of *Asparagus racemosus* Willd. rhizosphere

HPLC Samples			Retention time (Rt –minutes)							Metabolite (no.)
Plant Root extract (RE)			4.23	4.70	4.92	5.70	6.90	17.78	-	6
Plant Root washing (RW)			5.12	6.42	18.42	-	-	-	-	3
Soil Without Plant (SWP) - 1			-	-	-	-	-	-	-	
Reading	**Plant ↓**	**Dist. From Axis ↓**								
1	**1**	**1**	0.44	1.44	3.67	4.08	4.73	5.00	5.23	21
			5.58	6.04	6.42	6.38	7.17	7.56	7.82	
			8.42	8.86	10.83	11.68	13.06	14.67	16.59	
		2	0.16	037	0.69	1.20	1.76	2.50	2.78	20
			3.20	3.72	4.41	5.19	6.12	7.33	8.60	
			10.05	12.08	13.45	17.31	18.18	19.12	-	
	2	**1**	13.22	16.17	17.56	17.95	18.53	-	-	5
		2	6.62	6.84	14.61	17.29	-	-	-	4
	3	**1**	4.27	5.34	13.63	14.15	16.29	17.19	-	6
		2	5.32	7.29	9.08	14.33	17.23	-	-	5
Soil Without Plant -2			7.15	9.79	-	-	-	-	-	2
2	**1**	**1**	2.28	5.79	7.84	8.70	-	-	-	4
		2	2.95	5.17	6.12	7.07	18.17	-	-	5
	2	**1**	5.45	6.66	7.29	7.61	-	-	-	4
		2	4.38	5.21	6.16	17.31	-	-	-	4
	3	**1**	7.08	7.63	15.72	18.37	-	-	-	4
		2	4.98	6.25	7.08	18.42	-	-	-	4
Soil Without Plant -3			0.96	2.33	3.05	14.16	-	-	-	4
3	**1**	**1**	4.57	5.83	7.47	15.83	17.89	-	-	5
		2	5.20	6.02	6.30	6.74	18.18		-	5
		3	4.98	6.77	6.00	6.58	6.90	17.89	-	6
	2	**1**	5.31	6.19	18.29	-	-	-	-	3
		2	4.94	5.88	6.99	17.98	-	-	-	4
		3	4.76	5.03	6.34	6.88	7.58	18.2	-	6
	3	**1**	2.78	13.07	15.49	-	-	-	-	3
		2	4.78	7.23	7.57	14.62	17.15	18.86	-	6
		3	7.03	7.54	15.63	18.25	-	-	-	4

3.6.2. *Chlorophytum borivilianum* San. and Fern (Cb): Figure 3.49 shows all the chromatograms related to Cb rhizosphere analysis (Chromatograms in Figure 3.49 henceforth will be referred by respective extract names). For all the chromatograms, number of the peaks were considered to represent number of metabolites. Plant root extract (Figure 3.49 CbRE), showed presence of four metabolites named as 1 (yellow), 2 (green), 3 (pink) and 4 (blue) respectively. Table 3.12 shows, compilation of retention time of metabolites separated in all the Cb extracts. The plant root

washing showed presence of five metabolites one of which was coinciding with the root metabolite 2.

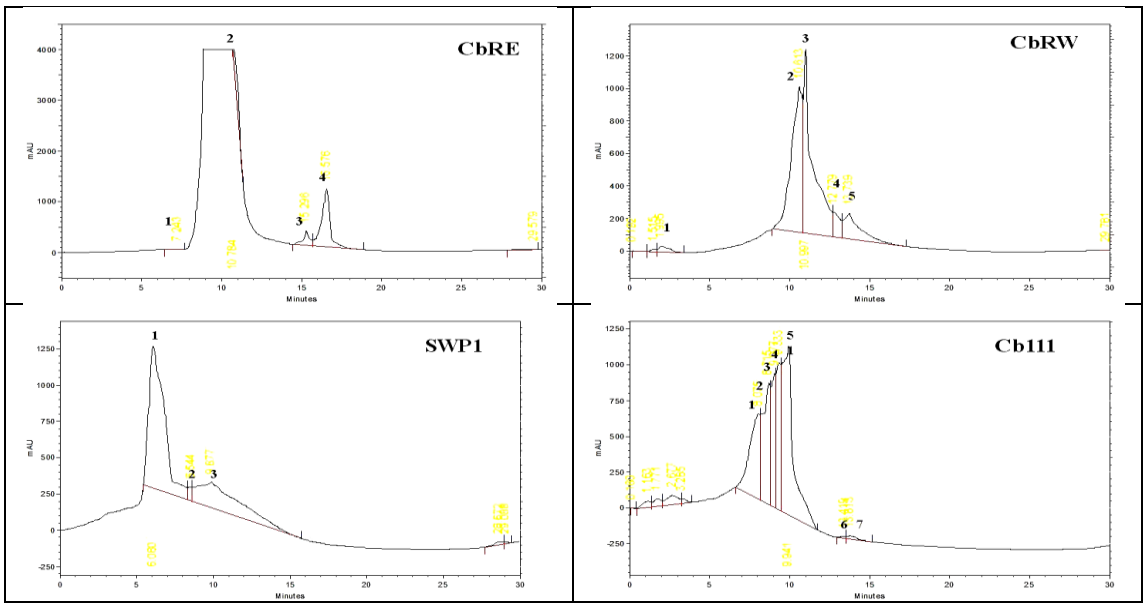
Reading one: The SWP1 showed presence of three peaks and elution time of none of them coincided with that of root metabolites indicating absence of any root metabolites. Figure 3.49, Cb111 and Cb112 showed presence of seven and six peaks respectively indicating presence of number of metabolites where in two metabolites from both were eluted similar to root metabolite 1 and 2. Extract Cb121 and Cb122 showed presence of two and four metabolites where in one of the metabolites in both appeared similar to the Cb root metabolite 2. Extract Cb131 and Cb132 were observed to contain four and five metabolites each. Two of the metabolite from Cb131 coincide with the root metabolite 1 and 2 and three metabolite from Cb132 showed resemblance with root metabolite 2, 3 and 4 in terms of elution time.

