EFFECT OF ANTIOXIDANT ON PESTICIDE INDUCED CYTOMORPHOLOGICAL CONSEQUENCES IN HEXAPLOID WHEAT (*TRITICUM AESTIVUM* L.)

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Dedicated

To

My Beloved Parents
Who Opened
My Eyes of Knowledge

DECLARATION

I hereby declare that the research work embodied in this thesis entitled **"EFFECT OF ANTIOXIDANT ON PESTICIDE INDUCED CYTOMORPHOLOGICAL CONSEQUENCES IN HEXAPLOID WHEAT (*TRITICUM AESTIVUM* L.)"** has been carried out by me for the degree of **Doctor of Philosophy** under the guidance of **Professor Dr. Md. Rezaul Karim** (Principal Supervisor), Department of Botany, University of Rajshahi and **Professor Dr. Golam Kabir** (Co-supervisor), Vice-Chancellor, Sheikh Hasina University, Netrokona Bangladesh.

I also declare that the result presented in this thesis is my own investigation and any part of this thesis work has not been submitted to elsewhere for any degree/diploma or for similar purpose.

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CERTIFICATE

This is to certify that **Afroja Akter** worked under our supervision as a Ph.D. Fellow, Roll No. 2012358503, Registration No: 2012358503, Session: 2019-2020, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh. It is our great pleasure to forward her thesis entitled **"EFFECT OF ANTIOXIDANT ON PESTICIDE INDUCED CYTOMORPHOLOGICAL CONSEQUENCES IN HEXAPLOID WHEAT (*TRITICUM AESTIVUM* L.)"** which is a bonafide record of research in the Department of Botany, University of Rajshahi, Bangladesh.

This work is original and has not been submitted so far in part or in full, for the award of any degree or diploma by any other institute in home or abroad. **Afroja Akter** has fulfilled all the requirements for submission of the thesis for the award of the degree of **Doctor of Philosophy**.

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The Authoress

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LIST OF ABBREVIATIONS

The following abbreviations have been used through the text:

AA	:	Ascorbic Acid
APx	:	Ascorbate Peroxidase
BADC	:	Bangladesh Agricultural Development Corporation
BARI	:	Bangladesh Agricultural Research Institute
cAPx	:	Cystosol Ascorbate Peroxidase
CAT	:	Catalase
CIMMYT	:	International Maize and Wheat Improvement Center
Cys	:	Cysteine
DAG	:	Day After Germination
DHA	:	De-hydro Ascorbate
DHAR	:	De-hydro Ascorbate Reductase
DMRT	:	Duncun's Multiple Range Test
DNA	:	De-oxyribo Nucleic Acid
EC	:	Emulsifiable Concentration
GPx	:	Gluthione peroxidase
GR	:	Gluthione Reductase
GSH	:	Glutathione
GST	:	Gluthione S-tranferase
ICV	:	Interphase Chromosome Volume

mAPx	:	Peroxisomes and glyoxisomes Ascorbate Peroxidase
MDA	:	Monodehydro Ascorbate
MDHAR	:	Monodehydro Ascorbate Reductase
MI	:	Mitotic Index
mitAPx	:	Mitochondria Ascorbate Peroxidase
NADPH	:	Nicotinamide Adenine Dinucleotide Phosphate
PMC	:	Pollen Mother Cell
PS-II	:	Pigment/Photo system-II
RCBD	:	Randomized Complete Block Design
RNS	:	Reactive Nitrogen Species
ROS	:	Reactive Oxygen Species
RSS	:	Reactive Sulfur Species
sAPx	:	Chloroplast Ascorbate Peroxidase
Se-Cys	:	Seleno Cysteine
SOD	:	Superoxide Dismutase
tAPx	:	Chloroplast thylakoid Ascorbate Peroxidase
WRC	:	Wheat Research Centre

ABSTRACT

To meet up the growing demand of huge number of population, high production of crop plants playing a major role in ensuring food security and food availability. But different kind of pests and diseases is affecting the production of crop plants adversely. Now-a-days pesticides have got a remarkable popularity in terms of pest management, plant protection and to increase the yield of the crop plants. Thus, crop plants are being directly affected with different sorts of adverse consequences. Mutagenic and carcinogenic effect of pesticides is managed by the use of antioxidant as it is used in case of mammals. The present study was designed to investigate the protective role of antioxidants against pesticide toxicity in wheat seeds. Wheat seeds treated with pesticides were used as control in this experiment and then pre- and post-treatment were operated using antioxidants. The seeds of pre- and post-treatment were designated as M_1 generation. Treated seeds were allowed to germinate in laboratory as well as in the field following three factors randomized complete block design and data were collected on cytological as well as growth and yield related characters. The M_2 seeds collected from M_1 plants (raised from the seeds treated previously) were left untreated and were sown in next year. Again data were collected on morphological and cytological characters from M2 generation to investigate the residual effect. Result obtained from M1 generation indicates that pesticides induced adverse morphological and cytological characters. At the same time pre- and post-treatment of antioxidants showed positive effect on reducing pesticide toxicity enhancing growth and yield related characters. The overall findings indicated that antioxidants have an antagonistic effect against pesticide induced cytomorphological consequences. Result, also revealed that pre-treatment showed more efficiency than that of post-treatment. Result of M2 generation showed that both pesticide and antioxidant had same sort of residual effect.

CHAPTER-I

INTRODUCTION

1. INTRODUCTION

1.1 General aspects of wheat (*Triticum aestivum* L.)

Wheat is one of the first domestic crops and has been one of the staples in the diet of many civilizations for more than 8,000 years. At present, it is cultivated in more land area than all major commercial crops such as rice, maize, potatoes and so on. It is grown on around 220 million hectares, with a higher cultivation area than any other crop and its world trade is greater than all the crops combined. While developing countries such as China and India are amongst the largest cultivators and exporter of wheat. The role of wheat, in its myriad forms as a staple in our day to day diet, can't be denied. It is considered as a dominant staple in most of the African and, West and Central Asian countries.

Various researches predict that the requirement of wheat is being expected to increase by at least 50% to 60% by the year 2050. Area expansion is likely to be a challenge and therefore, many countries have already started working towards increasing crop yield and production. China has drastically increased productivity per hectare, making it one of the leading countries in terms of productivity per hectare. Countries such as the U.S. and India already have launched initiatives aiming to double its productivity by the year 2030. While many countries are drawing long-term plans to improve productivity, cultivators at the ground level, still need to overcome challenges that can ensure viable crop yield and guarantee profits.

Now-a-days, the most fundamental activity of the people of Bangladesh is agriculture and the agriculture is very much focused on crop plants. Thus, it was earnest desire to find alternatives rather depending only rice for continuing food supply. Because at that time it was realized that rice alone could not meet the food requirements of the country (Banglapedia, 2006). Wheat was therefore chosen as an alternative winter food crop for Bangladesh. Since then wheat has become one of the popular food source of Bangladesh.

Wheat is now the second most important cereal crop in Bangladesh. It provides a source of income and food for millions of farm families. In Bangladesh all the varieties of wheat are semi dwarf spring type and they are grown successfully in the winter from mid October to mid November. For the last 25 years in Bangladesh, attention has been governed extensively on the higher yields of common wheat (*Triticum aestivum* L.).

1.2 Wheat production and uses of chemicals

Foley *et al.* (2011) reported that due to a rapidly growing population and to the lack of availability of new farmland, it will be necessary to continue to increase crop yield in the future. But Hossain and Teixeira (2013) found that "Even though existing wheat varieties in Bangladesh are high yielding, area and production did not increase sufficiently to match the growth in the human population". Due to increasing global population and changing diets in developing countries towards meat and milk products, demand for food production is projected to increase by 70% (Food and Agricultural Organization, 2009). Among the food crops, wheat is one of the most abundant sources of energy and proteins for the world population and its increased production is essential for food supply (Chhokar *et al.,* 2006). This phenomena created a great attention of scientists to develop wheat varieties in terms of increasing yield production and protection of plants from diseases, pests etc. for ensuring their production. As a result Wheat Research Centre (WRC), Bangladesh Agricultural Research Institute (BARI) and Bangladesh Agricultural Development Corporation (BADC) paid an intrinsic effort to develop high yielding wheat varieties with the collaboration of International Maize and Wheat Improvement Centre (CIMMYT). These included 'BARI Gom 19'(Sourov) and 'BARI Gom 20' (Gourab) released in 1998; 'BARI Gom 21'(Shatabdi) in 2000; 'BARI Gom 22' (Sufi), 'BARI Gom 23' (Bijoy) and 'BARI Gom 24' (Prodip) in 2005 (Pandit *et al.,* 2011); and 'BARI Gom 25' and 'BARI Gom 26' released in 2010 (BARI, 2012b) etc.

Apart from these activities, chemical treatment has become an accepted measure for developing yielding capacity, diseases and pest management capacity of the varieties. Progress in chemical crop protection has been extra-ordinary over the last 60 years, not only in the invention of new active ingredients, but also in the assessment of the behavior of these chemicals in the environment (Muller, 2002). Some tremendous benefits have been reported from the use of agrochemicals i. e. pesticides, herbicides, insecticides, weedicides etc. in Agriculture. Bureau Indian labour statistics (1998) reported that "Food grain production which stood at a mere 50 million tons in 1948-49, had increased almost fourfold to 198 million tones by the end of 1996-97. This was 19 million tons higher over 1995-96 production representing an increase of 10.5 percent". In wheat production applied pesticides which contributed to the growth of crop productivity as well food supply (Radosavac and Knezevic, 2017). According to Aktar *et al.* (2009) pesticides effects of suppressing pathogens on the plant contribute to the higher yield and quality. In the period 1988-1992, it becomes economically the most important pest in cereals, and up to 28% of cereals were chemically treated to decrease cereal leaf beetle's population (Stamenkovic, 2000: Jevtic *et al.*, 2002). Pesticides have been integral part of the process in which production losses have been remarkably decreased from the pests, weeds and diseases. Webster *et al.* (1999) stated that "considerable economic losses" would be suffered without pesticide use and quantified the significant increases in yield and economic margin that result from pesticide use. Warren (1998) also drew attention to spectacular increases in crop yields in the United States in the twentieth century. Estimates of global losses from pests for eight crops in some regions showed that pest-induced losses were more than 50% of attainable crop output (Oerke *et al.*, 1994).

According to Bahera and Singh (1999) weeds reduces yield of dry land crops by 37-79%. These undesirable plants compete with crop mainly for space, solar radiation, nutrients, water and carbondioxide. As a result they damage the crop and cause reduction in yield of

crop. Herbicides are compounds that designed to control the development of undesirable plant that may interfere with the growing of commercial crops (Blair *et al.,* 1990). So it is clear that many modern chemicals are being commonly used all over the world. Among them chlorinated pesticides are enjoying greatest popularity because of their low production cost, high insect toxicities, ease of application and long persistence.

1.3 Impact of chemicals

Although pesticides promise the effective mitigation of harmful bugs but unfortunately the risks associated with their use have surpassed their beneficial effects. It is evident that wide-spread use of pesticides in modern agriculture involved both direct and indirect danger (Metcalf, 1987; Woodruff *et al.,* 1994; Koh and Jeyaratnam, 1996; Van der Werf, 1996; Pimentel, 2005). There is now overwhelming evidence that some of these chemicals do pose a potential risk to humans and other life forms and unwanted side effects to the environment (Forget, 1993; Igbedioh, 1991; Jeyaratnam, 1985; Carson, 1962) also claimed detrimental effects of pesticides on the environment. Now it is well known that a significant fraction of pesticides are carcinogenic; for instance, 18% of all insecticides and 90% of all fungicides were found to be carcinogenic (National Research Council Committee, 1987) and the use of pesticides has many secondary consequences (Amer, 1996; Amer and Ali, 1968; Reddy and Rao, 1969; Alam *et al.,* 1977; Alam and Kabir, 1983, 1984; Kabir and Alam, 1986). Similarly herbicides also found to have some adverse effect to the non-target plants and organisms.

However, chemicals were used in USA more widely than in any country in the World, showed very minute quantities of residues in the soil. For example, if one kilogram of active ingredient of a chemical is applied and mixed thoroughly in the soil of 30 cm depth weighing 4 million kilograms, the chemical concentration would be 0.25 ppm. If it is mixed in the top 15cm of soil, the concentration would be 0.56 ppm. So it is clear that the mixing of chemical concentration in the soil is an important factor for its using. If it is not done

accurately then high concentration of residues can affect the plant growth and development (Rao and Murty (1980). In Bangladesh, most of the farmers are not aware of this subject matter and sometimes they can't understand the recommended doses and use them very discriminately. Besides, farmer do not get their desired results either due to have become resistant to some chemicals thus they increase the concentration of the chemicals than the recommended dose to combat the pest without anticipating any adverse effect. Radosavac and Knezevic (2017) also found that the farmers applied herbicides and insecticides more than recommended amount and insecticides less than recommended amount, what leads losses of yield, increase of costs, economic loss and negative effect on environment. The use of herbicides in modern agriculture exerts a threat on crop plant by a way of cytological damage to the cells of crop plant (Siddiqui *et al.,* 2008). Kumar and Jagannath (2015) found a significant reduction in the mitotic index of the dividing cells and the chromosomal abnormalities were increased as the concentration of herbicide increased. Another relevant factor is that low concentrations of many chemicals may not elicit acute detectable effects in organisms, but they may induce other damage, like genetic disorders and physiological alterations, which reduce life span in the long run (Poletta *et al.,* 2009). Kilingman (1947) also found much sterility and considerable morphological abnormalities in wheat spikelets treated by 2-4-D at or before the jointing stage resulted in greater reduction in yield. Chromosomal aberrations might be accepted as indicators of genetic damage induced by herbicides (Reddy and Reddy, 1985). Jalilian *et al.,* (2000) reported the cytogenetic effects of the pesticides. Some common pesticides (Thiodan, Folithion, Lebaycid, and Kitazin) caused a spectrum of cytogenetic abnormalities such as chromosome fragmentation, lagging of chromosomes, anaphase bridge formation as well as tri-polar and tetra-polar spindle formation in barley (Grover and Tyagi, 1980). Chromosomal aberrations have been considered as a reliable indicator of mutagenic activity, since there have been evidence for a correlation between chromosomal damage and toxic effects of a number of pesticides (Badar, 1983; Askin, 2006; Shehata *et al.,* 2008; Ozturk, 2008; Fisum and Goc Rasgele,

2009). Marcano and Carruyo (2004) reported that chemicals used in the agriculture could be transformed into mutagenic or carcinogenic agents by herbs or vegetables which are the first living beings in the food chain. Gul *et. al.* (2006) suggested that the 2-4-D induced chromosome exchange in immature embryos of wheat species is the genotoxicity of the herbicides. So it has become evident that these chemicals are causing manifold problems at morphological as well as cellular level of crop plants in terms of toxicity for long time.

According to Fenik *et al.* (2011) countless chemicals are environmentally stable, prone to bioaccumulation and toxic. Because some pesticides can persist in the environment, they can remain there for a years. Environmental contamination or occupational use can expose the general population to pesticides residues, including physical and biological degradation products present in the air, water and food (Mostafalou and Abdollahi, 2013). Sometimes rainfall can carry pesticides to surface water, contaminating rivers, lakes, and seas and taking these chemicals to distant place (Wilson, and Tisdell, (2001). According to Hernandez *et al.* (2013) a large quantity of pesticides is lost via spray drift, off-target deposition, run-off and photo-degradation, for instance which can have undesirable effect on some species, communities or ecosystems as a whole as well as on the humans. The high-risk groups expose to pesticides include production workers, formulators, sprayers, loaders and agricultural workers. Exposure to pesticides linked to negative effect of immune function, liver, intelligence, cardiovascular a respiratory function, reproductive abnormality cancer (Sarwar, 2016). Due to these secondary consequences of these chemicals consumers are facing health hazards. Worldwide, pesticide use has resulted in different cases of acute and chronic poisoning, with effects of varying hazard on human health, from mild effects to death (Navarro *et al.,* 2007). Several studies have highlighted the occurrence of chronic health hazards e.g. cancers, diabetes depression, neurological deficits, respiratory diseases and fertility problems, due to pesticides (European Food Safety Authority, 2013). The mutagenic and carcinogenic actions of herbicides, insecticides and fungicides on experimental animals is well known and several studies have shown that chronic exposure to

low levels of pesticides can cause mutations and/or carcinogenicity (Vainio *et al.,* 1991, Yu and Zhou, 2005, Bull *et al.,* 2006). Mutagenic efficiency of these chemicals to plants adversely affects the human population's fitness by increasing the frequencies of alleles with detrimental effects. Research findings suggest that these chemicals are cytotoxic, mutagenic and carcinogenic in mammalian cells. A high relation between mutagenecity and carcinogenecity makes the present practice of imperative to employ certain measures for protection against the effectiveness of these chemicals.

1.4 Generation of ROS/Free radicals

Throughout the entire life cycle plants are continuously exposed to various abiotic stresses and sometime multifaceted stresses. These stresses in turn causing the generation of various reactive oxygen species (ROS), such as singlet oxygen, superoxide, hydrogen peroxide, or hydroxyl radicals in cells (Sharma *et al.*, 2012; Das and Roychoudhury, 2014). Similarly the enormous use of pesticides leading free radicals mediated oxidative stresses in plant through abnormality, mutagenecity, carcinogenicity, cytotoxicity etc. Pesticide application caused oxidative stress to plants as a result of the generation of reactive oxygen species (Sharma *et al.,* 2018). The application of herbicides often resulted in growth reduction due to alterations in certain metabolic processes and can lead to oxidative stress in plants (Foyer *et al.,* 1995; Alla and Hassan, 2006). Environmental factors such as salinity, drought, chilling, metal toxicity, air pollution, UV-B radiation, and high doses of pesticides as well as pathogen infection lead to enhanced production of ROS in plant cell (Molassiotis *et al.,* 2016; Caverzan *et al.,* 2016). There are various evidences and there are also reports confirming that chemicals are mediating oxidative stress and imbalance between pro oxidant and antioxidant factors in plants. Similarly the ROS-mediated oxidative deterioration of macromolecules has been implicated as one of the major causative factors of many diseases, including cancer (Waris and Ahsan, 2006), atherosclerosis (Mugge and Kardiol, 1998), diabetes (Kaneto *et al.,* 2010), neurodegeneration (Polster, 2013), ageing (Jackson and McArdle, 2011) and so on.

1.5 Role of antioxidants

To detoxify these reactive oxygen intermediates, cells posses a vast network of antioxidants activity. An antioxidant prevents or slows down oxidation by donating an electron to unstable ROS, thereby oxidizing itself in place of susceptible cellular macromolecules. Halliwell *et al.* (2007) defined antioxidants as any substance that delays, prevents or removes oxidative damage to a target molecule. Therefore antioxidants are considered to be important components in defense against damage caused by the various oxidative stresses. According to Dunnet (2003) antioxidants have several mode of action such as a) free radical scavengers b) act as preventive antioxidants c) sequestrate elements by chelation and d) quench reactive oxygen species (ROS).

Investigations reveal that antioxidants are being reported to play an important role for modifying the mutagenecity and carcinogenecity.

1.6 Objectives

Now-a-day attempts are being made to look for prophylaxis of these types of chemical with the preparation of antioxidants in capsule or tablet form. Literatures reveal that the inhibition of mutagenesis and carcinogenesis by different natural and synthetic compounds suppress chromosomal aberrations in mammals induced by different mutagenic chemicals. As a result antioxidants consumption has got an accepted measure for avoiding such abnormalities in human society. With few exceptions, the large range of antioxidant defenses is surprisingly very similar in humans and plants (Arora *et al.,* 2002; Valko *et al.,* 2007; Halliwell, 2006; Mittler, 2002). But reports on anticlastogenic effect of antioxidant on plants induced by different chemical compounds are very much scanty.

Keeping this deficiency in mind the present study has been undertaken to gain the idea regarding the anticlastogenic effect of antioxidants on pesticide induced toxicity as well as aberrations at morphological and cellular level of wheat on the basis of following heads:

1. To investigate the effect of pre- and post-treatment of antioxidant on pesticides treated seeds of wheat.

2. To explore the role of antioxidant on reducing the mutagenic effect of pesticides induced seeds of wheat.

3. To observe the residual effect of pesticide and antioxidant.

CHAPTER-II

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Effect of antioxidant on different crops plants have been investigated by different workers in different time. The literature regarding the cytomorphological effect of antioxidant on wheat are not adequate and thus, a little information regarding this subject matter on wheat have been compiled here as follows under different heads:

2.1 Stresses of plant

Biochemical reaction is one of the driving forces take place within the cell organelles and help in sustaining plant life (Rahman, 2007). Plants cannot escape from adverse environmental conditions as well as biochemical reactions, and therefore, have evolved an array of mechanisms to protect themselves against various biotic and abiotic stresses around them. Plants encounter various abiotic stresses due to their sessile nature which include heavy metals, salt, drought, nutrient deficiency, light intensity, pesticide contamination, as well as extreme temperatures. In modern agriculture pesticides are being used in wide range throughout the world. Pesticides are a class of xenobiotic compounds that have been used to destroy or repel unwanted animals, insects and plants (weeds) for several decades. They consist a very large and diverse group of chemical compounds and their mixtures, namely herbicides, nematicides, insecticides, molluscicides, etc. In developing countries, they are used extensively and their application is expected to increase in coming years. In European Union, only for plant protection, more than 140000 tons of pesticides are being used every year. There are many categories according to which pesticides can be divided, e.g., depending on the organism to be combated, they can be divided into zoocides (combating animal pests), bacteriocides (against bacteria), herbicides (against weeds) and fungicides (against fungi). The division in terms of chemical structure can be also applied. Main groups are non-organic pesticides (arsenic and fluoride insecticides) and organic pesticides (chloroorganic, phosphoroorganic, carbaminianes, phenoxyacetic acid and triazyne

derivatives). Increasingly common use of pesticides even in food storage is a main reason why people are constantly exposed to their residues in food products and potable water. Only a part of pesticides used in EU is under the monitoring system and almost 50% of fruits, vegetables and crops are contaminated with pesticides and their residues (Commission staff working document, 2005). All classes of pesticides are of great global concern because they not only pollute our biosphere but also cause adverse effects on human health as well as crop yields (Khan *et al.,* 2020). They are also classified according to their hazardous effects, and all of them either fall into the category of highly (34.2%) or moderately (35.0%) poisonous (Damalas and Khan, 2017). In human beings, they have significant chronic health effects, including cancer, neurological effects, diabetes, respiratory diseases, fetal diseases, and genetic disorders. These health effects are different depending on the degree and type of exposure (Rastogi *et al.,* 2010) to various pesticides. In plants, the use of pesticides has direct or indirect effects on normal physiological and biochemical functions of plants. Their excessive use causes various toxicity symptoms in plants, leading to reduced seed germination, shoot length, root length and biomass, chlorosis, necrosis, retarded growth and photosynthetic efficiency (Sharma *et al.,* 2018; Vidyasagar *et al.,* 2009). As a result, plants abnormally develop, their growth is inhibited and ultimately their survival is threatened. Previous research has shown that pesticide-induced abnormal growth and development in plants is mostly due to their inability to take up the essential micro nutrients (Siddiqui and Ahmed, 2006) present in the growth media as well as their modes of action that may interfere with photosynthesis, thus causing chlorophyll degradation (Parween, *et al.,* 2016; Sharma *et al.,* 2015; Lei *et al.,* 2020; Conger and Fairchild, 1953). After application, pesticides pass through the degradation stage by numerous physiochemical reactions such as autolysis, photolysis, rearrangement, and inactivation upon binding to soil and macromolecules, and may cause the generation of reactive oxygen species (Mahmood *et al.,* 2014).

They are important from the environmental protection point of view, because they can disturb the natural balance of ecosystems. According to WHO pesticide residues in food are defined as a sum of chemical compounds present in food product as a result of pesticide usage (the parent substance and its degradation products). In addition to pesticides, smoke, mycotoxins, endocrine disrupting chemicals (such as polychlorinated biphenyl) and heavy metals (such as arsenic) have been listed as the most common human toxins. Toxins are poisons produced in living organisms of plants, animals and bacteria. Toxic substances (toxicants) are synthetic, toxic, man-made chemicals. These two groups of compounds also differ in the processes of production, distribution, heterogeneity of composition and increasing ubiquity at homes, in human and animal bodies and in the environment. They can cause biochemical damage in various ways. While there are many types of toxins, they tend to interfere with the normal activity of cells, making cells act differently than normal (Duke *et al.,* 2010). Carcinogens, such as pesticides, cause the multiplication of various cells to accelerate and cause cancer. Instead of disrupting certain processes in the body, many poisons, including pesticides, cause them to act differently, often with harmful effects. People deal with toxins with antidotes, either by building up immunity or by avoiding them. However, toxic substances such as pesticides exist on a massive scale and are associated with everyday economic, industrial and regulatory systems. This means that efforts to mitigate or eliminate the effects of pesticides must take into account these systems and the power dynamics that maintain them.

Problems with the use of pesticides are often associated with agriculture, forestry, and their accumulation as a result of spraying weeds growing along roads, railway lines, in gardens, parks and other urban areas (Lushchak *et al.,* 2018). One of the main factors connected with the risk that pesticides constitute to human health is their dose of exposure (Sabarwal *et al.*, 2018). Three categories of pesticide exposure can be distinguished: intentional (accidental/suicidal), occupational and non-occupational exposure (Davies, 1980). An intentional exposure is usually considered as a possibility to attempt suicide in developing

countries, because of an easy access to such chemicals especially in rural areas in China and South East Asian countries (Bertolote *et al.,* 2006). The second kind of pesticide exposure – occupational, is mainly considered as connected with pesticides transport and usage in crop protection, food storage and food sale. People such as dealers, farmers, applicators and sellers of fruit and vegetables are particularly exposed to pesticides, especially while mixing, loading, spraying and through a direct contact with fruits, vegetables and crops. In this case pesticides enter human organism through the respiratory tract and the skin (Shadnia, 2005). The third kind of pesticide exposure is non-occupational exposure, which is the most common way of the exposition. It occurs through the gastrointestinal tract with the consumption of fruits, vegetables and crops. It is rather difficult to exactly demonstrate the detailed cause and effect relationship associated with pesticide exposition and their possible hazardous effect. Chemical compound used as a pesticide should be characterized by high toxicity towards pests and simultaneously possibly low toxicity towards other organisms, especially human and aquatic organisms. They also should be accordingly durable, susceptible to biodegradation to such an extent that they do not accumulate in the environment (Bjorling-Poulsen *et al.,* 2008).

Given their specific properties, the ability to bioaccumulation and the high persistence in ecosystems, it seems obvious that their residues are found in living organisms (Bolognesi and Merlo, 2011). In order to estimate their effects in the environment, it is important to analyze their bio-concentration resulting from the exposure to these compounds in the diet (Katagi, 2010). The term “bio-concentration”, commonly used to describe the penetration and metabolism of pesticides in living organisms, contains three important aspects: physicochemical properties of an individual chemical compounds that are active substances of pesticides, physiological dispositions of the penetrated organism and environmental conditions surrounding the body. Pesticides can enter living organisms through various pathways, including: absorption through the skin, inhalation and through the digestive tract (Jabłonska-Trypuc *et al.,* 2017). There are many reports confirming the relationship between

exposure to pesticides and the occurrence of many chronic diseases such as various types of cancer, diabetes, neurodegenerative diseases, birth defects, endocrine disorders, respiratory problems, asthma, cardiovascular diseases, nephropathy, autoimmune diseases, chronic fatigue syndrome and aging (Abdollahi *et al.,* 2004; De Souza *et al.,* 2011; Mostafalou and Abdollahi, 2012). One of the main molecular mechanisms, by which pesticides affect living organisms influencing their health, is an increase in oxidative stress level. Oxidative stress has core importance in both abiotic and biotic stresses. It is produced when there is a critical disturbance between the production of antioxidative defense and reactive oxygen species (ROS).

Due to the inevitable exposure of humans to pesticides, which are common environmental toxins, there is a need for effective methods to reduce, and preferably eliminate, their harmful effects. Complementary and alternative solutions in the form of dietary antioxidants or functional foods can provide a safe way to prevent pesticide toxicity. In recent years, many plant derived dietary compounds, especially with antioxidative activity attract attention as chemo preventive substances, because of their ability to scavenge free radicals generated by environmental contaminants. Popular group of plant-derived compounds are flavonoids, which comprise a large, heterogenous group with benzopyran in their structure. They are present in vegetables, fruits and herbs as a secondary plant metabolite. They have free radical scavenging abilities and exhibit positive health effect, especially in cancer treatment and prevention (Rice-Evans *et al.,* 1995). Many compounds from the large group of plant hormones are tested for their antioxidative properties in human organism likewise. An example of such group of compounds is cytokinins, and well-known compound from this group considering its influence on oxidative stress parameters is kinetin. In human cells it acts as an antioxidant with chemo-protective properties (Jabłonska-Trypuc *et al.,* 2016). The mechanisms, by which pesticides may cause damage both on the cellular and tissue level usually involve oxidative stress reactions, especially generation of free radicals, lipid peroxides, oxidized proteins and oxidized sugars (Wang *et al.,* 2016; Lushchake *et al.,*

2005). Cellular enzymes, structural proteins, membranes components, sugars and nucleic acids are exposed to possible damage because of ROS (Reactive Oxygen Species) high reactivity (Jaganjac *et al.,* 2012; Zarkovic, 2018). The stimulatory influence of pesticides on lipid peroxidation and protein, DNA and sugars oxidation processes has been well documented, particularly in vivo (Semren *et al.,* 2018; Slaninova *et al.,* 2009). However, mechanisms, by which pesticides act on the cellular and molecular level are still not fully defined. Therefore, it is very important to study this phenomenon on a variety of levels and in many possible combinations, because the activity of pesticides in cells varies depending on their chemical structure, dose and time of exposure. It should be also mentioned that the literature data based on the mammalian experimental studies, which connect directly pesticides toxicity and the mechanisms of their action, especially oxidative stress, are very scarce.

The application of pesticides often results in growth reduction due to alterations in certain metabolic processes and can lead to oxidative stress in plants (Foyer *et al.,* 1995; Alla and Hassan, 2006). The production of free radicals and ROS is promoted by the herbicides, but these are then effectively scavenged by efficient regeneration of AA and GSH (Chang and Kao, 1997). Decreased GSH levels found in maize shoots enhanced their susceptibility to metolachlor (Farago *et al.,* 1993). According to Alla and Hassan (2007) plant tolerance to butachlor is related to GSH induction. As a result Reactive Oxygen Species (ROS) / free radicals are produced as a by-product of unavoidable metabolic process. From the last few years, a remarkable attention has been drawn towards the field of ROS or free radical chemistry for both plant and animal health point of view. Free radicals, reactive oxygen species and reactive nitrogen species are generated by plant and animal body by various endogenous and exogenous systems. There should be balance between free radicals and antioxidants for proper physiological function as their imbalance causes numerous diseases. Antioxidants are essential and important for plants and animals' sustenance. They are substances that protect cells from the damage caused by unstable molecules known as free radicals.

2.2 ROS/ Free radicals

Free radicals can be defined as reactive chemical species with one or more unpaired electron in its outer shell (Halliwell and Gutteridge, 2007; Bahorun *et al.,* 2006; Valko *et al.,* 2004; Valko *et al.,* 2007; Droge, 2002; Riley, 1994). They are formed due to homolytic cleavage of a chemical bond so that each atom may have one of paired electrons by cleavage of a radical to give another radical and also via redox reactions (Halliwell and Gutteridge, 2007; Bahorun *et al.,* 2006).

The radical might donate or accept an electron from other molecules in order to pair or it might simply join to the molecule, therefore, behaving as oxidant or reductant (Cheeseman and Slater, 1993). When these radicals give or take an electron or add to the anion to become a radical, the reactions generally continue as a chain reaction, in which, one radical begets another (Halliwell and Gutteridge, 1990). They are highly unstable and active towards chemical reactions with other molecules (Carocho and Ferreira, 2013) and are derived from three elements oxygen, nitrogen and sulfur, and thus, creating reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfur species (RSS). These include hydroxyl (OH•), superoxide (O_2•-), nitric oxide (NO•), nitrogen dioxide (NO_2•), peroxyl (ROO•) and lipid peroxyl (LOO•). Hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($1O_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite (ONOO-), dinitrogen trioxide (N_2O_3) and lipid peroxide (LOOH) are not the free radicals though they are generally known as oxidants and they can easily lead to free radical reactions in living organisms (Genestra, 2007). Majority of them are highly reactive species, thus, they are usually known as reactive oxygen species(ROS), which are capable to damage biological systems such as deoxyribonucleic acid (DNA), proteins, carbohydrates and lipids (Young and Woodside, 2001). Free radicals and other reactive species are derived either from normal essential metabolic processes or from external sources, such as exposure to x-rays, ozone, cigarette smoking, air pollutants, industrial chemicals, etc. Though a number of free radicals are formed in the body but the most important radicals are derivatives of oxygen better known as reactive oxygen species (Cheeseman and Slater, 1993).

2.3 Effects of free radicals/ROS

Free radicals are highly reactive if not inactivated. Their reactive nature is capable of damaging almost all types of bio-molecules including proteins, carbohydrates, lipids and nucleic acids. Free radicals are molecules, usually of oxygen, consisting of two atoms of oxygen. On combination, they balance each other. If these two atoms are separated, they form two free radicals, and this loss makes them unstable. As soon as these free radicals are created, they are checked by enzymes in the body, which are known as antioxidant enzymes, or nutrients in food that are known as antioxidant nutrients like vitamin C, vitamin E, beta-carotene and bio-flavonoids (Sharma, 1995). Trouble arises if the generation of free radicals is much more and enzymes are unable to handle it. These free radicals can destruct cell membranes, proteins and lipoproteins by a process known as lipid peroxidation. Proteins may also be damaged by ROS/NOS, leading to structural changes and loss of enzyme activity (Halliwell, 2007). They can attack DNA, leading to dysfunction, mutation and cancer. Jointly, it ultimately results in arthritis, emphysema in lungs and bronchitis, atherosclerosis in the blood vessels and ulcer in stomach, i.e., in each part of body these radicals can produce damage. They are also responsible for ageing (Bagchi and Puri, 1998). The body has several mechanisms to counteract these attacks by using DNA repair enzymes and/or antioxidants (Pancher *et al.*, 2007).

2.4 Antioxidant and it's mechanism against reactive oxygen species (ROS)/Free radicals

Antioxidant means "against oxidation". Oxidation is a chemical reaction that transfers electrons from a substance to oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. According to Halliwell and Gutteridge (1999) 'an antioxidant is any substance that when present at low concentrations com-pared with that of an oxidizable substrate significantly delays or inhibits oxidation of that substrate'. As a result cell or organ can be protected from damage caused by free radicals. They are sometimes called "free-radical scavengers".

In natural conditions, plants continuously produce several ROS during photosynthesis and respiration processes in cell organelles such as mitochondria, chloroplast, and peroxisomes. Thus, plants can maintain homeostasis by two different detoxification mechanisms involving non-enzymatic and enzymatic antioxidants (Mittler, 2002; Arbona *et al.,* 2003; Apel and Hirt, 2004; Sytar *et al.,* 2013). In plants, superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APx) are the main enzymatic antioxidants, whereas carotenoids, tocopherols, ascorbate, and glutathione are classified as the non-enzymatic antioxidants (Asada, 2000; Racchi, 2013). Racchi (2013) reported that SOD exists in various forms, such as Cu/ZnSOD, MnSOD, and FeSOD. Depending upon their affinity with the other ions in plants, each SODs are distributed in a different form in various plant organs such as chloroplasts (Cu/ZnSOD, FeSOD), cytosol (Cu/ZnSOD), and mitochondria (MnSOD). Primarily, SOD catalyzes the efficient removal of superoxide free radicals in chloroplasts as they are mainly generated in the photosystem I during the light reaction. CAT is located in the peroxisomes of plant cells, and its main role is the elimination of H_2O_2, which is produced by the SOD reaction. Another antioxidant, APx, also can remove H_2O_2; however, it is distributed in the preoxisomes as well as chloroplasts, cytosol, and mitochondrion (Racchi, 2013). Thus, APX can be found in different forms, such as cAPx (cytosol), mitAPx (mitochondria), sAPx (chloroplast stroma), mAPX (peroxisomes and glyoxisomes), and tAPx (chloroplast thylakoids) depending upon its location (Racchi, 2013). In the chloroplast, APx exist as sAPx and tAPx; the ratio of sAPx and tAPx in chloroplast differs according to the plant species and leaf senescence, and reveals different plant sizes. The cAPx is located in cytosol; thus, its plays a role in the elimination of H_2O_2, which is generated in cytosol. Therefore, all APxs are different in characteristics such as size, location, role, and amino acid sequences (Caverzan *et al.,* 2012).

Plants can induce defense responses against oxidative stress by activating the non-enzymatic antioxidants, which represent the second line of defense against ROS, hydrophilic molecules (ascorbate, glutathione), and lipophilic metabolites (carotenoids, α-tocopherol; Racchi,

2013; Suzuki *et al.,* 2014; Gowayed *et al.,* 2017). Ascorbate is a water-soluble antioxidant synthesized in mitochondria. It can translocate to other cell compartments by two different pathways. Normally, ascorbate can directly scavenge ROS ($^1O_2$, $2O_2^-$, and OH) in the cell. Furthermore, it is connected with the deep oxidase enzyme of violaxanthin, and acts as response matrix of APx (Szarka *et al.,* 2013). Due to its various roles, ascorbate is considered as the most powerful antioxidant in the plant cell (Gill and Tuteja, 2010; Racchi, 2013; Suzuki *et al.,* 2014). Glutathione is also an important water-soluble antioxidant, and plays an important role in scavenging $^1O_2$ and OH from chloroplasts (Sharma *et al.,* 2012). In addition, glutathione protects the thiol-groups of enzymes located in the chloroplast stroma and participates in the production of α-tocopherol and ascorbate (Xiang and Oliver, 1998; Sharma *et al.,* 2012; Racchi, 2013). Besides its role in detoxification of ROS, glutathione induces physiological responses such as the regulation of sulfur transport and expression of stress defense genes (Noctor *et al.,* 2002; Racchi, 2013). Carotenoids are a class of phenolic compounds distributed in various fruits and vegetables (Racchi, 2013). They can prevent lipid peroxidation by scavenging single oxide radical from chloroplasts (Kuhlbrandt *et al.,* 1994). Carotenoids are synthesized in plastids and consist of 40-carbon isoprenoids. According to Lu and Li (2008) carotenoids are classified as carotenes, which include the carbon and hydrogen atoms, and xanthophylls that contain the oxygenated form of carotenes (Wu *et al.,* 2017). The most important role of α-tocopherol is that it can eliminate $^1O_2$, $O^-2O_2^-$, and OH free radicals, which are generated in the thylakoid membranes; thus, it can prevent lipid peroxidation (Fryer, 2006; Kataria, 2017). α-tocopherol has adequate fluidity, enabling it to move easily within the lipid membrane. Thus, membrane safety is induced by the fluidity of α-tocopherol (Faltin *et al.,* 2010; Racchi, 2013).

2.5 Antioxidants and their mode of action

In contrast to the animal system, plants are more prone to oxidative damage due to an additional source of reactive oxygen intermediates that are acquired through photosynthesis. Endogenous ROS production via a number of mechanisms, such as cellular respiration, photosynthesis, organelle activity in the endoplasmic reticulum, and peroxisome and apoplastic activity, is an unavoidable phenomenon in plants and, to cope with this, plants have developed a sophisticated defense system comprising an array of enzymatic and non-enzymatic antioxidants. Plants are exposed to some stressful conditions, the balance between un-avoidable ROS generation and the antioxidant defense system is disturbed, leading to enhanced ROS generation and, ultimately, to oxidative stress. There are a variety of ROS produced in many different cellular and extracellular compartments, and these differ in their solubility, diffusibility and extent of toxicity. Therefore, plants need an array of different antioxidants that are capable of acting in different cellular compartments to detoxify ROS as soon as they are produced.

2.5.1 Superoxide dismutase (SOD)

Superoxide is the first species formed through the univalent reduction of oxygen in biological systems. SODs are a group of metallo enzymes and are considered to be enzymes of primary defense; they are widely present in all oxygen metabolizing cells. They effectively catalyze the dismutation of superoxide anions into oxygen and hydrogen peroxide (Alscher *et al.,* 2002). Depending upon the metal cofactor used, they are grouped into three major families: Cu/Zn SODs, Fe or Mn SODs and Ni SODs. In both plants and animals, the most common of the SODs is the Cu/Zn SOD, which is a soluble cytosolic protein (Tainer *et al.,* 1983). In plants, Fe SODs are mostly localized inside chloroplasts, while Mn SODs are abundantly found in mitochondria and peroxisomes. The last family is the Ni SODs, which have Ni as a cofactor; these are predominantly present in prokaryotes. All plant SODs are encoded by the nu-clear genome.

2.5.2 Ascorbate peroxidase (APx), glutathione reductase (GR), dehydroascorbate reductase (DHAR) and monodehyroascorbate reductase (MDHAR)

As a result of the spontaneous or enzymatic dismutation of superoxide, H_2O_2 is formed; this then needs further detoxification through several enzymatic and non-enzymatic anti-oxidants. The enzymes for H_2O_2 scavenging include CAT, various peroxidases and a series of four enzymes – APx, MDHAR, DHAR and GR – that belong to the AA–GSH cycle (Foyer and Halliwell, 1976; Nakano and Asada, 1981).

The AA–GSH cycle in plants has been studied in detail in chloroplasts and was named as the 'Asada–Halliwell pathway', but it is likely that all of the enzymes of this pathway also exist in the cytosol of both photo-synthetic and non-photosynthetic cells (Chen and Asada, 1989). The first step of this path-way involves the reduction of H_2O_2 to water by ascorbate peroxidase (APx) via the oxidation of ascorbate to monodehydroascorbate (MDA). APx is a haemo-protein and the cofactor haem acts as a site for oxidation–reduction reactions. MDA may give rise to dehydroascorbate (DHA). The reduction of MDA and DHA is catalysed by MDHAR and DHAR, respectively. DHAR uses GSH as reducing substrate and reduces DHA to ascorbate by converting GSH to disulfide, i.e. GSSG. The activities of these two ascorbate reducing enzymes are important in regenerating the ascorbate pool. Finally, the GSSG is reduced by GR to regenerate GSH at the expense of NADPH (reduced nicotinamide adenine dinucleotide phosphate), which acts as the electron donor. GR is a flavoprotein and has been well studied in plants, animals, humans and micro-organisms. It is crucial for maintaining a high GSH/GSSG ratio in the cell. In contrast to plants, in which the utilization of GSH for the direct reduction of H_2O_2 is not an import-ant process, animal glutathione peroxidases (GSH-PRXs) are very efficient and important both in the cytosol and the mitochondria (Yu, 1994).

2.5.3 Catalase (CAT)

CATs efficiently catalyse the decomposition of H_2O_2 to water and oxygen and do not even require a reducing substrate for their action. They are among the enzymes with the highest turnover number as one molecule can de-compose millions of molecules of H_2O_2 to oxygen and water each second (Goodsell, 2004). In animal cells, it is the cytosolic and peroxisomal CATs that primarily scavenge H_2O_2. In plants or, more specifically, in their leaf tissues, peroxisomal CATs are the major scavengers of H_2O_2 , and CAT activity has been found to be low in chloroplasts (Asada, 1994). However, CAT isoenzymes are recognized in mitochondria in maize (Scandalios et al., 1983). CATs are prone to photo-inactivation and require continuous de novo synthesis. Various stresses leading to protein inhibition or degradation may cause the inactivation of CATs in plants and animals.

2.5.4 Glutathione peroxidase (GPx)

GPx is an important selenium containing oxidoreductase in animals where it functions to reduce H_2O_2 to water and also effectively to reduce lipid hydro-peroxides to their corresponding alcohols. Its existence in plants has only recently been reported. In plants, the catalytic sites of the enzyme has a cysteine (Cys) residue rather than a selenocysteine (Se-Cys) residue, and the enzyme prefers thioredoxin as the electron source (Herbette *et al.*, 2002; Jung *et al.*, 2002; Navrot *et al.*, 2006). Several iso-enzymes of GPx are known to be distributed over different sub-cellular locations and with varying substrate specificity. In Populus tri-chocarpa, six genes coding for GPxs were identified, of which two are localized in the chloroplast and one is co-localized in the mitochondria (Navrot *et al.*, 2006). In humans, eight different isoforms of GPx (GPx1–8) have been identified, of which GPx1 is the most abundant in the cytoplasm and reduces H_2O_2. GPx3 is primarily present in plasma and GPx4 has a high affinity for lipid hydroperoxides. Glutathione (GSH) is an important low molecular weight thiol present in all living organisms. It is a tripeptide (a glutamylcysteinylglycine), and in plants it has been detected in virtually all cell compartments, including the cytosol, chloroplasts, endoplasmic reticulum, vacuoles and mitochondria. The nucleophilic nature of GSH, which is due to its thiol group, facilitates the

formation of mercaptide bonds with metals and reactions with selected electrophiles. Along with this reactivity, the relative stability and high water solubility of GSH makes it an ideal antioxidant to protect plants against stresses, including salt stress and oxidative stress (Alscher, 1989). Glutathione exists in two different forms in the living cells: reduced glutathione (GSH) and oxidized glutathione (GSSG). It is maintained almost exclusively in the reduced form in plants, hence glutathione is usually designated GSH. The relative proportion of GSH is normally greater under natural growth conditions. The physiological functions of glutathione have been mainly attributed to its reduced form in plants. Therefore, maintenance of a high proportion of GSH in plants is an essential requirement. Glutathione acts as a major antioxidant in plant cells and during its antioxidant reactions GSH is oxidized to GSSG. Further, plants have evolved mechanisms to maintain a high proportion of GSH, which is synthesized in both the chloroplasts and the cytosol of their leaves. The reduction of GSSG to GSH is catalyzed by GR, with the accompanying oxidation of NADPH (May *et al.*, 1998).

2.5.5 Additional proteins/enzymes

Glutathione S-transferase (GST) is a secondary defense enzyme that catalyzes the conjugation of the GSH molecule to a variety of chemical compounds and acts in the detoxification of herbicides. This conjugation is important in the synthesis of secondary metabolites, and GST also helps to prevent lipid peroxidation and DNA degradation. Thioredoxins also play redox regulatory roles in plants and protect organisms by scavenging reactive oxygen as well as regenerating oxidized proteins (Fernando *et al.*, 1992).

2.5.6 Carotenoids as antioxidants

Carotenoids are a group of more than 700 known lipid soluble tetra-terpenoids that are exclusively found in the plant kingdom. Two major subclasses of carotenoids are the xanthophyll (which contain oxygen) and the carotenoids (which are non-oxygenated and unsaturated hydrocarbons). Most carotenoids have antioxidant activity owing to their singlet oxygen and peroxyl radical quenching properties.

2.5.7 Vitamins as antioxidants

Several vitamins have antioxidant potential, including both those that are lipid soluble (A, E and K) and those that are water soluble (B and C) compound. The most important of all is ascorbate (vitamin C) which, together with GSH and other enzymatic antioxidants, excellently scavenges ROS and prevents the oxidative damage of macro-molecules (proteins, lipids and nucleic acids) in chloroplasts, mitochondria, peroxisomes and the cytosol and apoplast (Asensi-Faba-do and Munne-Bosch, 2010). Tocopherols (vitamin E) act as ROS quenchers in plastids, especially in thylakoid membranes, where they quench singlet oxygen and help to reduce lipid per-oxidation. In addition to vitamins C and E, vitamin B6 has also been proven to have potent antioxidant activity, which is mainly exerted via its potential to effectively scavenge singlet oxygen and superoxide anions. Sies *et al.* (1992) concluded that vitamins B6, C and E, in coordination with carotenoids, control singlet oxygen levels in chloroplasts and protect plants against photo-oxidative stress. Vitamin E acts at different locations in the chloroplast, such as reaction centre of photo-system II (PS-II) and inside the thylakoids, and effectively prevents lipid per-oxidation. In contrast to vitamin E, vitamins B6 and C are more complex in function owing to their multi-locational roles. Another vitamin B (B_1, thiamine) has also been shown to scavenge superoxide anions and hydroxyl radicals as evident from the studies conducted on Arabidopsis (Tunc-Ozdemir *et al.,* 2009). Under oxidative stress, the transcript levels of genes of the thiamine biosynthetic pathway were found to be up-regulated, leading to enhanced thiamine production. Although thiamine has been proved to have antioxidant properties, it still it is unclear whether it is involved directly or indirectly in the antioxidative defense system. However, a few reports suggest an indirect role of thiamine through the provision of more NAD(P)H under stress conditions. Finally, folates (vitamin B9), riboflavin (vitamin B_2), niacin (vitamin B_3) and phylloquinones (vitamin K_1), all are important regulators of the cellular redox state and hence are tightly involved in the complex network that makes up the anti-oxidative defense system.

2.5.8 Minerals as antioxidants

Minerals such as selenium (Se), copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) are involved in the antioxidant defense system either as a component of a wide variety of antioxidative enzymes and proteins, or because they are required to activate enzymes to make them functional. Cu, Zn, Fe and Mn are widely distributed in plants as a constituent of a very well-known enzyme of primary defence: SOD. As already noted, there are three main classes of SODs on the basis of the attached metal: Fe SODs (mostly located in plant chloroplasts) or Mn SODs (predominantly found in mitochondria and peroxisomes), and Cu/Zn SODs (present in chloroplasts, cytosols and extracellular spaces) and Ni SODs (which are primarily found in prokaryotes). In addition to being the constituents of metallo-enzymes, Cu and Zn ions also provide protection against oxidative stress by inducing cellular stress signaling to stabilize proteins and make them less prone to oxidation through mechanisms such as stimulation of anti-apoptotic phosphoinositide-3-kinase/Akt (protein kinase B) cascade (Klotz *et al.,* 2003). Selinium, being an important component of various antioxidant enzymes (e.g. GPx and thioredoxin reductase) is very well recognized as an essential micronutrient for humans, but recently its importance in the plant system has also been demonstrated. The antioxidant activity of Se as seleno-enzymes and seleno-proteins provides protection against oxidative stress caused by excess ROS and RNS. Plants convert Se mostly to Semethionine and this is incorporated in place of methionine into proteins, whereas Se-Cys and methyl-Se-Cys are not significantly incorporated into plant proteins. Recent studies have shown that Se, even at low concentrations, provides protection against various abiotic stresses, including low temperature, drought and metal toxicity by increasing the tolerance of plants to those stresses. In sorghum leaves, Se was also found to be protective against oxidative damage caused by high temperature stress (Djanaguiraman *et al.*, 2010).

2.5.9 Polyphenols as antioxidants

Polyphenols are polyhydroxylated phytochemicals that are widely distributed in plants and have diverse roles there in. They are potent antioxidants and are able to scavenge free radicals. Two major subclasses of antioxidant polyphenols are the flavonoids and the phenolic acids. Of the two, the flavonoids are more widely distributed and diversified in their roles.

Flavonoids are biosynthesized from two aromatic amino acids, phenylalanine and tyrosine, together with malonate. The basic framework of a flavonoid consists of 15 carbon atoms arranged in three rings (C_6-C_3-C_6), which are labeled as the A, B and C rings. There are various kinds of flavonoids, such as flavones, flavonols, flavanols, flavonones, isoflavones, anthocyanidins, etc. that differ from one another in the pattern of substitution of the C ring and the level of oxidation (Pietta, 2000). Flavonoids chelate metal ions and scavenge oxy radicals to form less reactive 'antioxidant radicals' that are further processed through dismutation and reduction (Samanta *et al.,* 2011). The antioxidative potential of flavonoids is supposed to be due to two structural elements: (i) conjugation of the 2,3-double bond and the 4-oxo group; and (ii) a 3′,4′- dihydroxy structure in the B ring (Croft, 2006). Kumar (2014) reported that Flavonoids promote antioxidant activity, cellular health and normal tissue growth and renewal throughout the body. They also work with vitamin C to reduce oxidative stress for the water based portion of the cell and may slow down some of the effects of aging. There are more than 4,000 unique flavonoids and they are most effective when several types are consumed together. Food sources include: cranberries, kale, beets, berries, red and black grapes, oranges, lemons, grapefruits and green tea (Banerjee *et al.,* 1993).

2.6 The role of antioxidants in plants

Stress perception and plant response interact via a huge network of molecules through high sensitivity detection and signal transduction pathways. Plants perceive stress signals in various ways, such as through receptors located on the plasma membrane, and intracellular or cytoskeleton-associated proteins. Transduction of the perceived signals is carried out by signal transduction cascades and results in altered gene expression programs that ultimately lead to metabolic adjustments and altered physiological responses. Salicylates (including salicylic acid, methyl salicylate and their glucosides) play an important role in signal perception and transmission in many plants (Shah and Klessig, 1999). Under normal conditions, the constitutive antioxidants in cells control the basal production of ROS, thereby maintaining their concentration. However, when plants are exposed to any adverse abiotic or biotic factors that induce the generation of ROS, this leads to a condition called oxidative stress in which there is an imbalance between ROS production and degradation. The role of the AA–GSH cycle in scavenging ROS, and especially H_2O_2, has been proven. The operation of the AA–GSH cycle involves sequential oxidation and re-reduction of AA (ascorbate) and GSH via four important enzymes – APx, MDHAR, DHAR and GR. The role of the AA–GSH cycle under conditions of biotic stress in plants is well documented (Baker *et al.,* 1995; De Gara, 2003).

The objective of this review is to make a state-of-the-art overview of pesticide uses, mechanisms of activity and their mutual interactions with plant and environment. For this purpose, a literature review was carried out focusing on new reports in the field of oxidative stress definition and parameters, pesticides induced oxidative stress and plant-derived compounds as antioxidants. Focusing mainly on the last 10 years articles considering mainly oxidative stress, plenty of articles were reviewed on the influence of pesticides on oxidative stress parameters on the cellular level. This review will discuss the potential benefits of antioxidant compounds in alleviating the side effects and toxicity associated with common environmental toxins such as pesticides.

The importance of this review is that it mainly aims to determine whether antioxidant can modulate the toxicity of environmental pollutants, thereby affecting health and the possibility of diseases generated by oxidative stress. Evidence will be provided that pesticide pollution increases oxidative stress level and plant-derived antioxidants may not play a role in neutralizing or buffering the effects of oxidizing pollutants.

This review summarizes also the most common sources of oxidative stress of plants, with an emphasis on pesticides and the potential to counter them with antioxidants. Due to extensive scientific data, not all studies could be included in this review. The reader is referred to the analysis of the references presented in the paper (and the references contained therein) in order to obtain details on the pesticides induced oxidative stresses and the scavenging role of antioxidants.

CHAPTER-III

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study was conducted in Professor Sultanul Alam Cytogenetic Laboratory and at research field of department of botany, University of Rajshahi, Rajshahi in two years trials from November, 2020 to March, 2021 and November 2021 to March, 2022 to find out the effect of antioxidant on pesticide induced cytomorphological consequences in hexaploid wheat. This chapter deals with the materials and methods that were used in carrying out the experiment.

3.1 Materials

3.1.1 Plant materials

The hexaploid wheat *(Triticum aestivum* L.) was used as testing material. Seeds were collected from Wheat Research Center of Bangladesh Agricultural Research Insitute (BARI), Shyampur, Rajshahi.

3.1.2 Mutagenic agent

One pesticide Tradelam 2.5 EC (Emulsifiable Concentration) was used as per recommended concentration for wheat with certain modification.

3.1.3 Antioxidant

To ascertain the anticlastogenic effect of the antioxidant comprised of vitamin and minerals three antioxidants prepared by different pharmaceuticals companies were used.