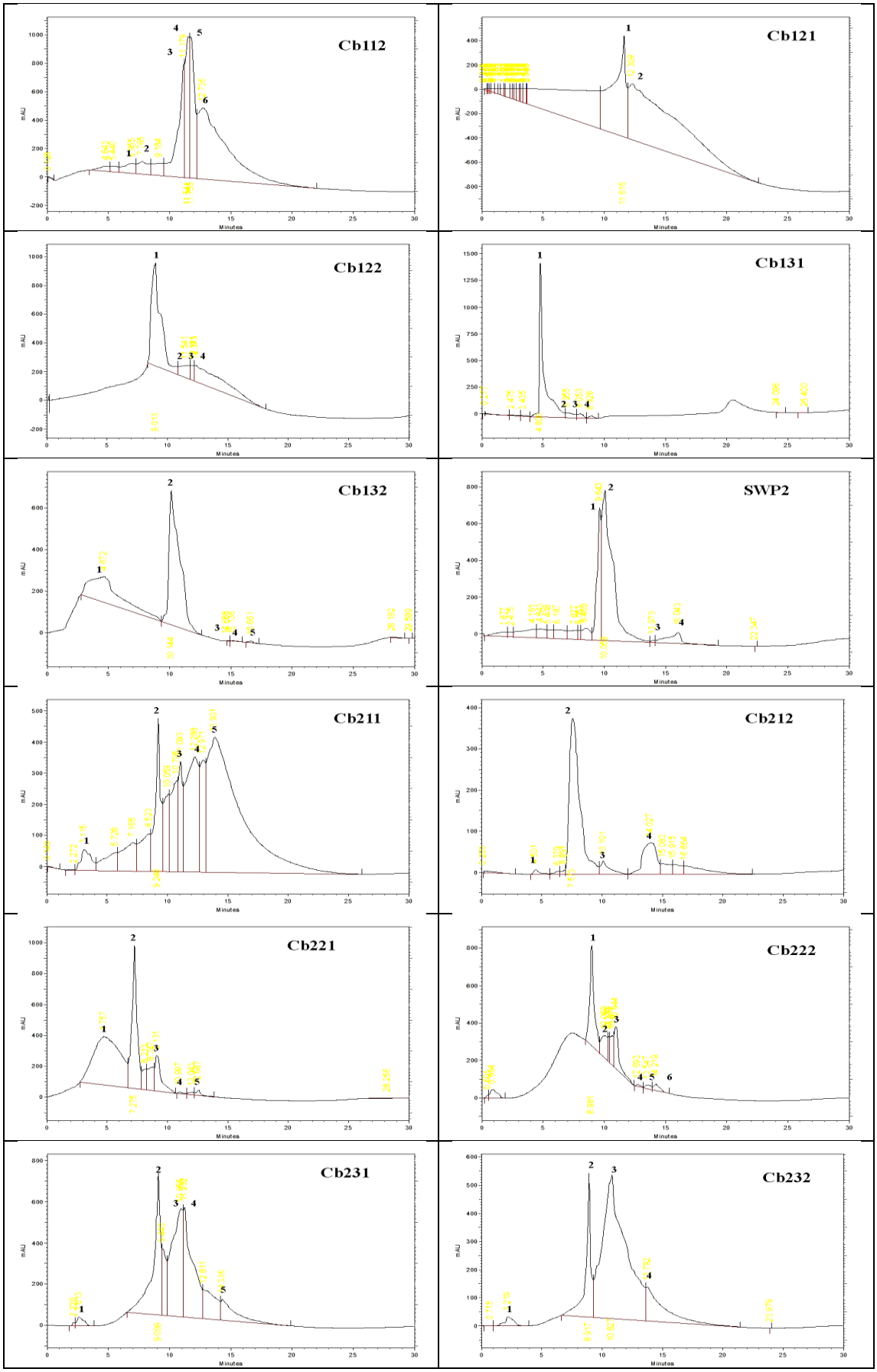
Reading two: SWP2 shows four peaks, with one of the peak having Rt similar to that of root metabolite 2, however the CbRE extract were isolated from root, the peak observed in SWP2 cannot be of the metabolite from the root (Figure 3.49). Extract Cb211 and Cb212 possessed five and four metabolites each, wherein in the Cb211 extract one of its metabolite was eluted at Rt similar to the Rt of root metabolite 2 and in the Cb212 extract three of its metabolite coincided in terms of retention time with the Rt of metabolite 1, 2 and 3 respectively. In extracts Cb 221 and Cb222, presence of five and six metabolites was detected, in which two of Cb221 metabolites were eluted similar to root metabolite 1 and 2 where as in Cb222 extract three of its metabolite were having retention time similar to that or root metabolite 1, 2 and 3 respectively. In extract Cb231 and Cb232, four and five metabolites were obtained. Cb231 showed three of its metabolite eluting at Rt similar to the root metabolite 1, 2,