Sl. No.	Antioxidants	Unite of Measurement	Forms
1	Antioxidant 1 (A_1)	Vitamin-C- 60mg, E.-30mg, Zinc(as zinc oxide)-15mg, Copper(as cupric oxide)-2mg, Lutein-6mg.	Capsule
2	Antioxidant 2 (A_2)	Vitamin-C- 60mg, E.-30mg, Zinc(as zinc oxide)-15mg, Copper(as cupric oxide)-2mg, Lutein-6mg.	Capsule
3	Antioxidant 3 (A_3)	Vitamin-C- 60mg, E.-30mg, Zinc(as zinc oxide)-15mg, Copper(as cupric oxide)-2mg, Lutein-6mg.	Capsule

3.2 Methods

3.2.1 Preparation of treatments

3.2.1.1 Pre-treatment

Here, the seeds were treated with different antioxidant in three duration (1 hour, 1.5 hour and 2 hour) prior to pesticide treatment.

3.2.1.2 Post-treatment

In this case, the seeds were treated first with the pesticide in three different duration (1 hour, 1.5 hour and 2 hour) prior to treatment with antioxidant.

3.2.1.3 Preparation of antioxidant concentration

10 tablets of each three antioxidant were mixed with 200 ml water for preparing antioxidant solution.

3.2.1.4 Preparation of pesticide concentration

12.5 ml, 12.5 ml and 25 ml pesticide were mixed with 250 ml, 125 ml and125 ml water for preparing 0.05%, 0.1% and 0.2% pesticide concentration respectively.

3.2.1.5 Control

Seeds were treated only with 0.05%, 0.1% and 0.2% pesticide concentration and kept under three different duration i.e. 1hour, 1.5 hour and 2 hour.

3.2.2 Design

A three (03) Factors Randomized Complete Block Design (RCBD) was employed with three replications.

Factor A- Antioxidant with values from 1 to 4

A_0 (Control, without antioxidant)

A_1 (Antioxidant 1)

A_2 (Antioxidant 2)

A_3 (Antioxidant 3)

Factor C – Pesticide concentration with values from 1 to 3

C_1 (0.05%)

C_2 (0.1%)

C_3 (0.2%)

Factor D - Duration with values from 1 to 3 were maintained for both antioxidant and pesticide.

D_1 (1 hour)

D_2 (1.5 hour)

D_3 (2 hour)

3.2.2.1 No. of treatments

In case of pre-treatment - 4 X 3 X 3 =36

In case of post-treatment - 4 X 3X 3=36

3.2.2.2 Treatments combination

Pre-treatment				Post-treatment			
$T_1 = A_0C_1D_1$	$T_{10} = A_1C_1D_1$	$T_{19} = A_2C_1D_1$	$T_{28} = A_3C_1D_1$	$T_{37} = A_0C_1D_1$	$T_{46} = A_1C_1D_1$	$T_{55} = A_2C_1D_1$	$T_{64} = A_3C_1D_1$
$T_2 = A_0C_1D_2$	$T_{11} = A_1C_1D_2$	$T_{20} = A_2C_1D_2$	$T_{29} = A_3C_1D_2$	$T_{38} = A_0C_1D_2$	$T_{47} = A_1C_1D_2$	$T_{56} = A_2C_1D_2$	$T_{65} = A_3C_1D_2$
$T_3 = A_0C_1D_3$	$T_{12} = A_1C_1D_3$	$T_{21} = A_2C_1D_3$	$T_{30} = A_3C_1D_3$	$T_{39} = A_0C_1D_3$	$T_{48} = A_1C_1D_3$	$T_{57} = A_2C_1D_3$	$T_{66} = A_3C_1D_3$
$T_4 = A_0C_2D_1$	$T_{13} = A_1C_2D_1$	$T_{22} = A_2C_2D_1$	$T_{31} = A_3C_2D_1$	$T_{40} = A_0C_2D_1$	$T_{49} = A_1C_2D_1$	$T_{58} = A_2C_2D_1$	$T_{67} = A_3C_2D_1$
$T_5 = A_0C_2D_2$	$T_{14} = A_1C_2D_2$	$T_{23} = A_2C_2D_2$	$T_{32} = A_3C_2D_2$	$T_{41} = A_0C_2D_2$	$T_{50} = A_1C_2D_2$	$T_{59} = A_2C_2D_2$	$T_{68} = A_3C_2D_2$
$T_6 = A_0C_2D_3$	$T_{15} = A_1C_2D_3$	$T_{24} = A_2C_2D_3$	$T_{33} = A_3C_2D_3$	$T_{42} = A_0C_2D_3$	$T_{51} = A_1C_2D_3$	$T_{60} = A_2C_2D_3$	$T_{69} = A_3C_2D_3$
$T_7 = A_0C_3D_1$	$T_{16} = A_1C_3D_1$	$T_{25} = A_2C_3D_1$	$T_{34} = A_3C_3D_1$	$T_{43} = A_0C_3D_1$	$T_{52} = A_1C_3D_1$	$T_{61} = A_2C_3D_1$	$T_{70} = A_3C_3D_1$
$T_8 = A_0C_3D_2$	$T_{17} = A_1C_3D_2$	$T_{26} = A_2C_3D_2$	$T_{35} = A_3C_3D_2$	$T_{44} = A_0C_3D_2$	$T_{53} = A_1C_3D_2$	$T_{62} = A_2C_3D_2$	$T_{71} = A_3C_3D_2$
$T_9 = A_0C_3D_3$	$T_{18} = A_1C_3D_3$	$T_{27} = A_2C_3D_3$	$T_{36} = A_3C_3D_3$	$T_{45} = A_0C_3D_3$	$T_{54} = A_1C_3D_3$	$T_{63} = A_2C_3D_3$	$T_{72} = A_3C_3D_3$

3.3 Seed treatment and germination

Seeds from the both pre and post treatment were washed frequently with tap water. The seeds for control were also washed in distilled water. Seeds from both treatment and control were allowed to germinate in the laboratory at room temperature and in pot. These seeds of pre and post treatment were designated as M1 generation. The M2 seeds collected from M1 plants (raised from the seeds treated previously) were left untreated and were sown in next year. Then data were collected for morphological and cytological study. This experiment was conducted in Professor S. Alam Cytogenetics Laboratory Department of Botany, University of Rajshahi, Rajshahi in the growing season of November, 2020 to March, 2021 and November 2021 to March, 2022.

3.4 Morphological features

For morphological studies plants raised from treated seeds in all the doses were grown in pots with three replications and data were recorded on following morphological characters.

3.4.1 Germination percentage

Some of the treated seeds (20 seeds) were placed on moist filter paper in petridishes and it was also made in earthen pots containing well manured soil following three replications for germination test. After three or four days of setting the experiment in laboratory and after seven days of sowing the seeds in pots germination percentages were estimated through following formula.

$$\% \text{ of germination} = \frac{\text{No. of germinated seed}}{\text{Total no. of seed}} \times 100$$

3.4.2 Coleoptile and root length

Data on coleoptile at 7 days after germination (DAG) and root length were recorded from the living materials only. Seedlings were picked up for recording the data on coleoptile and root length. These were measured in cm and root length was recorded from the first root at 7 days, 14 days and 25 days after germination (cm) in laboratory.

3.4.3 Plant height at 7 days, 14 days and 25 days after germination (cm) in laboratory and in pot

Plant height at different days after germination (DAG) both in laboratory and in pot were measured in centimeters (cm) by ruler, from the base of the stem (at the soil or petri dish surface) to the top of the canopy, or the highest part of the plant and values were recorded in register for analysis. Data were recorded on 10 (ten) plants from each three replications of every treatment.

3.4.4 Flag leaf length (cm)

Flag leaf length was measured as the distance from the base to the tip of the leaf. And data on flag leaf length were recorded on 10 (ten) plants from each three replications of every treatment in centimeter (cm) using ruler.

3.4.5 Number of tiller / plant

Number of tiller per plant were counted from 10 (ten) plants of each three replications of every treatment and data were recorded in register for analysis.

3.4.6 Number of spikelet / spike

After harvesting, 10 (ten) spike of each three replications of every treatment, number of spikelet were counted and data were recorded in register for analysis.

3.4.7 Number of seeds / spike

After harvesting 10 (ten) spike of each three replications of every treatment, number of seed were counted and data were recorded in register for analysis.

3.4.8 Length of inflorescence (with awn)

Length of inflorescence (with awn) was measured as the distance from the base to the tip of the awn. And data on length of inflorescence (with awn) were recorded on 10 (ten) plants from each three replications of every treatment in centimeter (cm) using ruler.

3.4.9 Length of inflorescence (without awn)

Length of inflorescence (without awn) was measured from the base to the tip of the inflorescence in centimeter (cm) using ruler. And data on length of inflorescence (without awn) were recorded on 10 (ten) plants from each three replications of every treatment.

3.4.10 100 seed weight (gm)

100 seeds were collected from each three replications of every treatment and weight was measured using electronic balance machine for data analysis.

3.4.11 Total seed weight (gm)

Total seed from each three replications of every treatment were collected and total seed weight was measured using electronic balance machine for data analysis.

3.5 Cytological features

3.5.1 Mitosis

3.5.1.1 Collection and fixation of root tips

Root tips of 1.0-1.5 cm in length were collected from each treatment and were fixed into 1:3 acetoalcohol and after 48 hours of fixation, they were transferred to 70% ethanol and stored in refrigerator until they were used. It was accomplished from germinating seeds in laboratory.

3.5.1.2 Staining of root tips

In order to collect mitotic data and to study interphase chromosome volume from root tip cells temporary slides were prepared by squash method of Haque *et al.* (1976) using haematoxylin with certain modifications as follows:

- After removing from 70% ethanol, the root tips were washed with frequent changes of distilled water for 5-6 minutes.
- The root tips were then transferred to 50% HCl for about 30 minutes to dissolve the middle lamella of cells.
- The root tips were washed again with distilled water for 5 minutes.
- After washing root tips with distilled water, mordanting was done by 2 % aqueous solution of iron alum (Ferric ammonium sulphate) for 15 minutes.
- Then root tips were washed again with frequent changes of distilled water for 7 minutes in order to complete removal of the mordanting fluid from the tissues.
- The root tips were stained with 0.5% haematoxylin stain for 30 minutes.
- Finally the root tips were washed with distilled water for 6 minutes.

3.5.1.3 Preparation of slides

One stained root tip was taken on to a clean slide and 1 -1.5mm portion of it was cut with a sharp blade and squashed by a plastic tapper in a drop of 0.5% acetocarmine. The cells were then covered with a cover glass and slight pressure was applied by a pencil eraser over the cover glass. The slide was warmed over an alcohol flame and gentle pressure was applied by fingertips over the cover glass keeping the slide duly wrapped in blotting paper. Slides were then observed under microscope and the data were recorded on mitosis abnormalities from different stages. Percentage of mitotic abnormality were estimated as follows.

$$\% \text{ of mitotic abnormality} = \frac{\text{Total no. of aberrant cells}}{\text{Total no. of cells in division}} \times 100$$

3.5.1.4 Mitotic index

After the preparation of temporary slide, dividing and non-dividing cells were counted under the microscope. A good number of cells were counted from three to four preparations. The mitotic index was obtained as follows:

$$\text{Mitotic Index (MI)} = \frac{\text{No. of dividing cells}}{\text{No. of studied cells (dividing + non-dividing)}} \times 100$$

3.5.1.5 Determination of interphase chromosome volume (ICV)

In order to calculate the interphase chromosome volume from root tip cells, nuclear volume was measured by oculometer and converted the values into micron (µ) with the help of a stage micrometer. The nuclear volume (NV) was calculated using the formula for a sphere, $NV = 4/3\ \pi r^3$ (Nayer *et al.,* 1970). The mean nuclear volume divided by the somatic chromosome number gave the interphase chromosome volume.

3.5.2 Meiosis

3.5.2.1 Collection and fixation of inflorescence

During the proper growth of the plant, young inflorescence from plants of various treatments along with control were collected and immediately fixed in modified carnoy's fixative (6:3:1: ethanol: chloroform: acetic acid). After 48 hours the ear heads were transferred to 70% ethanol and stored in the refrigerator for meiotic study.

3.5.2.2 Staining of anther and preparation of temporary slides

To collect data on meiotic abnormalities temporary slides were prepared from suitable anthers by acetocarmine smear technique. Young anther was placed on a clean slide and a drop of 2% acetocarmine was added. The anther wall was then crushed by a curved dissecting needle and the anther wall was removed. Then the pollen mother cells were covered with a cover glass, warmed gently over an alcohol flame and then a slight pressure was exerted by thumb or finger tip to spread out the chromosomes. Additional heating was applied as needed until the cytoplasm becomes clear. Care was also taken at the time of heating so as to prevent overheating. Cytological screening for chromosomal anomalies was carried out at all stages of meiosis from metaphase I to telophase II. Different abnormalities were recorded in the record book and photomicrographs were taken from the desired preparation. Meiotic abnormality was calculated following the formula given below.

$$\% \text{ of meiotic abnormality} = \frac{\text{Total no. of aberrant cells}}{\text{Total no. of cells in division}} \times 100$$

3.5.2.3 Collection of data on pollen sterility

A mature anther was taken on to a clean slide with a drop of 2% acetocarmine. The anther wall was then ruptured with the help of curved dissecting needle and pressure was applied to squeeze out its pollen grains in the fluid. The anther wall was then removed and the entire fluid containing pollen grains was covered with a cover glass and observed under

microscope. Stained pollen grains were counted as fertile. A total number of fertile and sterile pollen grains were scored and percentages were calculated as follows:

$$\% \text{ of pollen sterility} = \frac{\text{Total No. of sterile pollen grain}}{\text{Total No. of fertile + sterile pollen grain}} \times 100$$

3.5.2.4 Pollen abnormality

Conger and Fairchild (1953) were interested in establishing an improved method of pollen culture for cytological studies. For studying pollen cytology the procedure of Jagathesan and Sreenivasan (1966) was modified to fit wheat pollen grains. The complete procedure used was as follows:

1. Ripe anthers were collected at anthesis time and were treated with 0.2% colchicines solution in a small vial for 1hr at 26-28ºC.

2. Anthers were washed in water 2-4 minutes and then treated with a solution of 0.002M hydroxyquinoline for 1hr at 26-28ºC and they were washed in water for 10 minutes.

3. Anthers were fixed for 24 hours in Ostergren and Heneen's solution that was composed of methanol 60ml, chloroform 30ml, distilled water 20ml, picric acid 1gm and mercuric chloride 1gm. At the end of fixation period the material was stored for a period up to 6-8 weeks in 70% methyl alcohol in a refrigerator.

4. The anthers were hydrated in 70% and 35% methyl alcohol then washed by distilled water.

5. The anthers were hydrolyzed in 1N HCl for 20 minutes then washed in water.

6. The anthers were stained in leuco-basic fuchsin (Schiff's reagent) for 30-60 minutes.

7. The anthers were smeared into a drop of aceto-carmine stain and gently heated.

8. A cover glass was placed on the top of the material and gently tapped by keeping the slide between folds of a filter paper and were given uniform pressure to prevent the formation of fissures in pollen grain wall.

9. The cover slip was sealed with wax for longer study under the microscope.

10. For permanent preparations the sealed slides were stored in the refrigerator for 1/2 hour.

11. The wax was removed by a needle and the slide was immersed in a mixture of glacial acetic acid and n-butyl alcohol 1:1 to remove the cover glass.

12. The slide was transferred to n-butyl alcohol for two changes. The slides were kept for about two minutes in each change.

13. Canada balsam was used for mounting to get a permanent preparation.

$$\% \text{ of pollen abnormality} = \frac{\text{Total No. of abnormal pollen}}{\text{Total No. of normal + abnormal pollen}} \times 100$$

3.6 Data analysis

The findings from this experiment were statistically analyzed following two way Analysis of Variance (ANOVA) using MSTAT-C package program (versin 3.5) to assess the significant differences in the mean values of different treatments.

For analysis the data following statistical formula were used and these are as follows:

i) Mean:

$$\bar{X} = \frac{\sum x}{N}$$

Where,

$\bar{X}$=Mean

Σ= Summation

x = The individual reading from each plant or for each variety

N= Number of observations.

(ii) Standard deviation (Sd):

$$Sd = \sqrt{s^2} \text{ or } Sd = \sqrt{EMS}$$

Where,

S^2= Variance

EMS = Error mean square

CHAPTER-IV

RESULTS

4. RESULTS

The results obtained from the present investigation are described under following heads:

4.1 Result of M1 generation

4.1.1 Morphological features

In this investigation, various morphological characters as mentioned earlier were considered for morphological study.

4.1.1.1 Germination percentage

Data on germination percentage from laboratory and pot are presented in **Histogram 1-2 and Table 1.**

Pre-treatment: Treatment $A_3C_1D_1$ in laboratory and $A_3C_1D_2$ in pot exhibited highest germination percentage 94.67% and 86.00% respectively which were both significantly different from that of all other treatments. On the other hand treatment $A_0C_3D_3$ both in laboratory and in pot showed lowest germination percentage 39.33% and 37.00% respectively which were also significantly different from that of all other treatments.

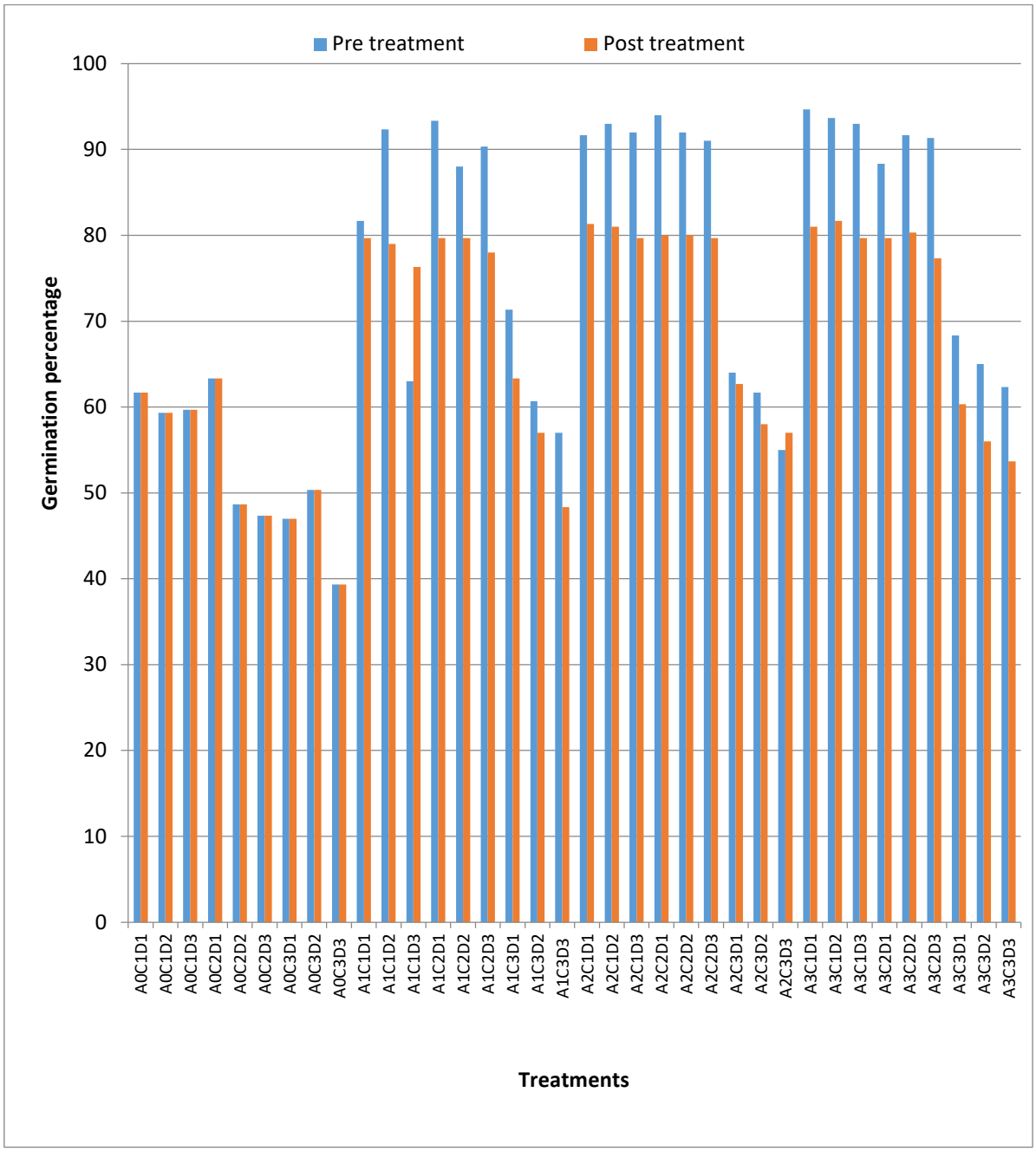
Post-treatment: Treatment $A_3C_1D_2$ in laboratory and $A_1C_1D_2$ in pot showed highest germination percentage 81.67% and 75.67% respectively which were both significantly different from that of all other treatments. On the other hand treatment $A_0C_3D_3$ both in laboratory and in pot showed lowest germination percentage 39.33% and 37.00% respectively which were significantly different from that of all other treatments.

4.1.1.2 Coleoptile length

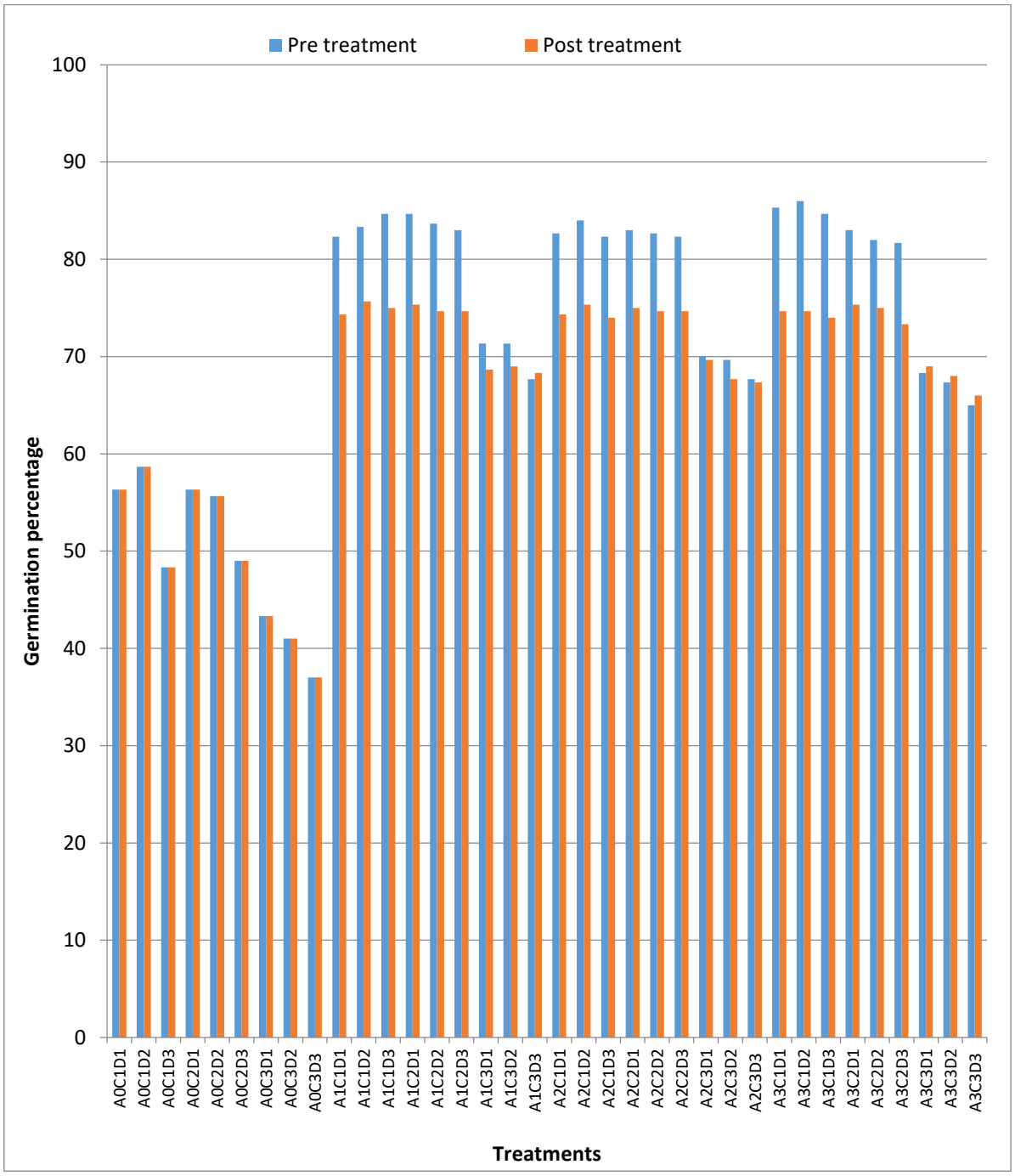
Collected data on coleoptile length are showed in **Histogram 3 and Table 1.**

Pre-treatment: It was observed that at 7 days after germination produced longest coleoptile (3.72cm) at $A_3C_3D_1$ **(Table 1)** and that was found statistically nonsignificant. The shortest coleoptile length (0.72cm) was found at $A_3C_3D_3$ which was also nonsignificant.

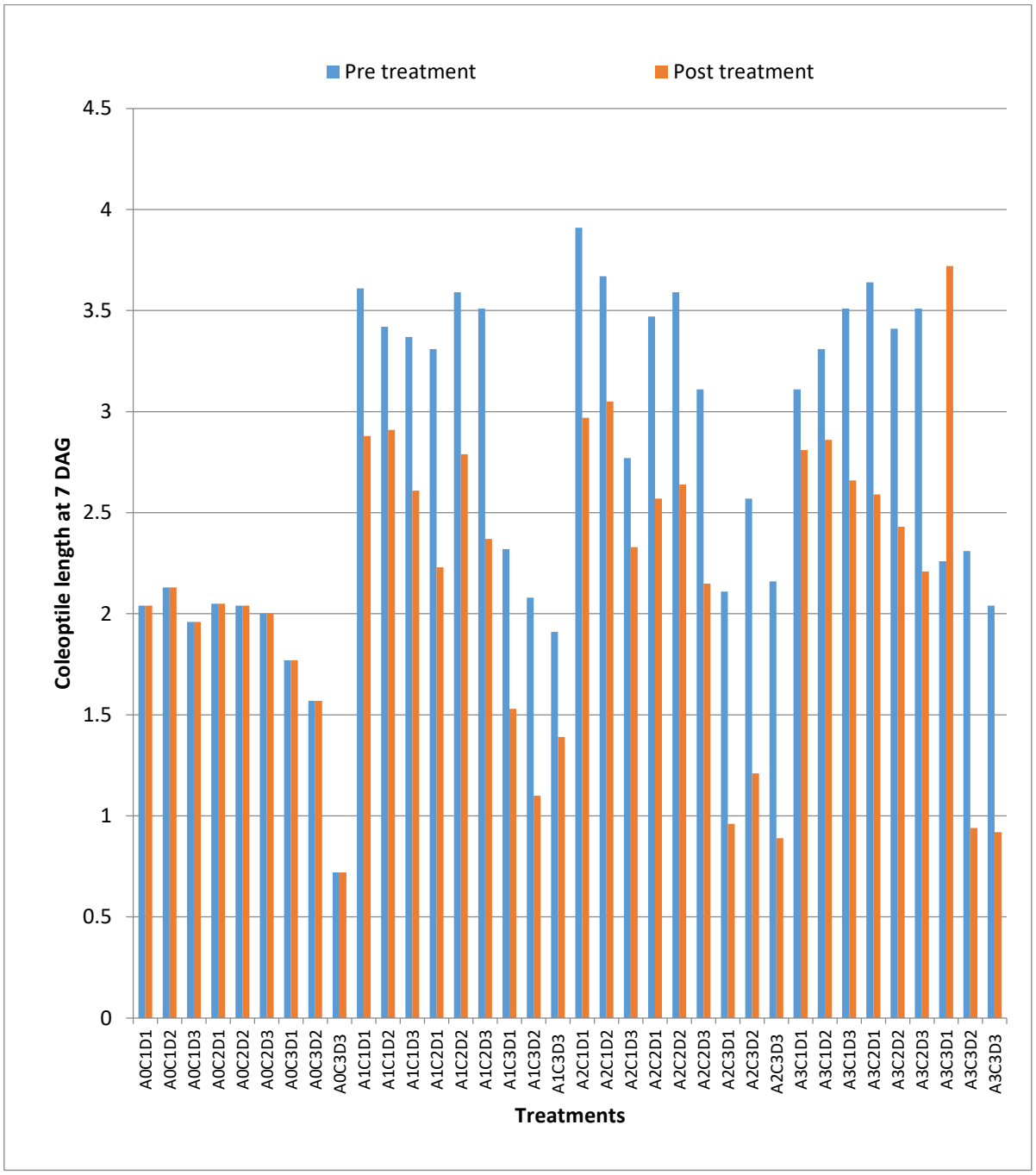
Post-treatment: At 7 days after germination (DAG) longest coleoptile (3.91cm) was found in treatment $A_2C_1D_1$ **(Table 1)** which was not significant. On the other hand shortest coleoptile (0.72cm) was found in treatment $A_0C_3D_3$ which was statistically not significant from that of all other treatments.



Histogram 1: Effect of antioxidant along with control on germination percentage of wheat induced with different concentration and duration of pesticide in laboratory in M1 generation.



Histogram 2: Effect of antioxidant along with control on germination percentage of wheat induced with different concentration and duration of pesticide in pot in M1 generation.



Histogram 3: Effect of antioxidant along with control on coleoptile length at 7 days after germination of wheat induced with different concentration and duration of pesticide in M1 generation.

Table 1: Effect of antioxidants along with control on germination % (in laboratory and in pot) of wheat induced with different concentration and duration of pesticide in M1 generation

Treatment	Pre-treatment			Post-treatment		
	Germination %		Coleoptile length (cm)	Germination %		Coleoptile length (cm)
	in laboratory	in pot	in laboratory	in laboratory	in pot	in laboratory
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
$A_0C_1D_1$	61.67 ± 0.58	56.33 ± 2.31	2.04 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
$A_0C_1D_2$	59.33 ± 1.53	58.67 ± 0.58	2.13 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
$A_0C_1D_3$	59.67 ± 1.53	48.33 ± 2.89	1.96 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
$A_0C_2D_1$	63.33 ± 1.53	56.33 ± 1.53	2.05 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
$A_0C_2D_2$	48.67 ± 2.31	55.67 ± 1.53	2.04 ± 0.05	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
$A_0C_2D_3$	47.33 ± 2.08	49.00 ± 3.61	2.00 ± 0.01	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
$A_0C_3D_1$	47.00 ± 2.65	43.33 ± 2.89	1.77 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
$A_0C_3D_2$	50.33 ± 1.53	41.00 ± 1.00	1.57 ± 0.12	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
$A_0C_3D_3$	39.33 ± 1.15	37.00 ± 1.73	0.72 ± 0.32	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
$A_1C_1D_1$	81.67 ± 2.89	82.33 ± 4.04	3.61 ± 0.19	79.67 ± 1.53	74.33 ± 0.58	2.88 ± 0.01
$A_1C_1D_2$	92.33 ± 2.52	83.33 ± 3.51	3.42 ± 0.41	79.00 ± 1.00	75.67 ± 2.08	2.91 ± 0.09
$A_1C_1D_3$	63.00 ± 2.89	84.67 ± 1.53	3.37 ± 0.54	76.33 ± 0.58	75.00 ± 1.00	2.61 ± 0.16
$A_1C_2D_1$	93.33 ± 2.89	84.67 ± 1.53	3.31 ± 0.09	79.67 ± 1.15	75.33 ± 0.58	2.23 ± 0.35
$A_1C_2D_2$	88.00 ± 2.65	83.67 ± 1.15	3.59 ± 0.44	79.67 ± 1.53	74.67 ± 0.58	2.79 ± 0.22
$A_1C_2D_3$	90.33 ± 0.58	83.00 ± 1.00	3.51 ± 0.47	78.00 ± 1.00	74.67 ± 0.58	2.37 ± 0.31
$A_1C_3D_1$	71.33 ± 1.15	71.33 ± 2.08	2.32 ± 0.36	63.33 ± 2.31	68.67 ± 2.08	1.53 ± 0.25
$A_1C_3D_2$	60.67 ± 0.58	71.33 ± 3.06	2.08 ± 0.23	57.00 ± 2.00	69.00 ± 1.73	1.10 ± 0.08
$A_1C_3D_3$	57.00 ± 3.46	67.67 ± 3.21	1.91 ± 0.31	48.33 ± 2.89	68.33 ± 3.51	1.39 ± 0.43
$A_2C_1D_1$	91.67 ± 1.53	82.67 ± 3.06	3.91 ± 0.03	81.33 ± 0.58	74.33 ± 1.53	2.97 ± 0.34
$A_2C_1D_2$	93.00 ± 1.73	84.00 ± 3.61	3.67 ± 0.13	81.00 ± 1.00	75.33 ± 1.53	3.05 ± 0.24
$A_2C_1D_3$	92.00 ± 2.00	82.33 ± 4.04	2.77 ± 0.46	79.67 ± 0.58	74.00 ± 2.00	2.33 ± 0.41
$A_2C_2D_1$	94.00 ± 1.00	83.00 ± 2.65	3.47 ± 0.45	80.00 ± 1.00	75.00 ± 1.00	2.57 ± 0.32
$A_2C_2D_2$	92.00 ± 1.00	82.67 ± 2.52	3.59 ± 0.13	80.00 ± 1.00	74.67 ± 0.58	2.64 ± 0.06
$A_2C_2D_3$	91.00 ± 1.73	82.33 ± 1.53	3.11 ± 0.28	79.67 ± 1.53	74.67 ± 0.58	2.15 ± 0.40
$A_2C_3D_1$	64.00 ± 3.61	70.00 ± 2.00	2.11 ± 0.86	62.67 ± 3.06	69.67 ± 1.53	0.96 ± 0.22
$A_2C_3D_2$	61.67 ± 1.53	69.67 ± 2.52	2.57 ± 0.48	58.00 ± 2.65	67.67 ± 3.06	1.21 ± 0.15
$A_2C_3D_3$	55.00 ± 4.00	67.67 ± 2.31	2.16 ± 0.56	57.00 ± 2.65	67.33 ± 2.52	0.89 ± 0.43
$A_3C_1D_1$	94.67 ± 0.58	85.33 ± 1.15	3.11 ± 0.11	81.00 ± 1.00	74.67 ± 1.53	2.81 ± 0.10
$A_3C_1D_2$	93.67 ± 0.58	86.00 ± 0.00	3.31 ± 0.42	81.67 ± 2.08	74.67 ± 0.58	2.86 ± 0.12
$A_3C_1D_3$	93.00 ± 1.73	84.67 ± 0.58	3.51 ± 0.53	79.67 ± 1.53	74.00 ± 1.00	2.66 ± 0.30
$A_3C_2D_1$	88.33 ± 0.58	83.00 ± 1.73	3.64 ± 0.32	79.67 ± 2.08	75.33 ± 0.58	2.59 ± 0.25
$A_3C_2D_2$	91.67 ± 2.89	82.00 ± 1.00	3.41 ± 0.42	80.33 ± 0.58	75.00 ± 1.00	2.43 ± 0.43
$A_3C_2D_3$	91.33 ± 0.58	81.67 ± 1.53	3.51 ± 0.62	77.33 ± 1.53	73.33 ± 0.58	2.21 ± 0.22
$A_3C_3D_1$	68.33 ± 5.77	68.33 ± 4.62	2.26 ± 0.09	60.33 ± 1.53	69.00 ± 0.00	3.72 ± 0.15
$A_3C_3D_2$	65.00 ± 5.00	67.33 ± 3.79	2.31 ± 0.57	56.00 ± 1.00	68.00 ± 1.00	0.94 ± 0.43
$A_3C_3D_3$	62.33 ± 2.52	65.00 ± 4.00	2.04 ± 0.48	53.67 ± 3.51	66.00 ± 2.65	0.92 ± 0.07
Level of significance	*	*	NS	**	*	NS
CV(%)	11.11	2.14	13.51	2.61	2.11	18.51

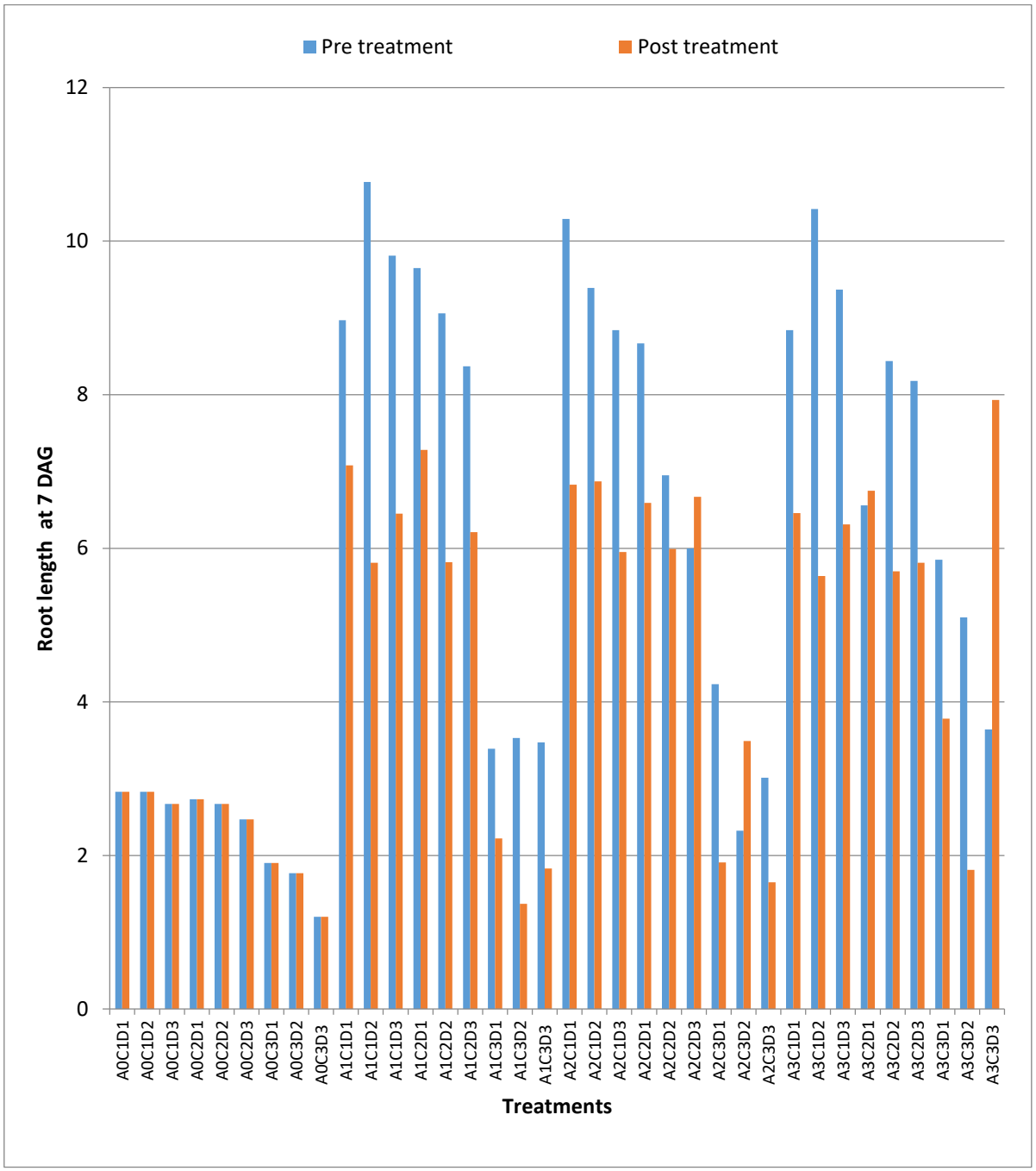
Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.1.1.3 Root length

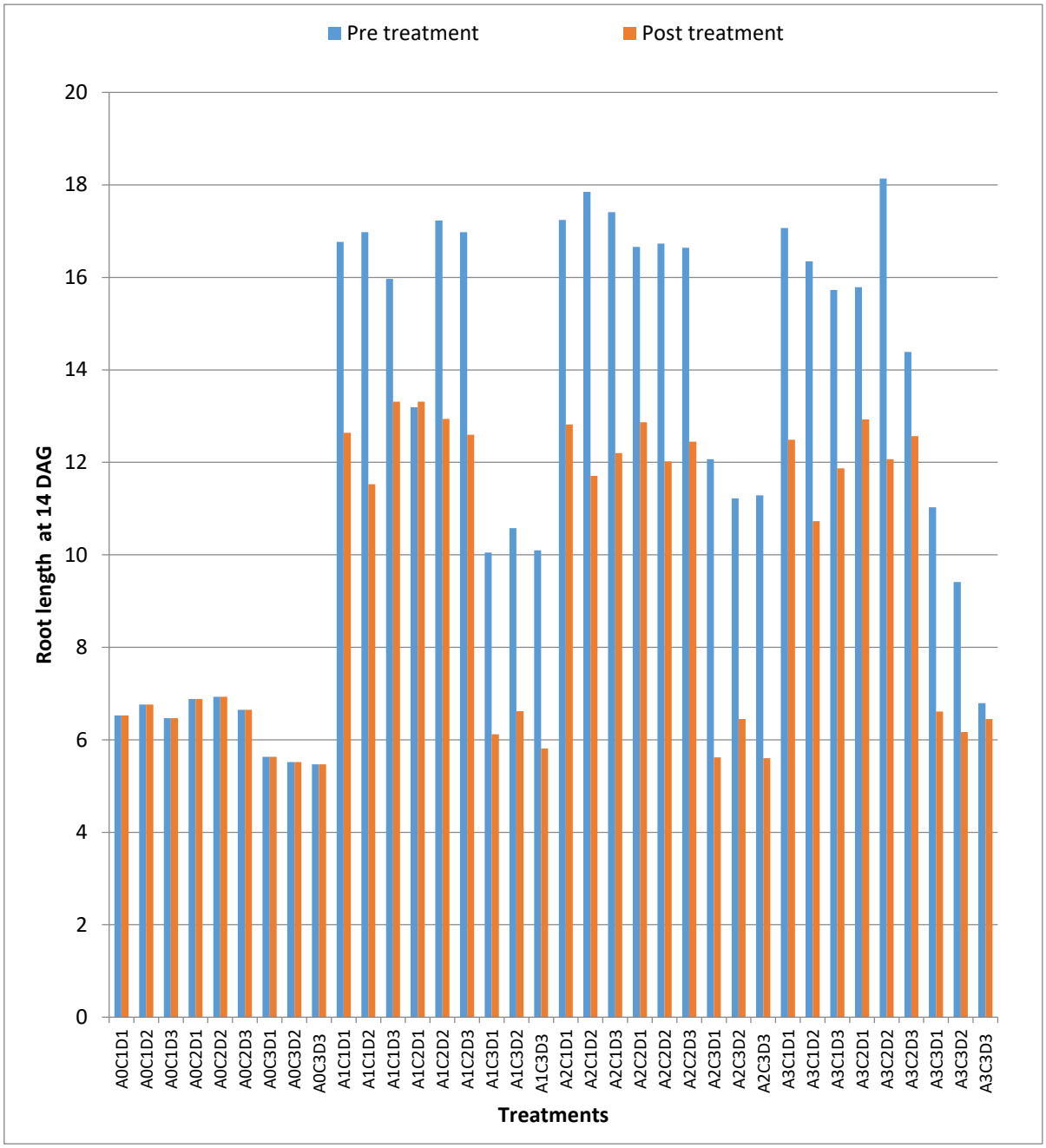
Root lengths at different days after germination are presented in **Histogram 4(a), 4(b), 4(c) and Table 2.**

Pre-treatment: At 7 days after germination (DAG) longest root (10.77cm) was found in treatment $A_1C_1D_2$ and shortest root (1.20cm) was observed at $A_0C_3D_3$ which were both statistically different from that of all other treatments. At 14 days after germination (DAG) longest root (18.14cm) was observed at $A_3C_2D_2$ and shortest root (5.47cm) was observed at $A_0C_3D_3$ which were both statistically different from that of all other treatments. At 25 days after germination (DAG) longest root (24.14cm) was observed at $A_1C_1D_3$. On the other hand shortest root (11.43cm) was observed at $A_0C_3D_3$ which was also statistically nonsignificant.

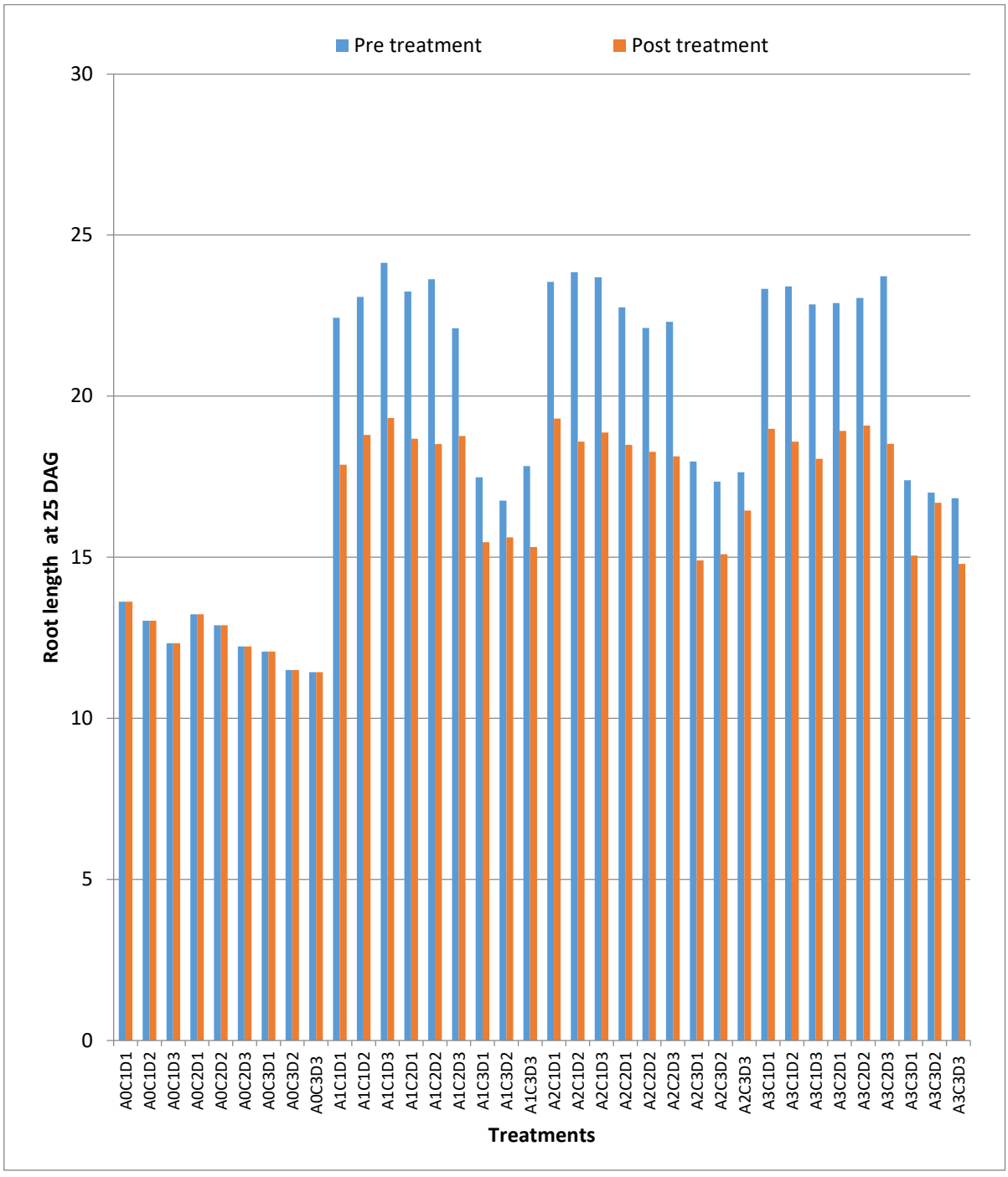
Post-treatment: At 7 days after germination (DAG) longest root (7.93 cm) was observed in treatment $A_3C_3D_3$ and shortest root (1.20cm) was observed at $A_0C_3D_3$ which were both statistically different from that of all other treatments. At 14 days after germination (DAG) longest root length (13.31cm) was observed at $A_1C_1D_3$ and $A_1C_2D_1$, and shortest root length (5.47cm) was observed at $A_0C_3D_3$ which were both statistically different from that of all other treatments. At 25 days after germination (DAG) highest value for root length (19.32cm) was observed at $A_1C_1D_3$ and lowest (11.43cm) was found at $A_0C_3D_3$ which were both statistically nonsignificant.



Histogram 4(a): Effect of antioxidant along with control on root length at 7 days after germination of wheat induced with different concentration and duration of pesticide in M1 generation.



Histogram 4(b): Effect of antioxidant along with control on root length at 14 days after germination of wheat induced with different concentration and duration of pesticide in M1 generation.



Histogram 4(c): Effect of antioxidant along with control on root length at 25 days after germination of wheat induced with different concentration and duration of pesticide in M1 generation.

Table 2: Effect of antioxidants along with control on root length (cm) at 7, 14 & 25 DAG of wheat induced with different concentration and duration of pesticide in M1 generation.

Treatment	Pre-treatment			Post-treatment		
	Root length (cm) 7 DAG	Root length (cm) 14 DAG	Root length (cm) 25 DAG	Root length (cm) 7 DAG	Root length (cm) 14 DAG	Root length (cm) 25 DAG
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	2.83 ± 0.70	6.53 ± 0.29	13.62 ± 0.33	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	2.83 ± 0.06	6.76 ± 0.23	13.03 ± 0.40	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	2.67 ± 0.42	6.47 ± 0.38	12.33 ± 0.14	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	2.73 ± 0.32	6.88 ± 0.07	13.23 ± 0.49	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	2.67 ± 0.25	6.93 ± 0.08	12.89 ± 0.09	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	2.47 ± 0.06	6.65 ± 0.10	12.23 ± 0.25	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	1.90 ± 0.10	5.63 ± 0.32	12.07 ± 0.55	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	1.77 ± 0.12	5.52 ± 0.51	11.50 ± 0.61	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	1.20 ± 0.26	5.47 ± 0.55	11.43 ± 0.49	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	8.97 ± 0.81	16.77 ± 0.78	22.43 ± 1.01	7.08 ± 0.68	12.64 ± 0.88	17.87 ± 0.32
A1C1D2	10.77 ± 0.39	16.98 ± 1.30	23.08 ± 0.60	5.81 ± 0.76	11.53 ± 1.31	18.80 ± 0.26
A1C1D3	9.81 ± 1.29	15.97 ± 0.96	24.14 ± 0.84	6.45 ± 0.53	13.31 ± 0.33	19.32 ± 0.55
A1C2D1	9.65 ± 0.50	13.19 ± 5.59	23.25 ± 1.69	7.28 ± 1.08	13.31 ± 0.13	18.68 ± 0.21
A1C2D2	9.06 ± 1.85	17.23 ± 0.61	23.63 ± 0.58	5.82 ± 0.62	12.94 ± 0.61	18.51 ± 0.49
A1C2D3	8.37 ± 0.73	16.98 ± 0.72	22.11 ± 0.46	6.21 ± 0.45	12.60 ± 0.65	18.76 ± 0.44
A1C3D1	3.39 ± 1.23	10.05 ± 0.37	17.48 ± 0.64	2.22 ± 0.85	6.12 ± 0.12	15.47 ± 1.82
A1C3D2	3.53 ± 1.29	10.58 ± 1.74	16.76 ± 1.36	1.37 ± 0.40	6.62 ± 0.51	15.62 ± 0.75
A1C3D3	3.47 ± 1.79	10.10 ± 0.64	17.83 ± 0.99	1.83 ± 1.22	5.81 ± 0.63	15.32 ± 0.52
A2C1D1	10.29 ± 3.21	17.24 ± 0.62	23.55 ± 0.51	6.83 ± 1.25	12.82 ± 0.78	19.30 ± 0.51
A2C1D2	9.39 ± 1.14	17.85 ± 0.20	23.85 ± 0.90	6.87 ± 1.09	11.71 ± 1.42	18.59 ± 0.51
A2C1D3	8.84 ± 0.94	17.41 ± 0.66	23.69 ± 1.40	5.95 ± 0.89	12.20 ± 0.83	18.87 ± 0.63
A2C2D1	8.67 ± 0.32	16.66 ± 0.60	22.76 ± 1.74	6.59 ± 0.92	12.87 ± 0.81	18.49 ± 0.60
A2C2D2	6.95 ± 1.68	16.73 ± 0.65	22.12 ± 0.50	5.99 ± 0.28	12.02 ± 0.18	18.27 ± 0.63
A2C2D3	6.00 ± 0.85	16.64 ± 1.21	22.31 ± 0.70	6.67 ± 0.23	12.45 ± 1.16	18.13 ± 0.98
A2C3D1	4.23 ± 1.31	12.07 ± 1.54	17.97 ± 0.04	1.91 ± 0.16	5.62 ± 0.54	14.90 ± 0.86
A2C3D2	2.32 ± 0.54	11.22 ± 0.92	17.35 ± 0.67	3.49 ± 0.29	6.45 ± 0.39	15.09 ± 2.26
A2C3D3	3.01 ± 1.59	11.29 ± 1.38	17.64 ± 1.11	1.65 ± 0.65	5.60 ± 0.48	16.45 ± 2.91
A3C1D1	8.84 ± 1.63	17.07 ± 0.63	23.33 ± 1.14	6.46 ± 0.61	12.49 ± 0.45	18.99 ± 0.28
A3C1D2	10.42 ± 1.80	16.35 ± 1.32	23.41 ± 1.35	5.64 ± 0.59	10.73 ± 0.51	18.59 ± 0.52
A3C1D3	9.37 ± 1.74	15.73 ± 1.89	22.85 ± 0.75	6.31 ± 1.40	11.87 ± 0.93	18.06 ± 0.10
A3C2D1	6.56 ± 0.16	15.79 ± 0.55	22.89 ± 0.91	6.75 ± 0.22	12.93 ± 0.60	18.92 ± 0.19
A3C2D2	8.44 ± 0.73	18.14 ± 0.70	23.05 ± 1.00	5.70 ± 0.55	12.07 ± 0.79	19.09 ± 0.63
A3C2D3	8.18 ± 1.57	14.39 ± 0.62	23.72 ± 0.95	5.81 ± 0.08	12.57 ± 1.34	18.52 ± 0.68
A3C3D1	5.85 ± 1.29	11.03 ± 1.52	17.39 ± 0.45	3.78 ± 0.96	6.61 ± 1.39	15.05 ± 1.70
A3C3D2	5.10 ± 0.29	9.41 ± 1.49	17.01 ± 1.31	1.81 ± 1.38	6.17 ± 0.89	16.69 ± 0.88
A3C3D3	3.64 ± 0.33	6.79 ± 0.32	16.83 ± 0.56	7.93 ± 0.32	6.45 ± 2.02	14.79 ± 2.09
Level of significance	*	*	NS	*	*	NS
CV(%)	19.32	10.46	4.57	14.55	8.27	5.55