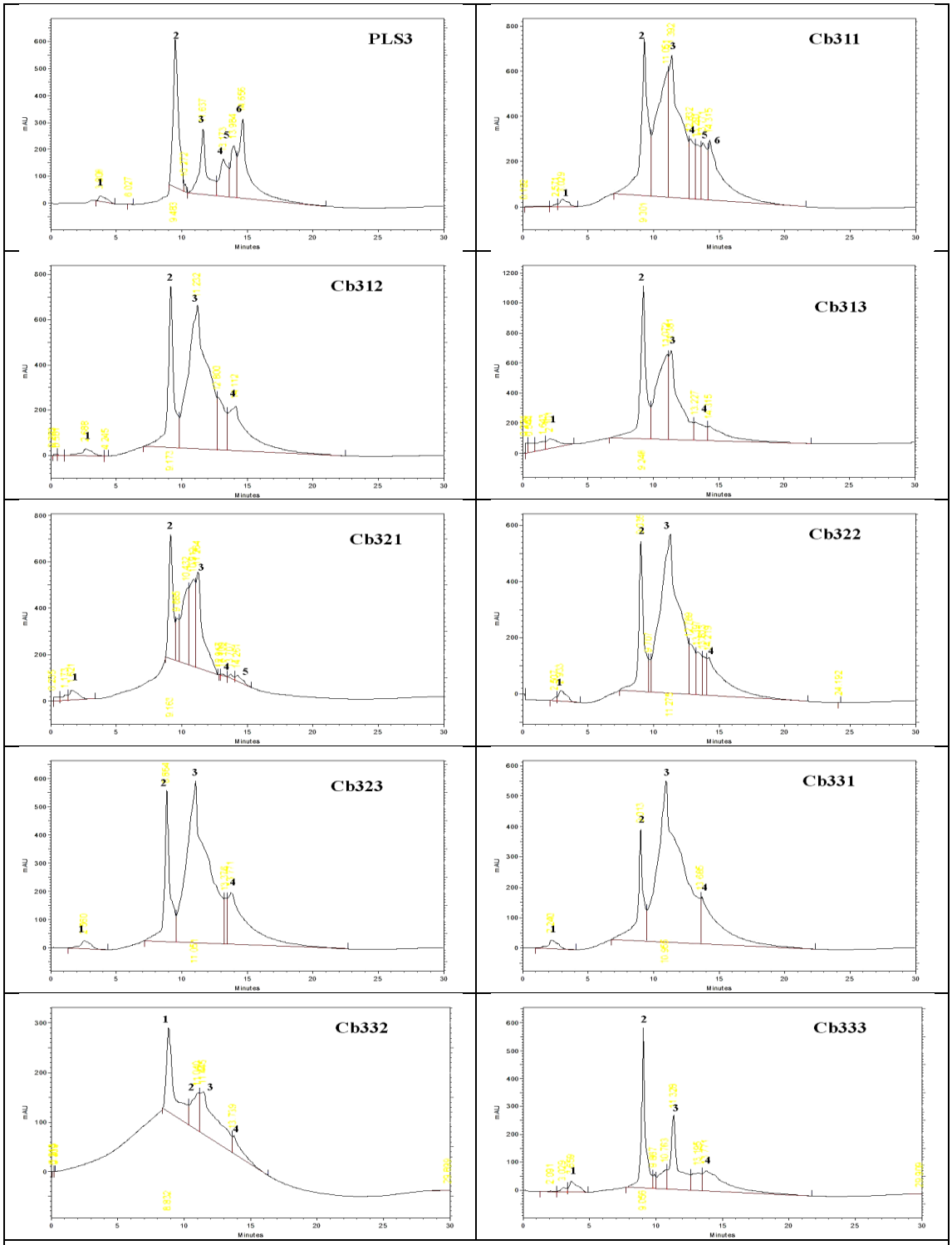
3 and Cb232 showed two of its metabolite eluting at Rt similar to the root metabolite 1 and 2.