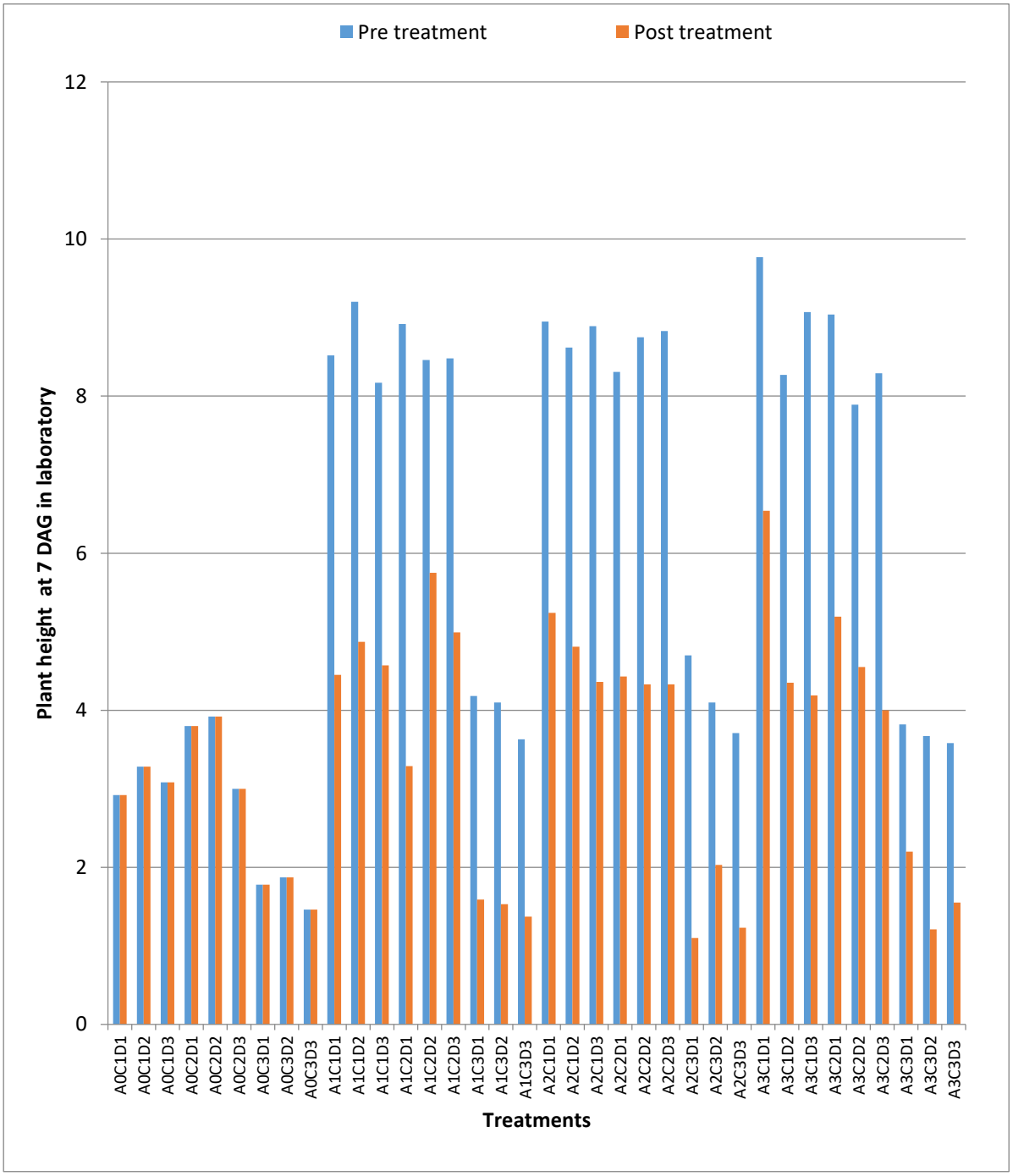
Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.1.1.4 Plant height in laboratory

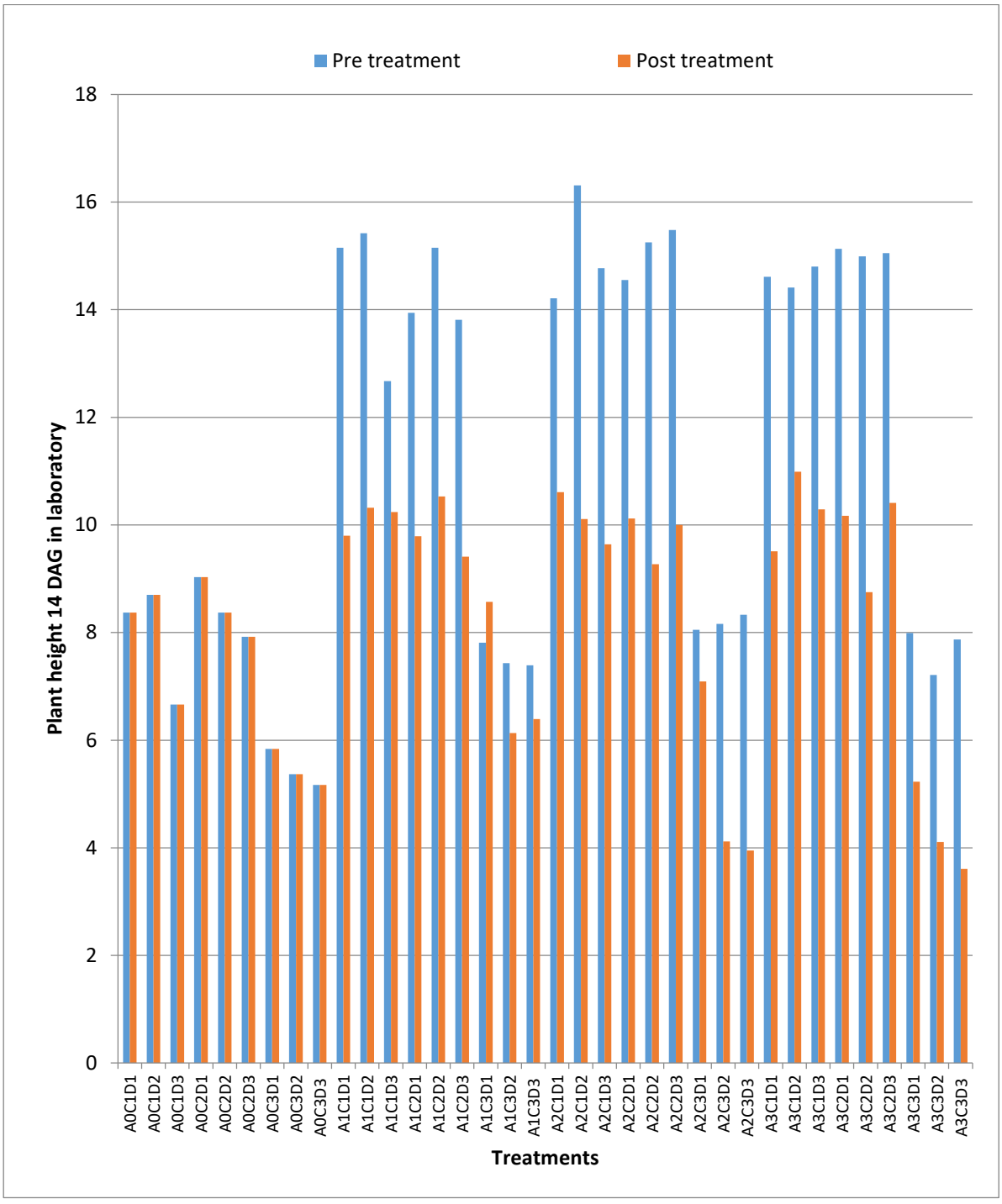
Plant height at different days after germination (DAG) in laboratory is presented in **Histogram 5(a), 5(b), 5(c) and Table 3.**

Pre-treatment: At 7 days after germination treatment (DAG) $A_3C_1D_1$ showed highest value (9.77cm) for plant height was not significantly different from all other treatments. On the other hand lowest value (1.46cm) was found at $A_0C_3D_3$ which was statistically non-significant. At 14 days after germination (DAG) highest value (16.31cm) was observed at $A_2C_1D_2$ and lowest value (5.17) was observed at $A_0C_3D_3$ both were significantly different from that of all other treatments. At 25 days after germination treatment $A_1C_2D_1$ showed highest plant height (25.36cm) and treatment $A_0C_3D_3$ showed lowest plant height (10.48cm) which were both significantly different from that of all other treatments.

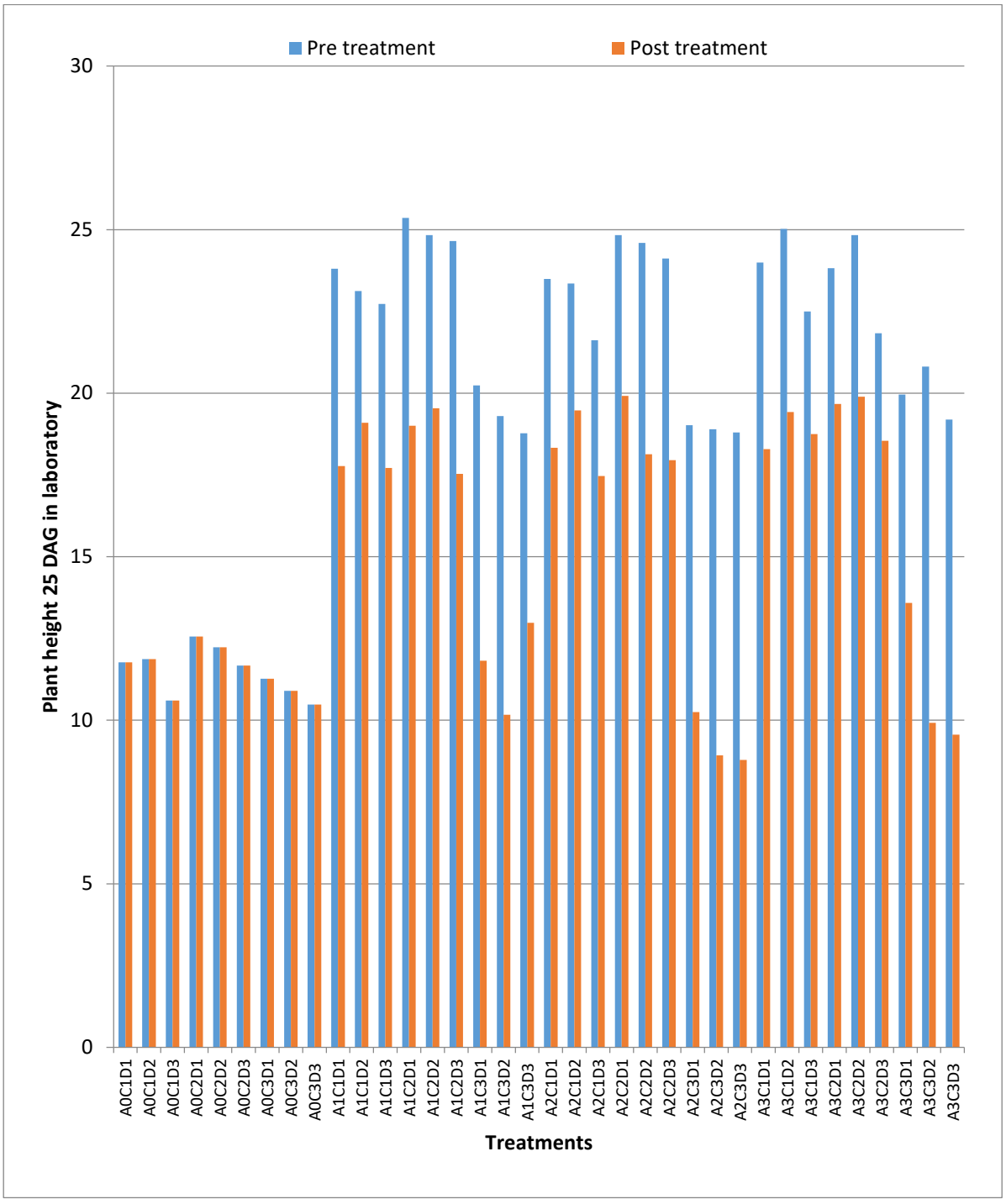
Post-treatment: At 7 days after germination (DAG) treatment $A_3C_1D_1$ was observed highest value for plant height (6.54cm) and $A_2C_3D_1$ was observed lowest value (1.10cm) both were significantly nonsignificant. At 14 days after germination (DAG) highest plant height (10.99cm) was observed at $A_3C_1D_2$ and lowest (3.61cm) was observed at $A_3C_3D_3$ which were both significantly different from that of all other treatments. At 25 days after germination (DAG) maximum plant height (19.92cm) was found at $A_2C_2D_1$ and minimum value (8.79cm) at $A_2C_3D_3$ both were significantly different from that of all other treatments.



Histogram 5(a): Effect of antioxidant along with control on plant height (cm) at 7 days after germination of wheat induced with different concentration and duration of pesticide in laboratory in M1 generation



Histogram 5(b): Effect of antioxidant along with control on plant height (cm) at 14 days after germination of wheat induced with different concentration and duration of pesticide in laboratory in M1 generation.



Histogram 5(c): Effect of antioxidant along with control on plant height (cm) at 25 days after germination of wheat induced with different concentration and duration of pesticide in laboratory in M1 generation.

Table 3: Effect of antioxidants along with control on plant height (cm) at 7, 14 & 25 DAG in laboratory of wheat induced with different concentration and duration of pesticide in M1 generation

Treatment	Pre-treatment			Post-treatment		
	Plant height (cm) in lab			Plant height (cm) in lab		
	7 DAG	14 DAG	25 DAG	7 DAG	14 DAG	25 DAG
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	2.92 ± 0.14	8.37 ± 1.03	11.77 ± 0.58	Same as pre-treatment	Same as pretreatment	Same as pretreatment
A0C1D2	3.28 ± 0.20	8.70 ± 0.72	11.87 ± 0.32	Same as pre-treatment	Same as pretreatment	Same as pretreatment
A0C1D3	3.08 ± 0.38	6.66 ± 0.67	10.60 ± 0.17	Same as pre-treatment	Same as pretreatment	Same as pretreatment
A0C2D1	3.80 ± 0.36	9.03 ± 1.05	12.56 ± 0.39	Same as pre-treatment	Same as pretreatment	Same as pretreatment
A0C2D2	3.92 ± 0.14	8.37 ± 0.96	12.23 ± 0.90	Same as pre-treatment	Same as pretreatment	Same as pretreatment
A0C2D3	3.00 ± 0.50	7.92 ± 0.83	11.67 ± 0.42	Same as pre-treatment	Same as pretreatment	Same as pretreatment
A0C3D1	1.78 ± 0.30	5.84 ± 0.12	11.27 ± 0.38	Same as pre-treatment	Same as pretreatment	Same as pretreatment
A0C3D2	1.87 ± 0.32	5.37 ± 0.53	10.90 ± 0.36	Same as pre-treatment	Same as pretreatment	Same as pretreatment
A0C3D3	1.46 ± 0.46	5.17 ± 0.70	10.48 ± 0.42	Same as pre-treatment	Same as pretreatment	Same as pretreatment
A1C1D1	8.52 ± 1.47	15.15 ± 1.76	23.80 ± 0.37	4.45 ± 0.62	9.80 ± 0.55	17.77 ± 0.67
A1C1D2	9.20 ± 1.71	15.42 ± 0.37	23.12 ± 0.48	4.87 ± 1.07	10.32 ± 0.57	19.09 ± 0.71
A1C1D3	8.17 ± 1.25	12.67 ± 0.82	22.73 ± 0.44	4.57 ± 0.86	10.24 ± 0.42	17.71 ± 0.72
A1C2D1	8.92 ± 1.60	13.94 ± 1.42	25.36 ± 0.45	3.29 ± 1.76	9.79 ± 0.68	19.00 ± 0.39
A1C2D2	8.46 ± 0.37	15.15 ± 0.85	24.83 ± 0.34	5.75 ± 0.80	10.53 ± 0.81	19.54 ± 0.35
A1C2D3	8.48 ± 0.44	13.81 ± 1.45	24.65 ± 0.62	4.99 ± 0.76	9.41 ± 0.50	17.53 ± 0.66
A1C3D1	4.18 ± 0.28	7.81 ± 0.45	20.24 ± 0.42	1.59 ± 0.37	8.57 ± 1.08	11.82 ± 0.94
A1C3D2	4.10 ± 0.42	7.43 ± 0.27	19.30 ± 0.17	1.53 ± 0.48	6.13 ± 1.89	10.17 ± 0.10
A1C3D3	3.63 ± 0.04	7.39 ± 0.49	18.77 ± 0.62	1.37 ± 0.92	6.39 ± 2.16	12.98 ± 5.41
A2C1D1	8.95 ± 0.20	14.21 ± 0.56	23.49 ± 0.27	5.24 ± 0.27	10.61 ± 0.81	18.33 ± 0.49
A2C1D2	8.62 ± 1.45	16.31 ± 0.31	23.35 ± 0.35	4.81 ± 0.41	10.11 ± 0.31	19.47 ± 0.38
A2C1D3	8.89 ± 0.81	14.77 ± 1.20	21.62 ± 0.60	4.36 ± 0.50	9.64 ± 1.56	17.47 ± 0.23
A2C2D1	8.31 ± 0.62	14.55 ± 1.33	24.83 ± 0.52	4.43 ± 0.62	10.12 ± 0.83	19.92 ± 0.07
A2C2D2	8.75 ± 0.44	15.25 ± 1.05	24.59 ± 0.74	4.33 ± 0.27	9.27 ± 1.86	18.13 ± 0.49
A2C2D3	8.83 ± 0.34	15.48 ± 0.18	24.12 ± 0.84	4.33 ± 0.86	10.00 ± 1.13	17.95 ± 0.90
A2C3D1	4.70 ± 0.94	8.05 ± 0.14	19.02 ± 0.68	1.10 ± 0.56	7.09 ± 1.28	10.25 ± 0.38
A2C3D2	4.10 ± 0.36	8.16 ± 0.81	18.90 ± 0.49	2.03 ± 0.83	4.12 ± 0.50	8.93 ± 0.79
A2C3D3	3.71 ± 0.47	8.33 ± 0.28	18.80 ± 0.14	1.23 ± 0.97	3.95 ± 0.71	8.79 ± 0.50
A3C1D1	9.77 ± 0.13	14.61 ± 0.69	23.99 ± 0.13	6.54 ± 1.21	9.51 ± 0.43	18.29 ± 0.37
A3C1D2	8.27 ± 0.93	14.41 ± 1.15	25.02 ± 0.30	4.35 ± 0.08	10.99 ± 0.69	19.42 ± 0.28
A3C1D3	9.07 ± 0.67	14.80 ± 0.82	22.50 ± 0.76	4.19 ± 1.28	10.29 ± 1.12	18.75 ± 0.54
A3C2D1	9.04 ± 0.64	15.13 ± 2.37	23.82 ± 0.21	5.19 ± 0.68	10.17 ± 0.41	19.67 ± 0.41
A3C2D2	7.89 ± 0.78	14.99 ± 1.76	24.83 ± 0.69	4.55 ± 1.29	8.75 ± 0.74	19.89 ± 0.23
A3C2D3	8.29 ± 1.49	15.05 ± 1.67	21.83 ± 0.66	4.00 ± 0.79	10.41 ± 0.49	18.54 ± 0.23
A3C3D1	3.82 ± 0.68	7.99 ± 0.04	19.96 ± 0.89	2.20 ± 0.63	5.23 ± 0.51	13.59 ± 4.74
A3C3D2	3.67 ± 0.33	7.21 ± 0.56	20.81 ± 0.82	1.21 ± 1.00	4.11 ± 1.31	9.92 ± 0.07
A3C3D3	3.58 ± 0.56	7.87 ± 0.30	19.19 ± 0.49	1.55 ± 0.15	3.61 ± 0.36	9.56 ± 0.21
Level of significance	NS	*	*	NS	*	*
CV(%)	12.67	8.92	2.64	11.67	11.63	8.72

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.1.1.5 Plant height in pot

The plant height in pot at different days after germination were recorded which are shown in **Fig. 1**, **Histogram 6(a), 6(b), 6(c) and Table 4.**

Pre-treatment: At 7 days after germination (DAG) treatment $A_1C_2D_2$ gave the maximum plant height (10.67cm) and minimum plant height (3.60cm) was found at $A_0C_2D_3$ which were both significantly different from that of all other treatments, At 14 days after germination (DAG) $A_2C_2D_2$ was showed the highest plant height (20.71cm) and the lowest plant height (10.10cm) was found at $A_0C_3D_3$. Which were significantly non significant. At 25 days after germination (DAG) highest plant height (34.53cm) was observed at $A_3C_1D_1$ and lowest plant height (21.50cm) was observed at $A_0C_3D_3$ which were both significantly different from that of all other treatments.

Post-treatment: At 7 days after germination (DAG) maximum plant height (9.14cm) was found in treatment $A_3C_1D_2$ and minimum (3.60cm) was found at $A_0C_2D_3$ which were significantly different from that of all other treatments. At 14 days after germination (DAG) highest plant height (19.19cm) was observed at $A_3C_1D_3$ and the lowest plant height (10.10cm) was found at $A_0C_3D_3$ which were significantly nonsignificant. At 25 days after germination (DAG) maximum plant height (31.58cm) was found at $A_2C_2D_3$ and minimum (21.50cm) was found at $A_0C_3D_3$ which were both significantly different from that of all other treatments.

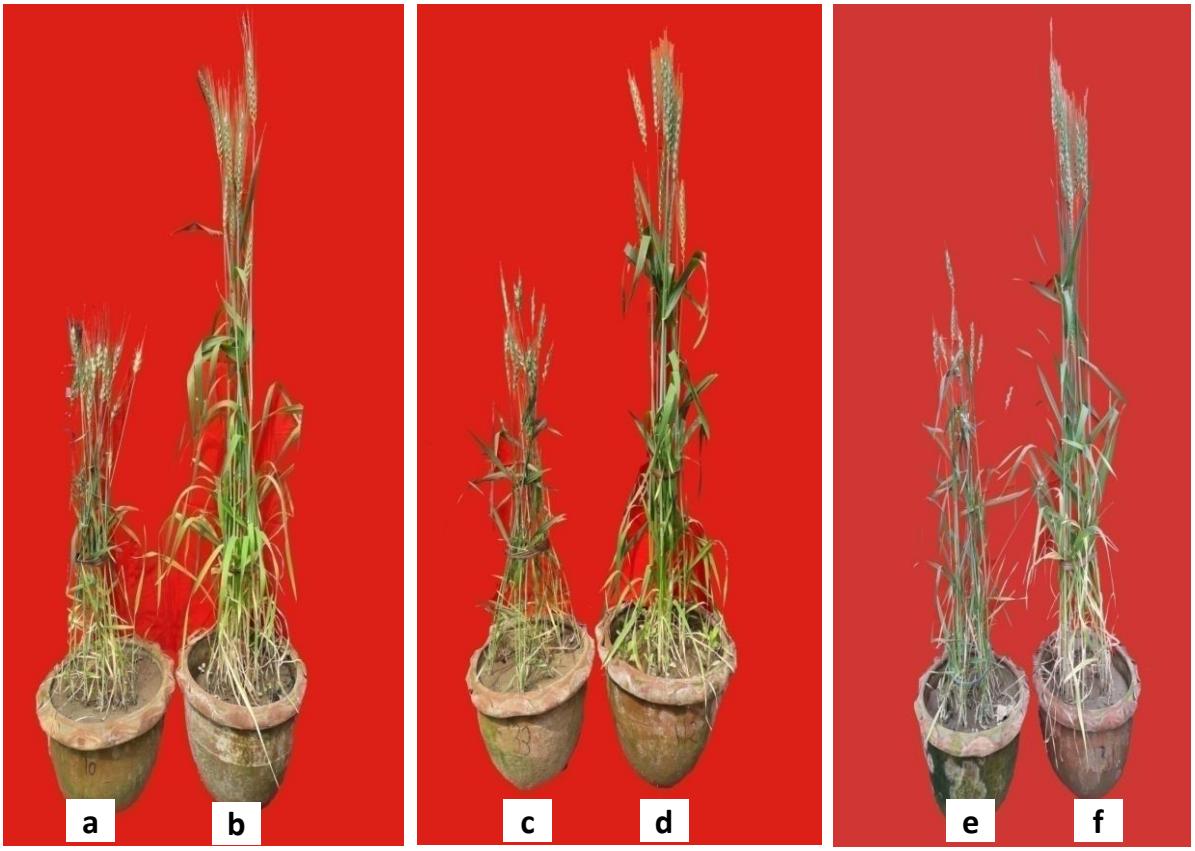
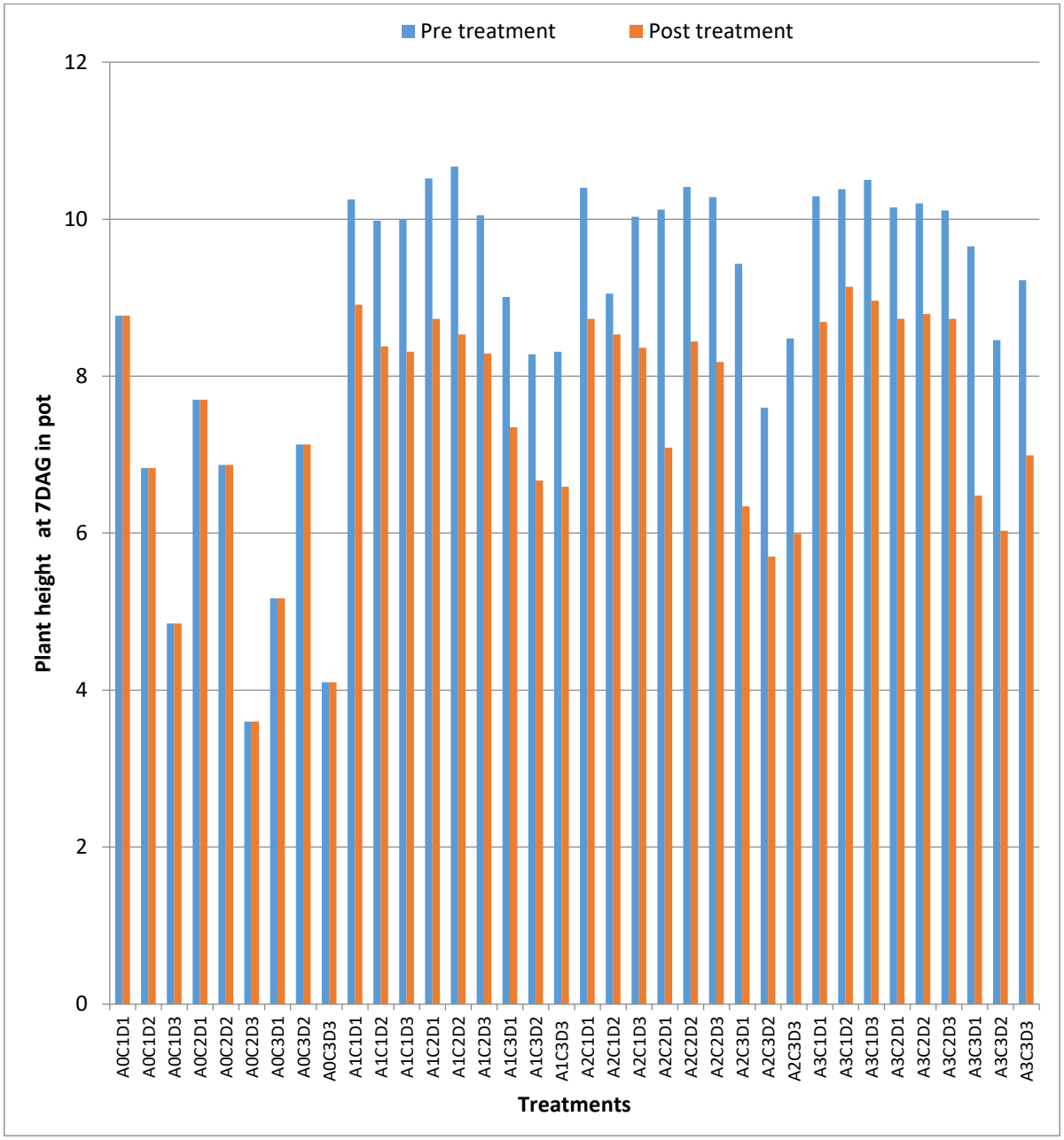
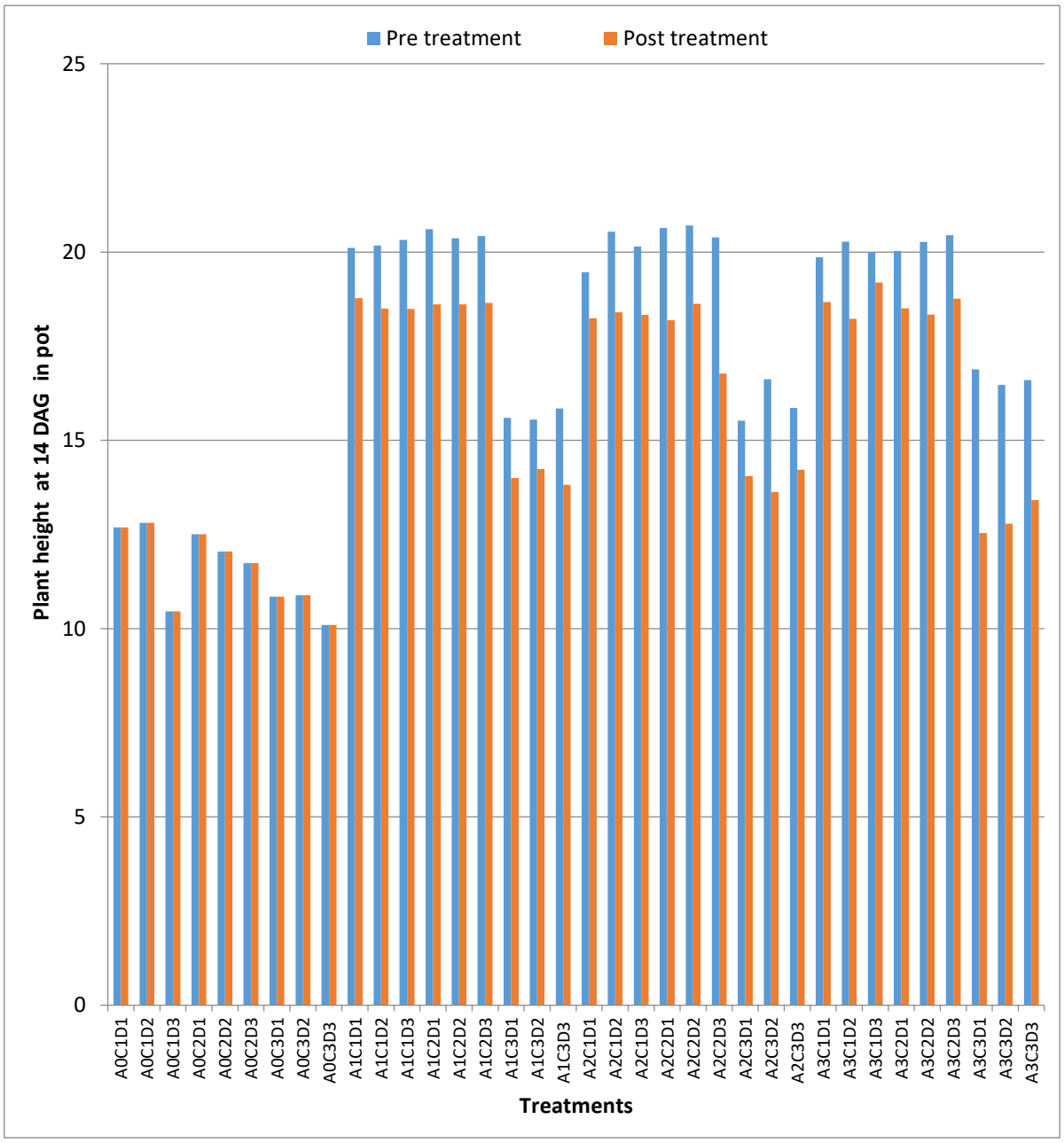


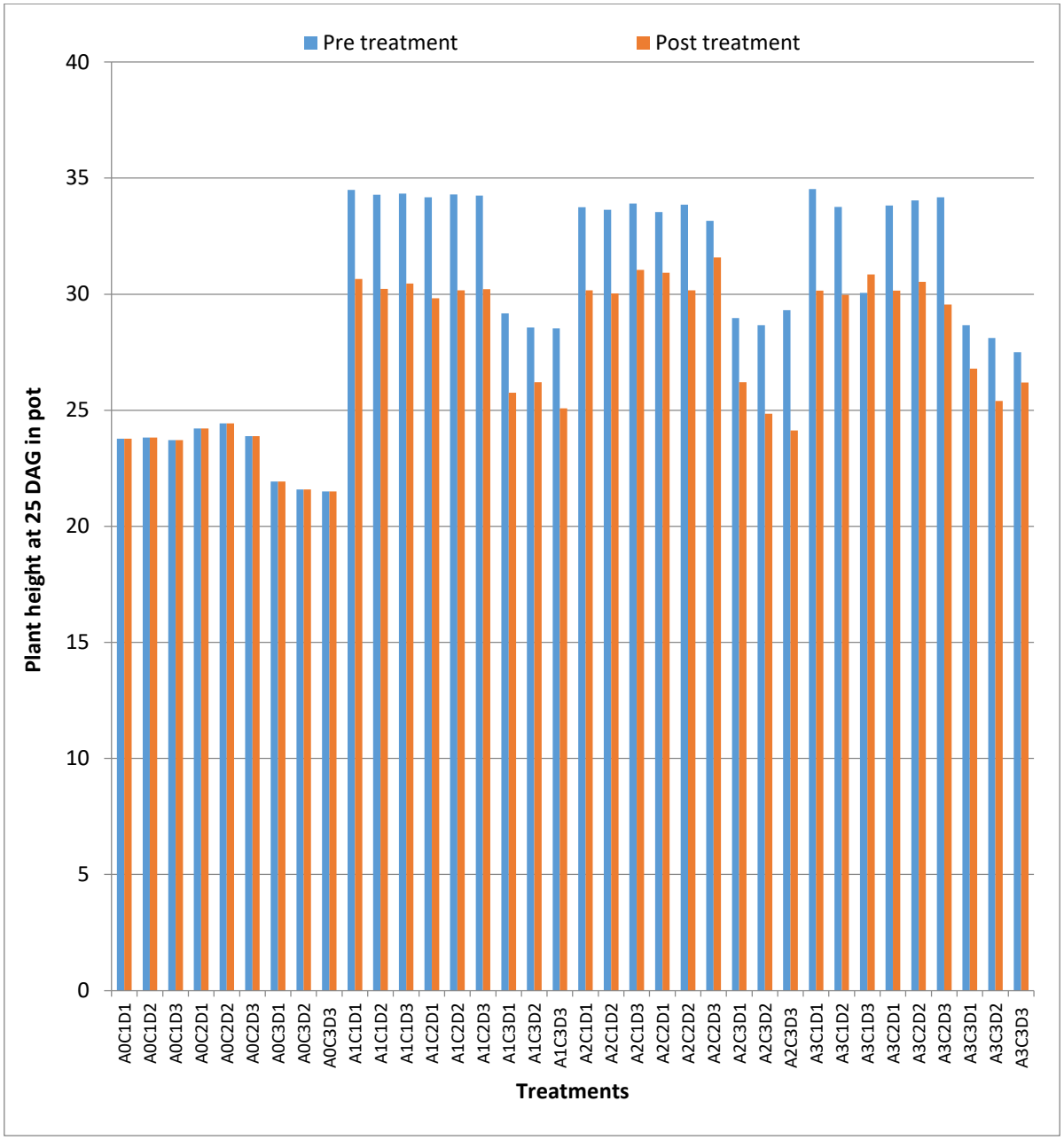
Fig. 1: Phenotypic effect due to post- and pre-treatment with pesticide and antioxidant in M1 generation. (a) Post-treatment (b) Pre-treatment (c) Post-treatment (d) Pre-treatment (e) Post-treatment (f) Pre-treatment.



Histogram 6(a): Effect of antioxidant along with control on plant height (cm) at 7 days after germination of wheat induced with different concentration and duration of pesticide in pot in M1 generation.



Histogram 6(b): Effect of antioxidant along with control on plant height (cm) at 14 days after germination of wheat induced with different concentration and duration of pesticide in pot in M1 generation.



Histogram 6(c): Effect of antioxidant along with control on plant height (cm) at 25 days after germination of wheat induced with different concentration and duration of pesticide in pot in M1 generation.

Table 4: Effect of antioxidants along with control on plant height (cm) at 7, 14 & 25 DAG in pot of wheat induced with different concentration and duration of pesticide in M1 generation.

Treatment	Pre-treatment			Post-treatment		
	Plant height (cm) in pot			Plant height (cm) in pot		
	7 DAG	14 DAG	25 DAG	7 DAG	14 DAG	25 DAG
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	8.77 ± 0.32	12.69 ± 1.02	23.77 ± 0.40	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	6.83 ± 0.76	12.81 ± 0.12	23.82 ± 0.71	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	4.85 ± 0.25	10.46 ± 0.47	23.71 ± 0.20	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	7.70 ± 0.48	12.51 ± 0.45	24.22 ± 0.46	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	6.87 ± 0.15	12.05 ± 0.18	24.43 ± 0.40	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	3.60 ± 0.26	11.74 ± 0.42	23.88 ± 0.13	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	5.17 ± 0.31	10.85 ± 0.12	21.93 ± 0.12	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	7.13 ± 0.25	10.89 ± 0.19	21.59 ± 0.96	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	4.10 ± 0.70	10.10 ± 0.13	21.50 ± 0.50	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	10.25 ± 0.22	20.11 ± 0.26	34.49 ± 0.25	8.91 ± 0.83	18.78 ± 0.97	30.66 ± 0.33
A1C1D2	9.98 ± 0.16	20.17 ± 0.53	34.28 ± 0.66	8.38 ± 0.44	18.50 ± 0.44	30.23 ± 1.09
A1C1D3	10.00 ± 0.10	20.32 ± 0.17	34.33 ± 0.55	8.31 ± 0.17	18.49 ± 0.22	30.46 ± 0.92
A1C2D1	10.52 ± 0.28	20.61 ± 0.24	34.17 ± 0.54	8.73 ± 0.17	18.61 ± 0.22	29.82 ± 0.71
A1C2D2	10.67 ± 0.15	20.37 ± 0.35	34.29 ± 0.63	8.53 ± 0.26	18.61 ± 0.65	30.16 ± 0.42
A1C2D3	10.05 ± 0.09	20.43 ± 0.45	34.25 ± 0.32	8.29 ± 0.44	18.65 ± 1.15	30.21 ± 0.46
A1C3D1	9.01 ± 0.11	15.60 ± 0.45	29.18 ± 0.98	7.35 ± 0.09	14.00 ± 0.14	25.75 ± 1.20
A1C3D2	8.28 ± 0.26	15.55 ± 0.11	28.57 ± 0.69	6.67 ± 0.22	14.24 ± 0.62	26.21 ± 0.99
A1C3D3	8.31 ± 0.32	15.85 ± 0.72	28.53 ± 0.46	6.59 ± 0.41	13.82 ± 1.61	25.08 ± 0.25
A2C1D1	10.40 ± 0.19	19.46 ± 0.56	33.74 ± 0.83	8.73 ± 0.17	18.24 ± 1.15	30.16 ± 0.28
A2C1D2	9.05 ± 0.48	20.54 ± 0.13	33.63 ± 1.04	8.53 ± 0.29	18.40 ± 0.38	30.03 ± 0.67
A2C1D3	10.03 ± 0.16	20.15 ± 0.06	33.90 ± 0.73	8.36 ± 0.37	18.33 ± 0.78	31.04 ± 0.23
A2C2D1	10.12 ± 0.11	20.64 ± 0.05	33.54 ± 0.83	7.09 ± 0.31	18.19 ± 0.22	30.92 ± 0.33
A2C2D2	10.41 ± 0.08	20.71 ± 0.39	33.85 ± 0.78	8.44 ± 0.47	18.63 ± 0.20	30.17 ± 0.25
A2C2D3	10.28 ± 0.15	20.39 ± 0.06	33.16 ± 0.84	8.18 ± 0.32	16.77 ± 1.98	31.58 ± 2.06
A2C3D1	9.43 ± 0.48	15.52 ± 0.69	28.97 ± 0.66	6.34 ± 0.39	14.05 ± 1.07	26.21 ± 0.44
A2C3D2	7.60 ± 0.38	16.62 ± 0.58	28.66 ± 0.70	5.70 ± 0.52	13.63 ± 1.35	24.85 ± 0.51
A2C3D3	8.48 ± 0.87	15.86 ± 0.41	29.31 ± 0.88	5.99 ± 0.61	14.22 ± 1.78	24.13 ± 0.18
A3C1D1	10.29 ± 0.08	19.86 ± 0.24	34.53 ± 0.56	8.69 ± 0.56	18.67 ± 0.45	30.15 ± 0.40
A3C1D2	10.38 ± 0.20	20.28 ± 0.55	33.76 ± 0.36	9.14 ± 0.41	18.23 ± 0.20	29.97 ± 0.40
A3C1D3	10.50 ± 0.17	20.00 ± 0.23	30.06 ± 6.69	8.96 ± 0.58	19.19 ± 0.37	30.85 ± 0.84
A3C2D1	10.15 ± 0.19	20.03 ± 0.52	33.82 ± 0.70	8.73 ± 0.54	18.51 ± 0.51	30.15 ± 0.91
A3C2D2	10.20 ± 0.20	20.27 ± 0.96	34.04 ± 1.59	8.79 ± 0.37	18.34 ± 0.57	30.53 ± 0.43
A3C2D3	10.11 ± 0.37	20.45 ± 0.50	34.17 ± 0.71	8.73 ± 0.43	18.76 ± 0.46	29.55 ± 0.59
A3C3D1	9.65 ± 0.22	16.89 ± 2.04	28.66 ± 1.30	6.48 ± 0.57	12.54 ± 0.46	26.79 ± 0.78
A3C3D2	8.46 ± 1.39	16.47 ± 0.41	28.11 ± 1.10	6.03 ± 0.59	12.79 ± 0.87	25.40 ± 1.04
A3C3D3	9.22 ± 0.33	16.60 ± 1.15	27.50 ± 1.64	6.99 ± 0.15	13.41 ± 2.10	26.19 ± 1.18
Level of significance	**	NS	*	**	NS	*
CV(%)	4.68	3.45	4.45	5.88	5.40	2.82

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.1.1.6 No. of tiller/plant

Histogram 7 and Table 5 reveals the result on no. of tiller/plant.

Pre-treatment: $A_3C_2D_1$ produced highest value for no. of tiller/ plant (2.47) which was statistically non-significant. On the other hand minimum value for no. of tiller/plant (1.03cm) was found at $A_0C_3D_3$ which was statistically identical with that of all other treatments.

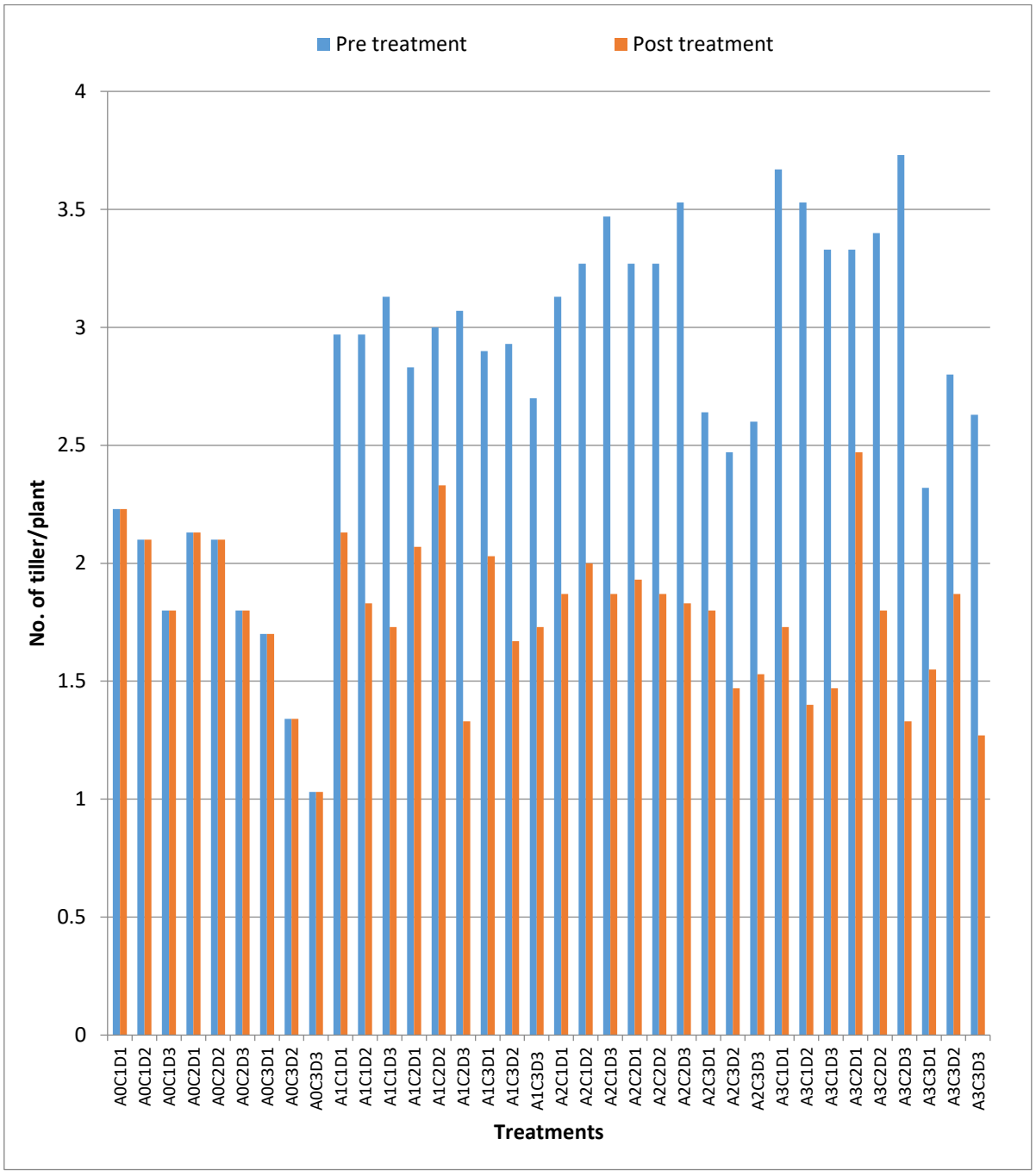
Post-treatment: Highest value for no. of tiller/plant (3.73cm) was observed at $A_3C_2D_3$ and lowest value for no. of tiller/plant (1.03cm) was found at $A_0C_3D_3$ both were statistically similar with that of all other treatments.

4.1.1.7 No. of spikelet/spike

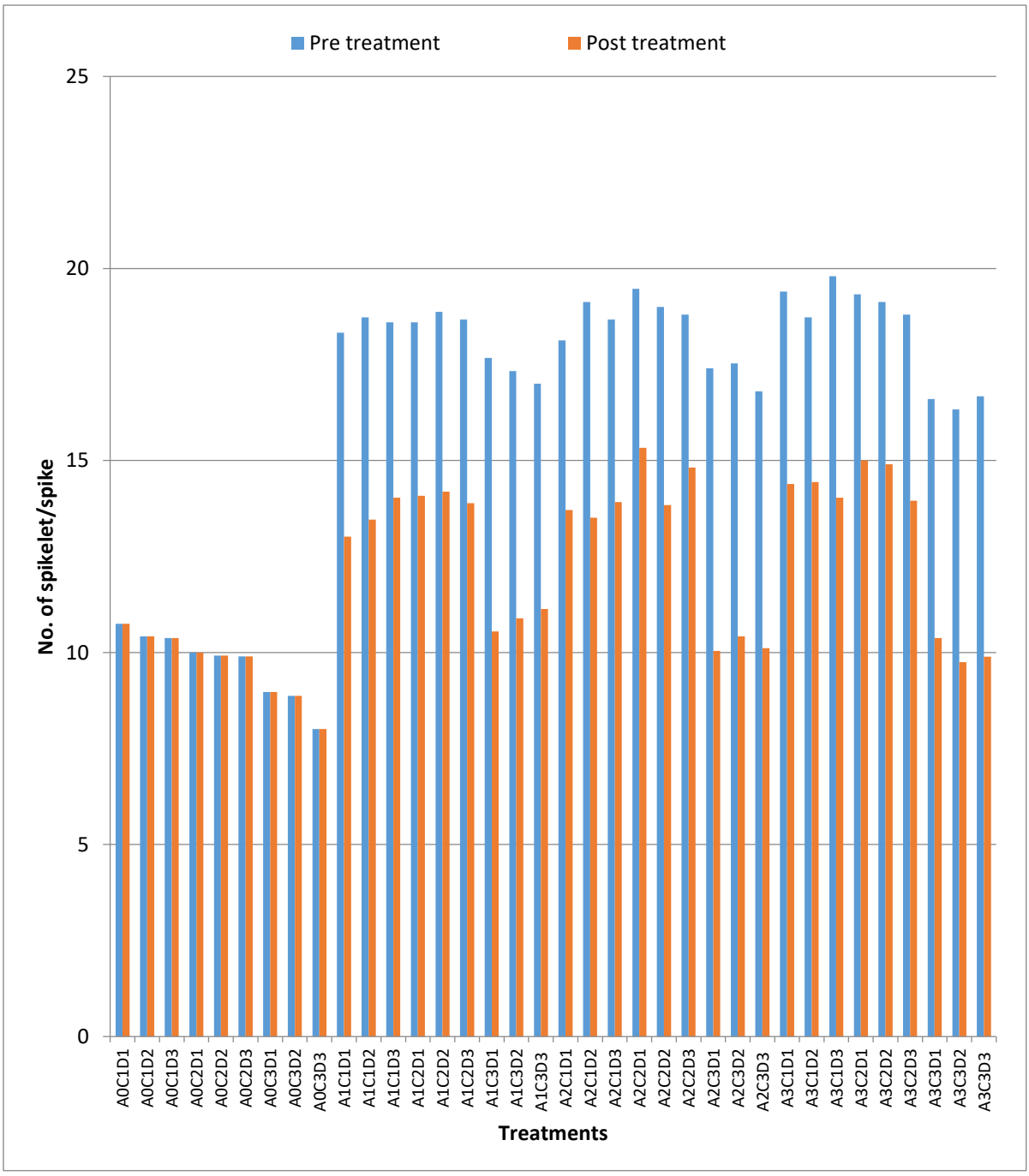
Histogram 8 and Table 5 showing the result on no. of spikelet/spike.

Pre-treatment: $A_3C_1D_3$ produced maximum value for no. of spikelet/spike (19.80) which was statistically different from that of all other treatments. Minimum value for no. of spikelet/spike (8.01) was found at $A_0C_3D_3$ which was also statistically different from that of all other treatments.

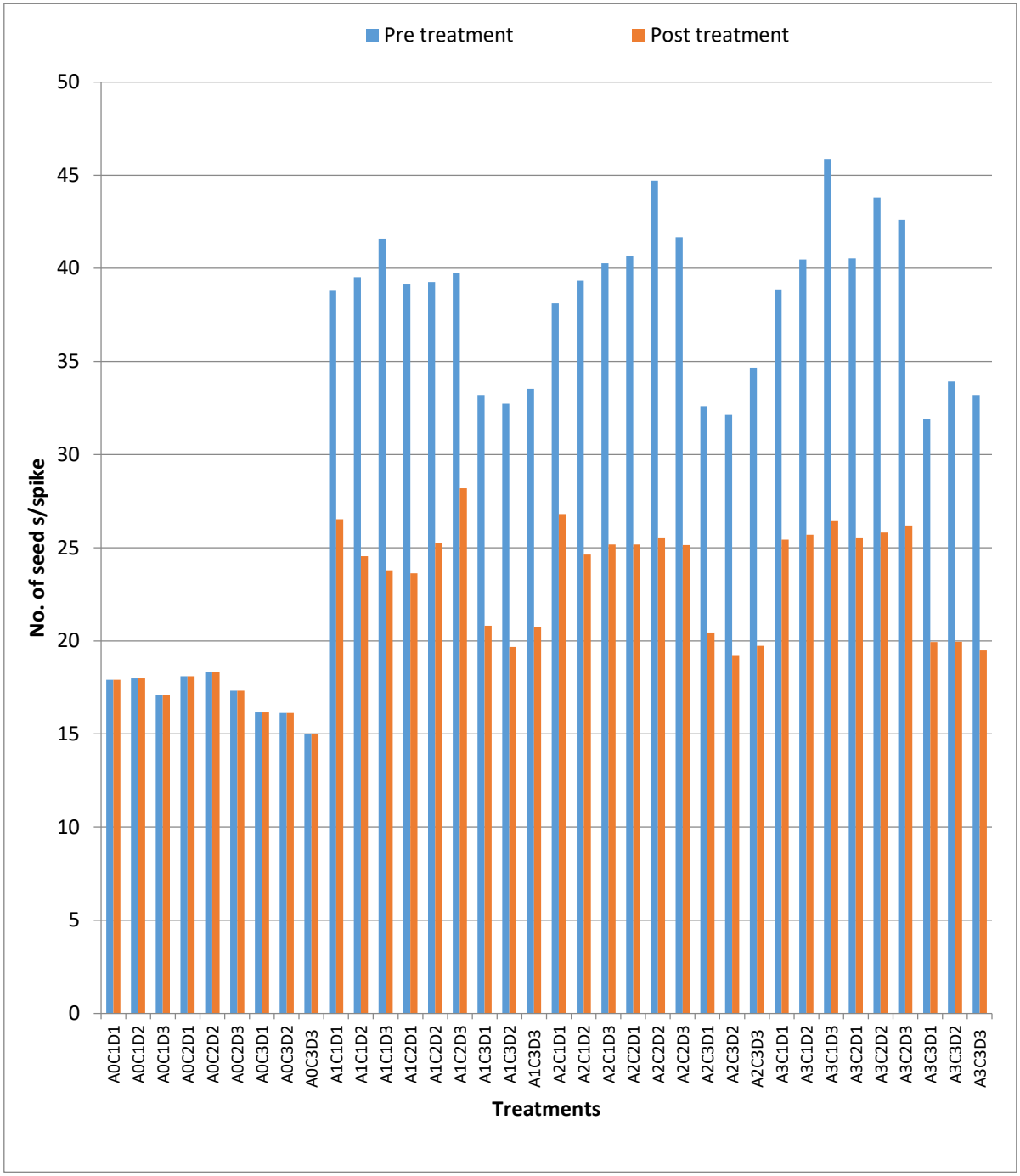
Post-treatment: Maximum value for no. of spikelet/spike (15.33) was found at $A_2C_2D_1$ and minimum value for no. of spikelet/spike (8.01) was found at $A_0C_3D_3$ which were both statistically different from that of all other treatments.



Histogram 7: Effect of antioxidant along with control on no. of tiller/plant of wheat induced with different concentration and duration and duration of pesticide in M1 generation.



Histogram 8: Effect of antioxidant along with control on no. of spikelet/spike of wheat induced with different concentration and duration and duration of pesticide in M1 generation.



Histogram 9: Effect of antioxidant along with control no. of seeds/spike of wheat induced with different concentration and duration of pesticide in M1 generation.

Table 5: Effect of antioxidants along with control on no. of tiller/plant, no. of spikelet/spike and no. of seeds/spike of wheat induced with different concentration and duration of pesticide in M1 generation.