Reading three: SWP3 shows total six peaks with three of them having similar Rt as that of root metabolite Rt, however they cannot be the root metabolites as the sample were devoid of plant roots (Figure 3.49). Extract Cb311, Cb312 and Cb313 showed seven, four and four metabolites respectively, of which three from each were similar to metabolite 1, 2 and 3 from the Cb root. Extract Cb321, Cb322 and Cb323 showed five, four and four metabolites. For Cb321 and Cb322 three of their metabolites were eluting at Rt similar to root metabolite 1, 2 and 3. For Cb323 two of its metabolite were eluting like that of root metabolite 1 and 2. For Extracts Cb331, Cb332 and Cb333 each showed presence of four, three and four metabolites. Extract Cb331 and Cb333 showed two for their metabolite eluting at Rt similar to the metabolite 1 and 2 of Cb root and Cb332 had only one such metabolite, that was eluted similar to metabolite 2 of root.

Figure 3.49: HPLC Chromatograms from rhizosphere analysis of *Chlorophytum borivilianum* San. and Fern.




Cb112
Cb121
Cb122
Cb131
Cb132
SWP2
Cb211
Cb212
Cb221
Cb222
Cb231
Cb232
mAU
Minutes



Note: **SWP** -Soil Without Plant (from Reading1,2,3), **CbRE**-Ar root extracts, **CbRW**-Ar root washing, Numerals in sequence **(e.g.Cb111)** starting from left, first numeral- Reading number, second numeral- plant number, third numeral – distance of probe from plant axis

Table 3.12: Retention time of the metabolites separated in HPLC analysis of *Chlorophytum borivilianum* San. and Fern. rhizosphere

HPLC Samples			Retention time (Rt –minutes)							Metabolites (no.)
Plant Root extract			7.24	10.78	15.29	16.57	-	-	-	4
Plant Root washing			1.99	10.61	10.99	12.77	-	-	-	4
Soil Without Plant- 1			6.08	8.54	9.87	-	-	-	-	3
Reading	**Plant ↓**	**Dist. From Axis ↓**								
1	**1**	**1**	8.07	8.71	9.07	9.33	9.94	13.41	13.81	7
		2	6.95	7.76	11.17	11.54	11.75	12.73	-	6
	2	**1**	11.61	12.30	-	-	-	-	-	2
		2	9.01	11.54	12.18	12.37	-	-	-	4
	3	**1**	4.80	6.95	8.05	8.92	-	-	-	4
		2	4.67	10.14	14.68	15.13	16.66	-	-	5
Soil Without Plant- 2			9.64	10.69	11.97	16.04	-	-	-	4
2	**1**	**1**	3.11	9.24	11.09	12.28	13.93	-	-	5
		2	4.50	7.57	10.0	14.02	-	-	-	4
	2	**1**	4.75	7.27	9.13	10.99	12.58	-	-	5
		2	8.98	10.06	10.94	12.69	13.54	14.21	-	6
	3	**1**	2.61	9.09	10.95	11.23	14.33	-	-	5
		2	2.21	8.91	10.82	13.79	-	-	-	4
Soil Without Plant- 3			3.80	9.48	11.63	13.17	13.98	14.65	-	6
3	**1**	**1**	3.02	9.30	11.05	11.39	12.82	13.77	14.31	7
		2	2.68	9.17	11.23	14.11	-	-	-	4
		3	2.10	9.24	11.38	14.31	-	-	-	4
	2	**1**	1.62	9.16	11.26	13.70	14.26	-	-	5
		2	2.93	9.03	11.27	14.29	-	-	-	4
		3	2.56	8.86	11.05	13.71	-	-	-	4
	3	**1**	2.24	9.01	10.95	13.68	-	-	-	4
		2	8.83	11.0	11.44	13.73	-	-	-	3
		3	3.65	9.05	11.32	13.77	-	-	-	4