Treatment	Pre-treatment			Post-treatment		
	No. of Tiller/plant	No. of Spikelet/spike	No. of Seeds/spike	No. of Tiller/plant	No. of Spikelet/spike	No. of Seeds/spike
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	2.23 ± 0.38	10.75 ± 0.25	17.90 ± 0.98	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	2.10 ± 0.26	10.42 ± 0.07	17.98 ± 0.48	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	1.80 ± 0.10	10.38 ± 0.10	17.08 ± 0.71	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	2.13 ± 0.06	10.00 ± 0.02	18.09 ± 0.14	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	2.10 ± 0.17	9.92 ± 0.08	18.32 ± 1.15	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	1.80 ± 0.10	9.90 ± 0.04	17.33 ± 0.87	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	1.70 ± 0.20	8.97 ± 0.06	16.15 ± 1.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	1.34 ± 0.15	8.87 ± 0.03	16.13 ± 0.59	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	1.03 ± 0.12	8.01 ± 0.01	15.00 ± 0.11	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	2.13 ± 0.31	18.33 ± 0.31	38.80 ± 3.02	2.97 ± 0.21	13.02 ± 0.35	26.53 ± 1.85
A1C1D2	1.83 ± 0.65	18.73 ± 0.76	39.53 ± 1.92	2.97 ± 0.21	13.46 ± 1.25	24.55 ± 0.51
A1C1D3	1.73 ± 0.61	18.60 ± 0.35	41.60 ± 4.80	3.13 ± 0.61	14.03 ± 0.92	23.79 ± 0.45
A1C2D1	2.07 ± 0.42	18.60 ± 0.20	39.13 ± 0.99	2.83 ± 0.38	14.08 ± 1.51	23.63 ± 0.78
A1C2D2	2.33 ± 0.61	18.87 ± 0.90	39.27 ± 5.08	3.00 ± 0.35	14.19 ± 0.53	25.27 ± 1.52
A1C2D3	1.33 ± 0.31	18.67 ± 0.64	39.73 ± 7.22	3.07 ± 0.46	13.89 ± 1.30	28.19 ± 4.69
A1C3D1	2.03 ± 0.06	17.67 ± 0.90	33.20 ± 2.77	2.90 ± 0.10	10.55 ± 0.73	20.81 ± 0.73
A1C3D2	1.67 ± 0.42	17.33 ± 0.99	32.73 ± 7.99	2.93 ± 0.12	10.89 ± 0.14	19.67 ± 1.53
A1C3D3	1.73 ± 0.92	17.00 ± 0.87	33.53 ± 0.95	2.70 ± 0.46	11.13 ± 0.65	20.75 ± 0.66
A2C1D1	1.87 ± 0.31	18.13 ± 0.61	38.13 ± 2.00	3.13 ± 0.31	13.71 ± 0.53	26.81 ± 2.72
A2C1D2	2.00 ± 0.40	19.13 ± 0.64	39.33 ± 3.06	3.27 ± 0.92	13.51 ± 0.92	24.63 ± 1.48
A2C1D3	1.87 ± 0.23	18.67 ± 0.99	40.27 ± 4.39	3.47 ± 0.23	13.92 ± 1.90	25.17 ± 1.27
A2C2D1	1.93 ± 0.50	19.47 ± 1.33	40.67 ± 4.69	3.27 ± 0.64	15.33 ± 1.11	25.17 ± 1.61
A2C2D2	1.87 ± 0.31	19.00 ± 0.72	44.71 ± 2.05	3.27 ± 0.58	13.84 ± 0.67	25.51 ± 1.82
A2C2D3	1.83 ± 0.55	18.80 ± 0.20	41.67 ± 2.04	3.53 ± 0.12	14.82 ± 0.98	25.14 ± 1.56
A2C3D1	1.80 ± 0.53	17.40 ± 0.35	32.60 ± 1.74	2.64 ± 0.34	10.04 ± 0.12	20.45 ± 0.51
A2C3D2	1.47 ± 0.31	17.53 ± 1.14	32.13 ± 2.21	2.47 ± 0.31	10.42 ± 0.82	19.23 ± 0.69
A2C3D3	1.53 ± 0.42	16.80 ± 0.69	34.67 ± 0.61	2.60 ± 0.20	10.11 ± 0.31	19.73 ± 0.64
A3C1D1	1.73 ± 0.42	19.40 ± 1.25	38.87 ± 2.14	3.67 ± 0.12	14.39 ± 1.30	25.43 ± 1.39
A3C1D2	1.40 ± 0.20	18.73 ± 0.61	40.47 ± 5.64	3.53 ± 0.12	14.44 ± 0.77	25.69 ± 0.60
A3C1D3	1.47 ± 0.12	19.80 ± 1.83	45.87 ± 5.08	3.33 ± 0.46	14.03 ± 0.60	26.43 ± 1.69
A3C2D1	2.47 ± 0.50	19.33 ± 0.50	40.53 ± 4.96	3.33 ± 0.23	15.00 ± 0.61	25.51 ± 1.75
A3C2D2	1.80 ± 0.35	19.13 ± 0.42	43.80 ± 3.34	3.40 ± 0.40	14.90 ± 0.40	25.81 ± 1.72
A3C2D3	1.33 ± 0.12	18.80 ± 0.20	42.60 ± 3.80	3.73 ± 0.23	13.95 ± 1.08	26.20 ± 2.31
A3C3D1	1.55 ± 0.28	16.60 ± 0.87	31.93 ± 1.36	2.32 ± 0.98	10.38 ± 0.28	19.93 ± 1.61
A3C3D2	1.87 ± 0.42	16.33 ± 0.23	33.93 ± 3.52	2.80 ± 0.20	9.75 ± 0.37	19.95 ± 0.93
A3C3D3	1.27 ± 0.12	16.67 ± 0.50	33.20 ± 2.95	2.63 ± 0.75	9.89 ± 0.17	19.48 ± 0.48
Level of significance	NS	*	*	NS	*	*
CV(%)	11.06	4.34	9.81	14.46	6.27	5.63

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.1.1.8 No. of seeds/spike

Pre-treatment: Histogram 9 and Table 5 reveals that maximum value for no. of seeds/spike (45.87) was found at treatment $A_3C_1D_3$ which was statistically different from that of all other treatments. Minimum value for no. of seeds/spike (15.00) was found at $A_0C_3D_3$ which was also statistically different from that of all other treatments.

Post-treatment: Histogram 14 and Table 5 reveals that maximum value for no. of seeds/spike (28.19) was found at $A_1C_2D_3$ and Minimum value for no. of seed/ spike (15.00) was found at $A_0C_3D_3$ which were statistically different from that of all other treatments.

4.1.1.9 Flag leaf length

Histogram 10 and Table 6 reveals the result on no. of tiller/plant.

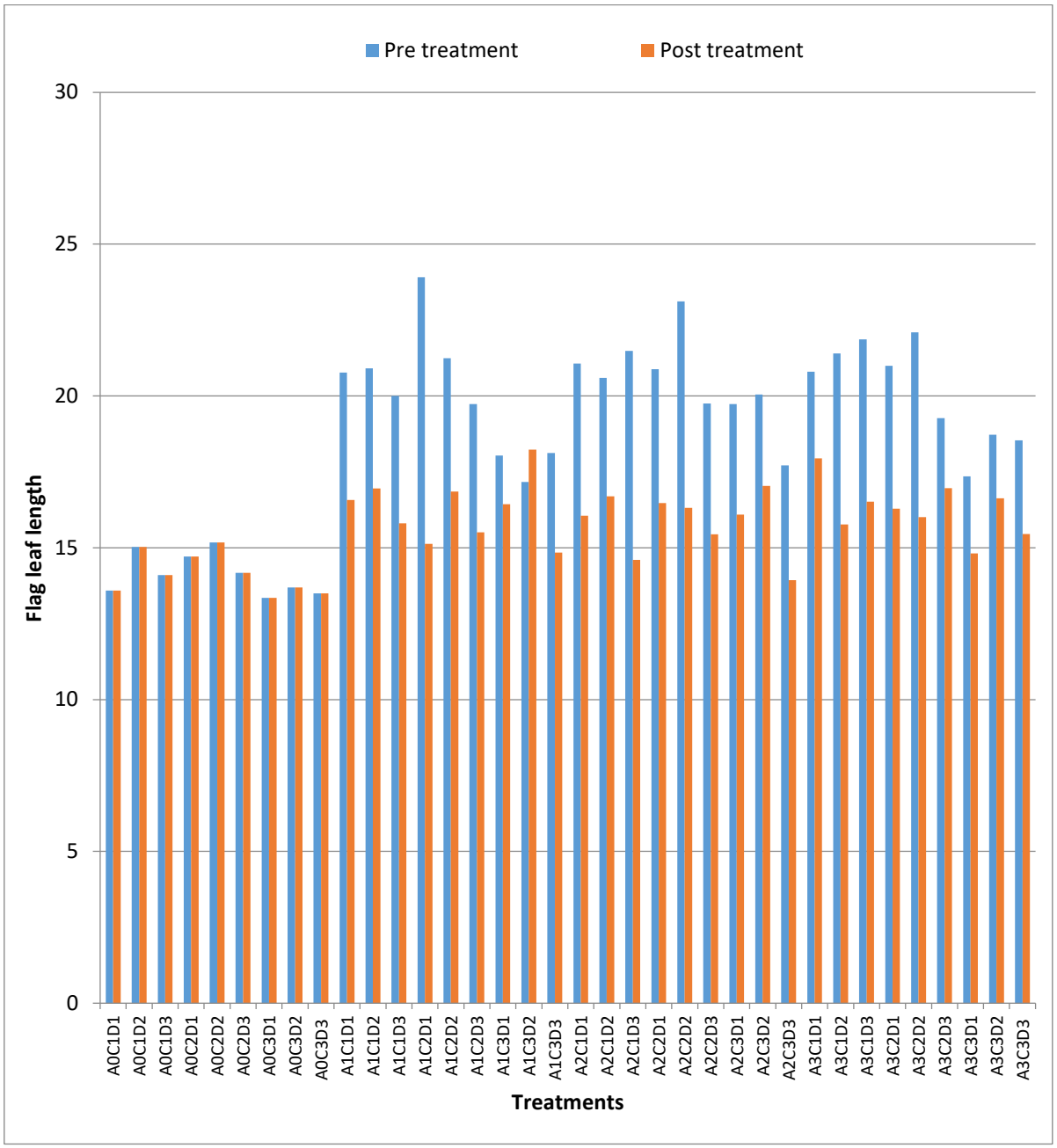
Pre-treatment: Treatment $A_1C_2D_1$ produced the highest flag leaf length (23.91cm) and shortest flag leaf length (13.35cm) was found due to $A_0C_3D_1$ which were both statistically different from that of all other treatments.

Post-treatment: Longest flag leaf length (18.23cm) was observed due to $A_1C_3D_2$ was statistically different from that of all other treatments. On the other hand shortest flag leaf length (13.35cm) was found at $A_0C_3D_1$ which was also statistically different from that of all other treatments.

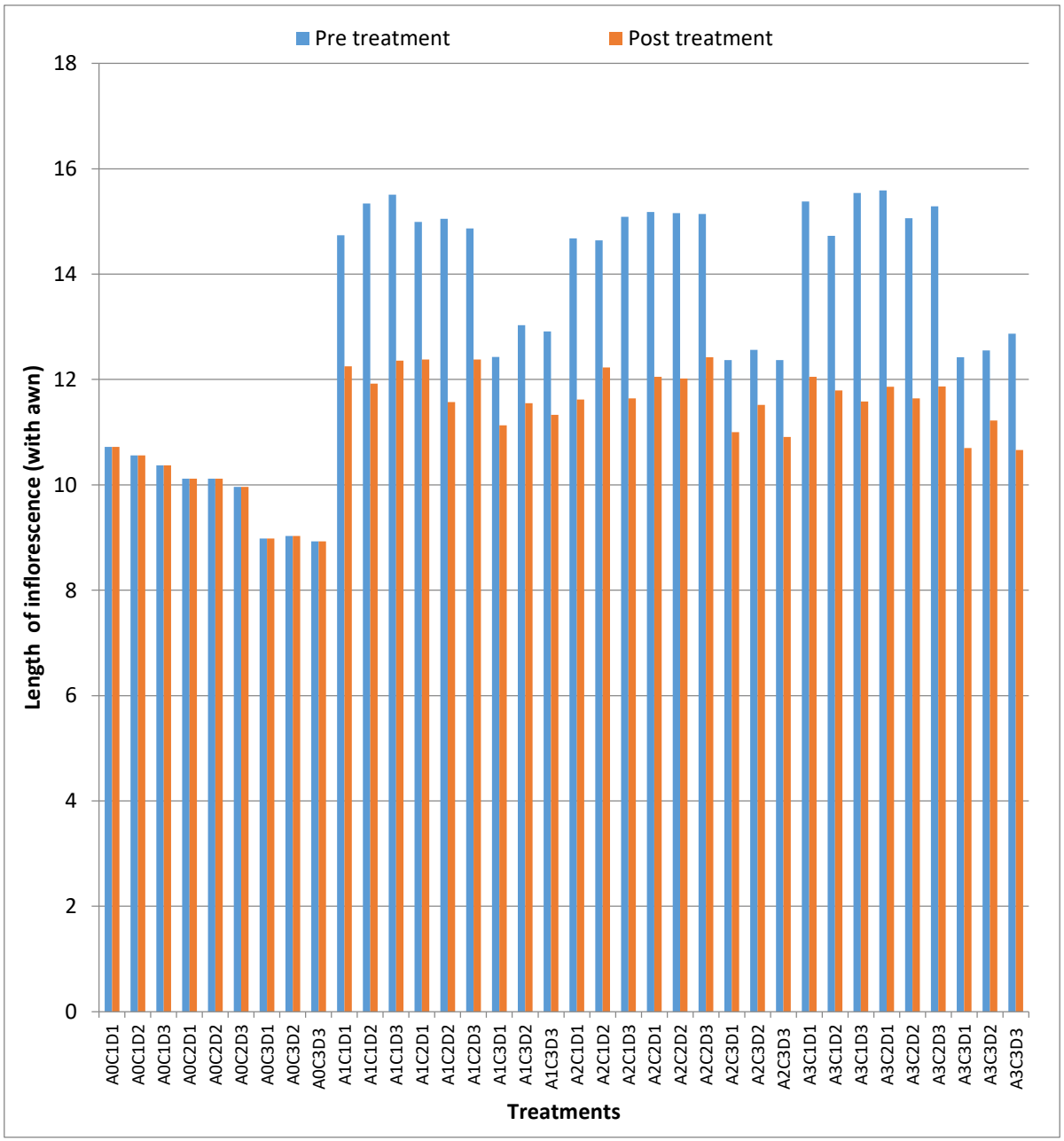
4.1.1.10 Length of inflorescence (with awn) and length of inflorescence (without awn)

Pre-treatment: Histogram 11-12 and Table 6 indicated that longest inflorescence with awn (15.59cm) was observed at $A_3C_2D_1$ and inflorescence without awn (10.35cm) was found at $A_3C_2D_3$ which were significantly different from that of all other treatments. Lowest length of inflorescence with awn (8.93cm) and without awn (4.40cm) was found at $A_0C_3D_3$ which were statistically different from that of all other treatments.

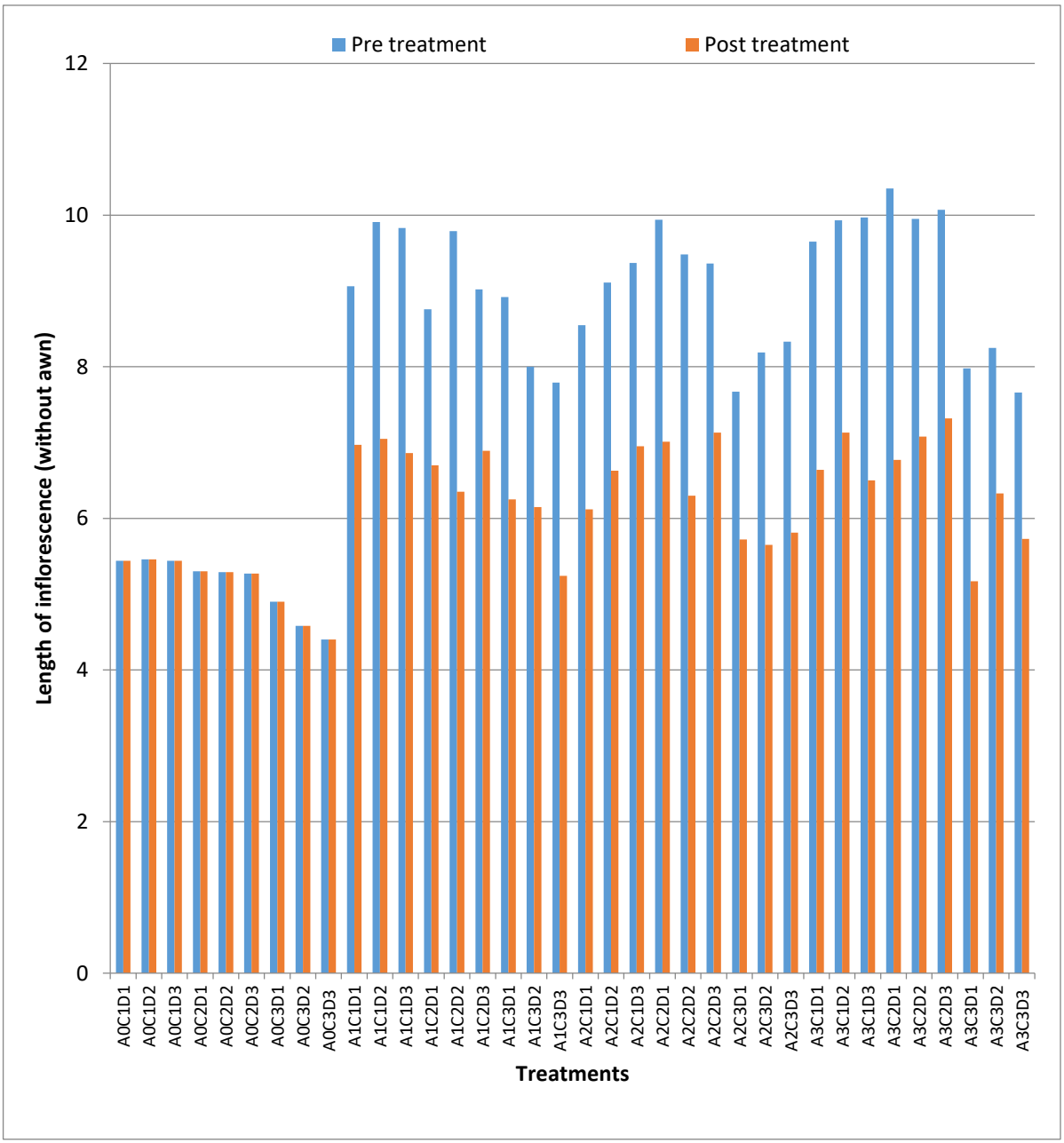
Post-treatment: Maximum value for inflorescence length with awn (12.38cm) was found at $A_1C_2D_3$ and Maximum value for inflorescence length without awn (7.32cm) was found at $A_3C_2D_3$ which were statistically different from that of all other treatments. Minimum value for inflorescence length both with awn (8.93cm) and without awn (4.40cm) were found at $A_0C_3D_3$ which were statistically different from that of all other treatments (**Histogram 11-12 and Table 6)**.



Histogram 10: Effect of antioxidant along with control on flag leaf length of wheat induced with different concentration and duration and duration of pesticide in M1 generation.



Histogram 11: Effect of antioxidant along with control on length of inflorescence (with awn) of wheat induced with different concentration and duration of pesticide in M1 generation.



Histogram 12: Effect of antioxidant along with control length of inflorescence (without awn) of wheat induced with different concentration and duration of pesticide in laboratory in M1 generation.

Table 6: Effect of antioxidants along with control on flag leaf length (cm), length of inflorescence (cm) with & without awn of wheat induced with different concentration and duration of pesticide in M1 generation.

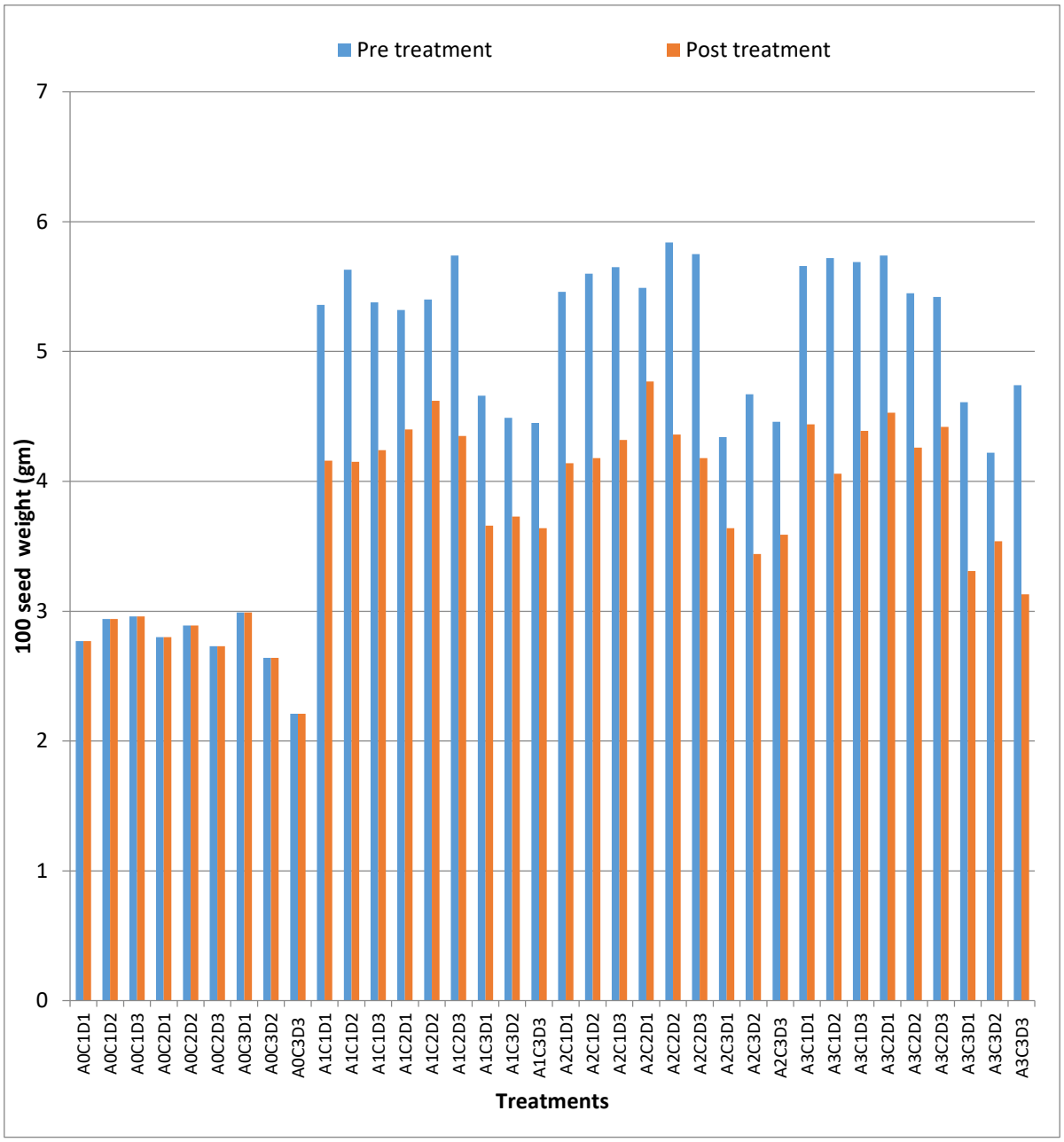
Treatment	Pre-treatment			Post-treatment		
	Flag leaf length(cm)	Length of inflorescence (cm) with awn	Length of inflorescence (cm) without awn	Flag leaf length (cm)	Length of inflorescence (cm) with awn	Length of inflorescence (cm) without awn
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	13.59 ± 0.60	10.72 ± 0.16	5.44 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	15.03 ± 0.31	10.56 ± 0.05	5.46 ± 0.08	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	14.10 ± 1.15	10.37 ± 0.13	5.44 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	14.71 ± 0.63	10.12 ± 0.12	5.30 ± 0.03	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	15.17 ± 0.14	10.12 ± 0.12	5.29 ± 0.03	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	14.17 ± 0.14	9.96 ± 0.05	5.27 ± 0.03	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	13.35 ± 0.37	8.98 ± 0.02	4.90 ± 0.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	13.69 ± 0.63	9.03 ± 0.12	4.58 ± 0.02	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	13.50 ± 0.66	8.93 ± 0.06	4.40 ± 0.11	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	20.77 ± 0.86	14.74 ± 1.08	9.06 ± 0.49	16.57 ± 0.40	12.25 ± 0.26	6.97 ± 0.37
A1C1D2	20.91 ± 0.21	15.34 ± 0.55	9.91 ± 1.00	16.95 ± 0.74	11.92 ± 0.35	7.05 ± 0.82
A1C1D3	20.00 ± 0.76	15.51 ± 0.40	9.83 ± 0.45	15.80 ± 0.22	12.36 ± 0.44	6.86 ± 0.07
A1C2D1	23.91 ± 0.19	14.99 ± 0.66	8.76 ± 0.46	15.13 ± 0.56	12.38 ± 0.19	6.70 ± 0.39
A1C2D2	21.24 ± 1.91	15.05 ± 0.68	9.79 ± 0.41	16.85 ± 1.33	11.57 ± 0.41	6.35 ± 0.06
A1C2D3	19.73 ± 0.27	14.87 ± 0.38	9.02 ± 1.06	15.51 ± 0.33	12.38 ± 0.47	6.89 ± 1.02
A1C3D1	18.04 ± 1.09	12.43 ± 0.44	8.92 ± 0.64	16.43 ± 0.82	11.13 ± 0.33	6.25 ± 0.45
A1C3D2	17.17 ± 1.50	13.03 ± 0.80	8.00 ± 0.83	18.23 ± 1.12	11.55 ± 0.18	6.15 ± 0.24
A1C3D3	18.12 ± 1.48	12.91 ± 0.13	7.79 ± 0.50	14.84 ± 0.63	11.33 ± 0.14	5.24 ± 0.28
A2C1D1	21.07 ± 0.85	14.68 ± 0.53	8.55 ± 0.31	16.05 ± 1.24	11.62 ± 0.55	6.12 ± 0.48
A2C1D2	20.59 ± 0.38	14.64 ± 0.60	9.11 ± 0.66	16.69 ± 0.81	12.23 ± 1.06	6.63 ± 0.22
A2C1D3	21.48 ± 1.32	15.09 ± 0.83	9.37 ± 0.88	14.60 ± 1.28	11.64 ± 0.41	6.95 ± 0.80
A2C2D1	20.88 ± 1.68	15.18 ± 1.01	9.94 ± 0.47	16.47 ± 0.58	12.05 ± 0.52	7.01 ± 0.91
A2C2D2	23.11 ± 1.17	15.16 ± 0.17	9.48 ± 1.01	16.31 ± 1.31	12.02 ± 0.12	6.30 ± 0.49
A2C2D3	19.75 ± 1.10	15.14 ± 0.50	9.36 ± 0.68	15.44 ± 1.49	12.42 ± 0.35	7.13 ± 0.60
A2C3D1	19.73 ± 0.56	12.37 ± 0.48	7.67 ± 0.39	16.09 ± 0.45	11.00 ± 0.64	5.72 ± 0.60
A2C3D2	20.05 ± 0.69	12.56 ± 0.14	8.19 ± 0.75	17.04 ± 1.44	11.52 ± 0.62	5.65 ± 0.44
A2C3D3	17.71 ± 0.62	12.37 ± 0.40	8.33 ± 0.26	13.93 ± 0.84	10.91 ± 0.88	5.81 ± 0.64
A3C1D1	20.80 ± 0.64	15.38 ± 0.42	9.65 ± 0.34	17.94 ± 1.36	12.05 ± 0.77	6.64 ± 0.22
A3C1D2	21.40 ± 1.37	14.73 ± 0.64	9.93 ± 0.22	15.77 ± 3.00	11.79 ± 0.45	7.13 ± 0.59
A3C1D3	21.86 ± 2.03	15.54 ± 1.10	9.97 ± 0.56	16.52 ± 1.48	11.58 ± 0.53	6.50 ± 0.46
A3C2D1	20.99 ± 0.46	15.59 ± 0.16	10.35 ± 0.32	16.29 ± 1.06	11.86 ± 0.48	6.77 ± 0.54
A3C2D2	22.09 ± 0.28	15.06 ± 0.74	9.95 ± 0.70	16.01 ± 1.68	11.64 ± 0.53	7.08 ± 0.50
A3C2D3	19.27 ± 1.25	15.29 ± 0.40	10.07 ± 0.87	16.96 ± 1.85	11.87 ± 0.83	7.32 ± 1.22
A3C3D1	17.35 ± 0.93	12.42 ± 0.64	7.98 ± 0.31	14.81 ± 1.04	10.70 ± 0.74	5.17 ± 0.49
A3C3D2	18.72 ± 1.22	12.55 ± 0.77	8.25 ± 0.88	16.63 ± 1.16	11.22 ± 0.49	6.33 ± 0.23
A3C3D3	18.54 ± 2.41	12.87 ± 0.60	7.66 ± 0.80	15.45 ± 0.56	10.66 ± 0.40	5.73 ± 0.59
Level of significance	*	*	*	*	*	*
CV(%)	5.63	4.12	7.06	6.97	4.11	8.24

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

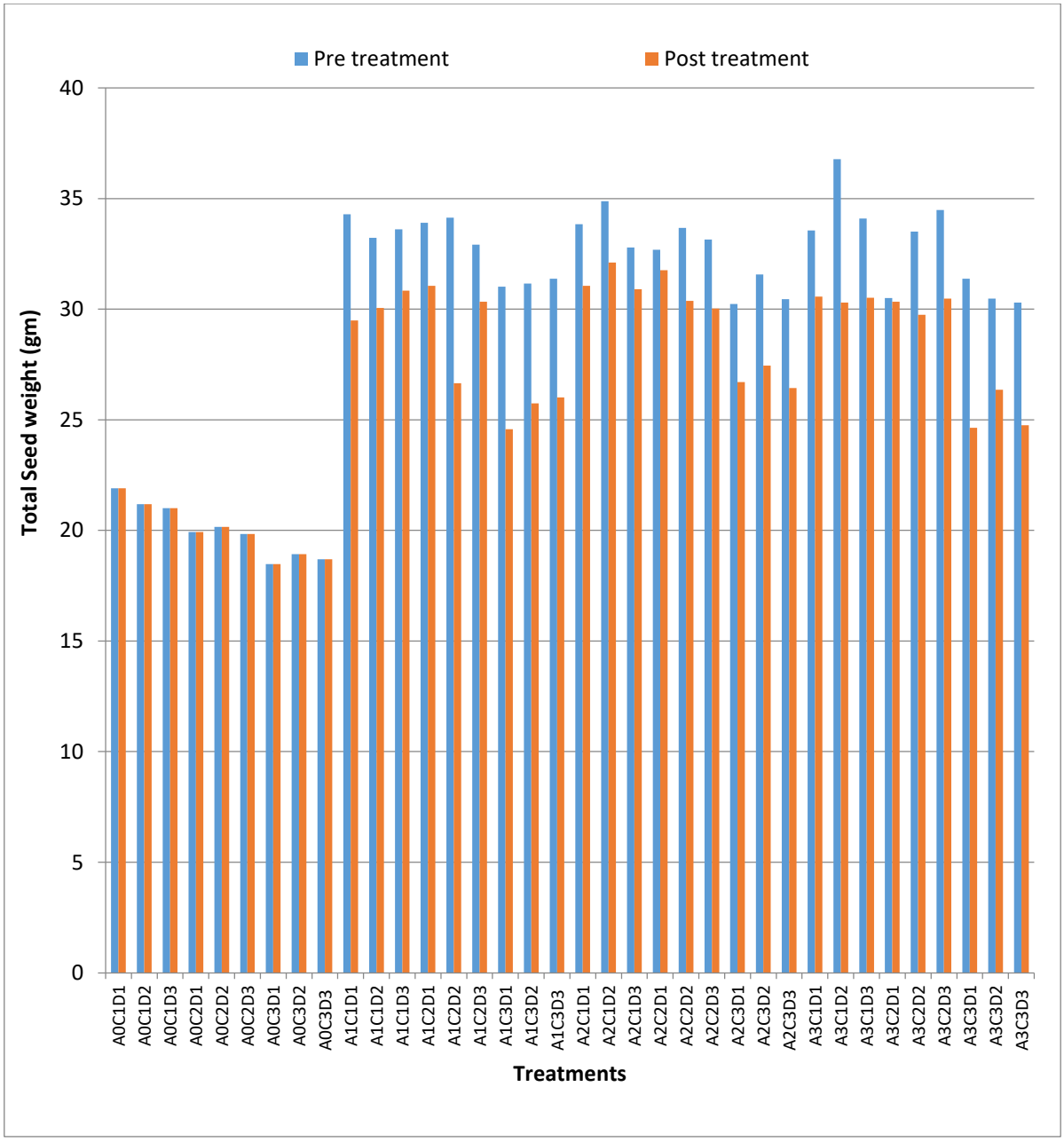
4.1.1.11 100 seed weight and total seed weight

Pre-treatment: $A_2C_2D_2$ produced highest value for 100 seed weight (5.84gm) and lowest value for 100 seed weight (2.21gm) was found at $A_0C_3D_3$ which was statistically different from that of all other treatments. Maximum value for total seed weight (36.78gm) was found at $A_3C_1D_2$ and minimum value for total seed weight (18.47gm) was found at $A_0C_3D_1$ which was statistically different from that of all other treatments (**Histogram 13-14 and Table 7)**.

Post-treatment: $A_2C_2D_1$ produced highest value for 100 seed weight (4.77gm) and lowest value for 100 seed weight (2.21gm) was found at $A_0C_3D_3$ which was statistically different from that of all other treatments. Maximum value for total seed weight (32.11gm) was found at $A_2C_1D_2$ and minimum value for total seed weight (18.47gm) was found at $A_0C_3D_1$ which was statistically different from that of all other treatments (**Histogram 13-14 and Table 7)**.



Histogram 13: Effect of antioxidant along with control 100 seed weight (gm) of wheat induced with different concentration and duration and duration of pesticide in M1 generation.



Histogram 14: Effect of antioxidant along with control on total seed weight (gm) of wheat induced with different concentration and duration of pesticide in M1 generation.

Table 7: Effect of antioxidants along with control on 100 seed weight and total seed weight of wheat induced with different concentration and duration of pesticide in M1 generation.

Treatment	Pre-treatment		Post-treatment	
	100 seed weight (gm)	Total seed weight (gm)	100 seed weight (gm)	Total seed weight (gm)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	2.77 ± 0.39	21.90 ± 0.14	Same as pre-treatment	Same as pre-treatment
A0C1D2	2.94 ± 0.07	21.18 ± 0.58	Same as pre-treatment	Same as pre-treatment
A0C1D3	2.96 ± 0.08	21.01 ± 0.66	Same as pre-treatment	Same as pre-treatment
A0C2D1	2.80 ± 0.30	19.92 ± 0.94	Same as pre-treatment	Same as pre-treatment
A0C2D2	2.89 ± 0.10	20.16 ± 0.81	Same as pre-treatment	Same as pre-treatment
A0C2D3	2.73 ± 0.37	19.84 ± 0.55	Same as pre-treatment	Same as pre-treatment
A0C3D1	2.99 ± 0.00	18.70 ± 0.60	Same as pre-treatment	Same as pre-treatment
A0C3D2	2.64 ± 0.34	18.92 ± 0.80	Same as pre-treatment	Same as pre-treatment
A0C3D3	2.21 ± 0.26	18.47 ± 0.61	Same as pre-treatment	Same as pre-treatment
A1C1D1	5.36 ± 0.23	34.29 ± 0.66	4.16 ± 0.44	29.49 ± 0.75
A1C1D2	5.63 ± 0.29	33.22 ± 1.02	4.15 ± 0.17	30.05 ± 0.65
A1C1D3	5.38 ± 0.46	33.61 ± 0.20	4.24 ± 0.63	30.84 ± 1.17
A1C2D1	5.32 ± 0.23	33.90 ± 0.49	4.40 ± 0.34	31.05 ± 0.64
A1C2D2	5.40 ± 0.35	34.13 ± 0.62	4.62 ± 0.44	26.65 ± 6.17
A1C2D3	5.74 ± 0.22	32.92 ± 0.91	4.35 ± 0.05	30.34 ± 3.04
A1C3D1	4.66 ± 0.87	31.01 ± 0.91	3.66 ± 0.19	24.57 ± 1.99
A1C3D2	4.49 ± 0.42	31.16 ± 0.35	3.73 ± 0.25	25.74 ± 3.07
A1C3D3	4.45 ± 0.28	31.38 ± 0.65	3.64 ± 0.16	26.01 ± 0.04
A2C1D1	5.46 ± 0.37	33.84 ± 0.95	4.14 ± 0.09	31.05 ± 1.14
A2C1D2	5.60 ± 0.10	34.88 ± 1.75	4.18 ± 0.22	32.11 ± 0.59
A2C1D3	5.65 ± 0.32	32.78 ± 1.58	4.32 ± 0.12	30.90 ± 0.72
A2C2D1	5.49 ± 0.32	32.68 ± 2.76	4.77 ± 0.25	31.76 ± 2.38
A2C2D2	5.84 ± 0.08	33.67 ± 1.09	4.36 ± 0.22	30.37 ± 0.73
A2C2D3	5.75 ± 0.11	33.15 ± 0.95	4.18 ± 0.13	30.03 ± 0.63
A2C3D1	4.34 ± 0.33	30.23 ± 0.54	3.64 ± 0.09	26.70 ± 2.29
A2C3D2	4.67 ± 0.35	31.57 ± 0.93	3.44 ± 0.40	27.45 ± 1.35
A2C3D3	4.46 ± 0.39	30.45 ± 0.67	3.59 ± 0.38	26.43 ± 1.05
A3C1D1	5.66 ± 0.43	33.56 ± 1.30	4.44 ± 0.12	30.57 ± 1.25
A3C1D2	5.72 ± 0.08	36.78 ± 3.03	4.06 ± 0.39	30.29 ± 0.98
A3C1D3	5.69 ± 0.12	34.10 ± 1.09	4.39 ± 0.22	30.52 ± 1.10
A3C2D1	5.74 ± 0.08	30.50 ± 0.57	4.53 ± 0.01	30.33 ± 0.53
A3C2D2	5.45 ± 0.29	33.51 ± 1.06	4.26 ± 0.44	29.74 ± 0.76
A3C2D3	5.42 ± 0.28	34.48 ± 1.43	4.42 ± 0.35	30.48 ± 1.18
A3C3D1	4.61 ± 0.13	31.37 ± 0.43	3.31 ± 0.29	24.63 ± 1.27
A3C3D2	4.22 ± 0.20	30.48 ± 0.72	3.54 ± 0.47	26.35 ± 0.94
A3C3D3	4.74 ± 0.12	30.29 ± 1.25	3.13 ± 0.15	24.75 ± 1.85
Level of Significance	*	*	*	*
CV(%)	6.42	3.77	7.80	6.12

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.1.2 Cytological study

4.1.2.1 % of mitotic abnormality

Mitotic study of the root tip cells of wheat emerged from the seeds treated with different treatments showed various types of chromosomal abnormalities shown in **Fig. 2, Histogram 15 and Table 8.**

Most of the abnormalities were characterized by chromosome fragments, lagging chromosomes, bridges etc. In case of certain treatments some cells showed diverse types of chromosomal abnormalities such as single anaphase bridge, double anaphase bridge, multiple anaphase bridge, single bridge with fragment, single bridge with lagging chromosome, double bridges with fragment and/or lagging chromosome, multiple bridges with fragment and/or lagging chromosome and fragments together.

Pre-treatment: Highest percentage of mitotic abnormality (24.00%) was observed at $A_0C_3D_3$ and the lowest percentage of mitotic abnormality (7.33%) was found at $A_3C_1D_2$ and $A_3C_2D_1$ which were statistically different from that of all other treatments.

Post-treatment: Maximum value for percentage of mitotic abnormality (24.00%) was observed due to treatment $A_0C_3D_3$ and minimum value for percentage of mitotic abnormality (12.33%) was due to treatment $A_3C_1D_2$ which were statistically different from that of all other treatments.

4.1.2.2 Mitotic index

The mitotic index studied from the root tip cells of different treatments are shown in **Histogram 16 and Table 8**.

Pre-treatment: Mitotic index was found to be highest (52.33%) in the root emerged from the seeds of $A_2C_2D_3$ treatment and the value was found to be significantly different from that of all other treatments. On the other hand, the lowest value for mitotic index (11.67%) was observed at $A_0C_3D_3$ which was also statistically different from that of all other treatments.

Post-treatment: Highest value for mitotic index (46.00%) was observed at $A_1C_1D_2$ and lowest value for mitotic index (11.67%) was observed at $A_0C_3D_3$ which were both statistically significant when compared to that of all other treatments.

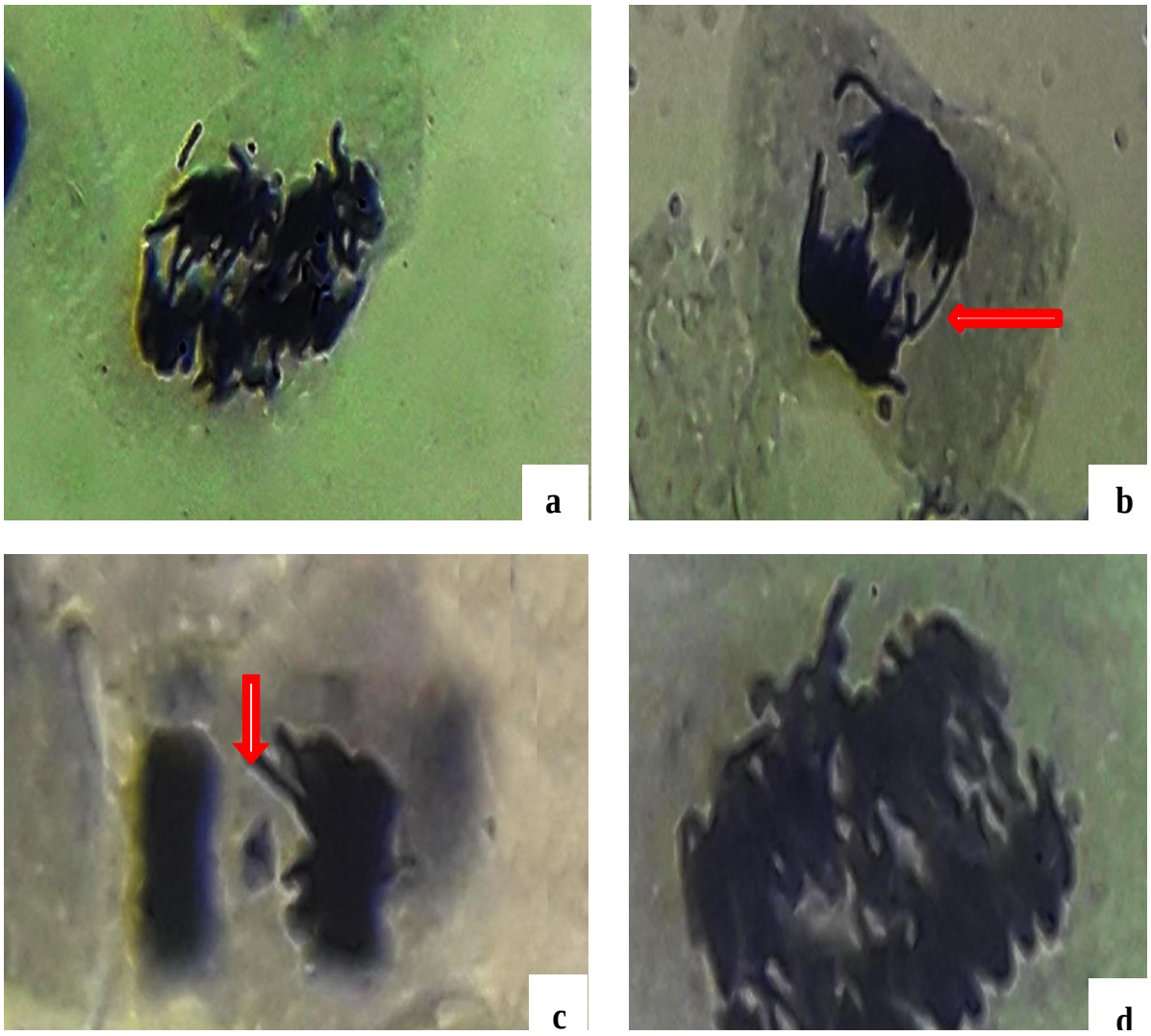


Fig. 2(a): Different types of mitotic abnormalities of wheat induced with different concentration and duration of pesticide in M1 generation a) Irregular distribution of anaphase chromosome b) Chromosome bridge at late anaphase c) Fragment at telophase d) Slow and complex movement.

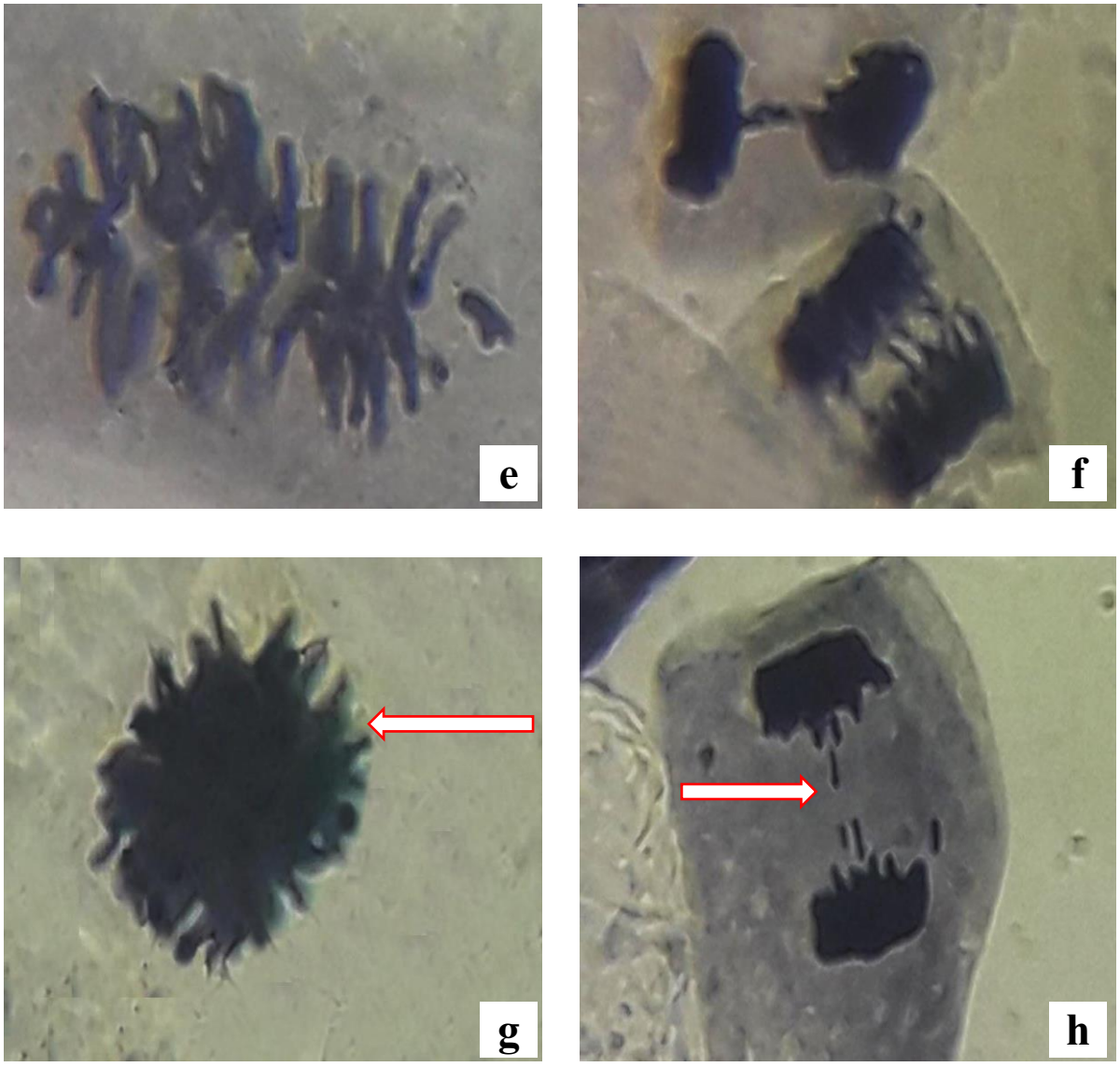
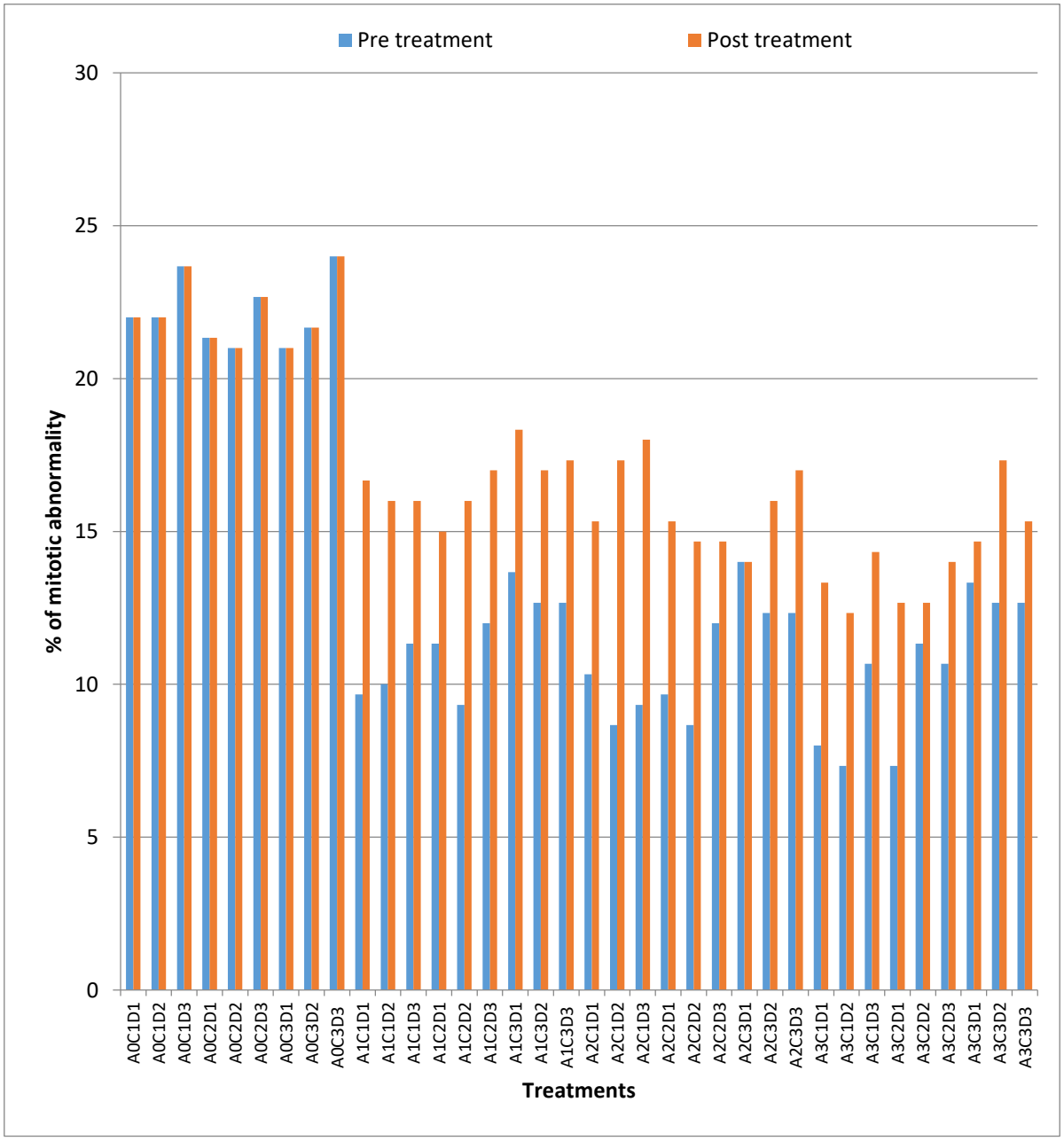
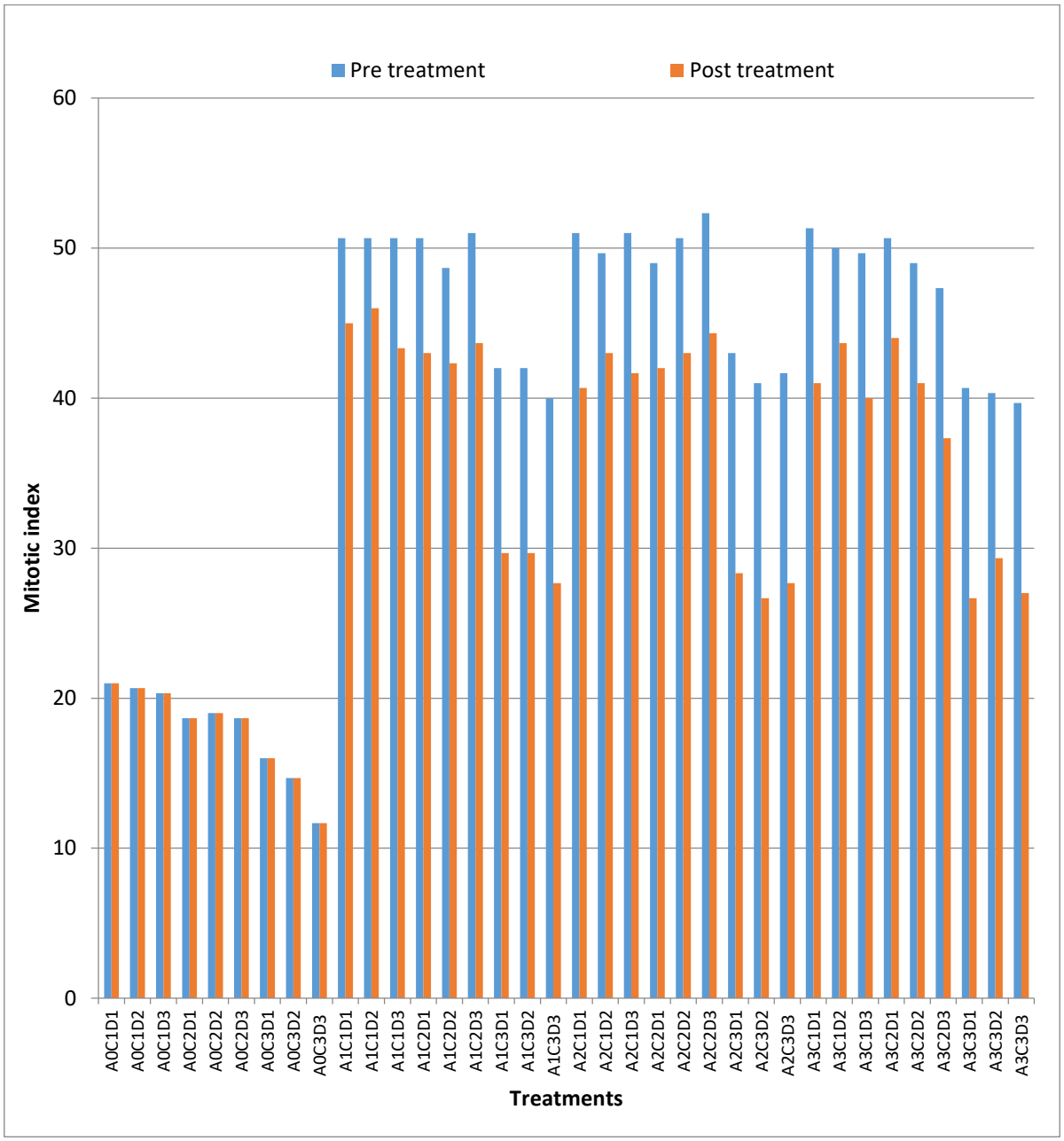


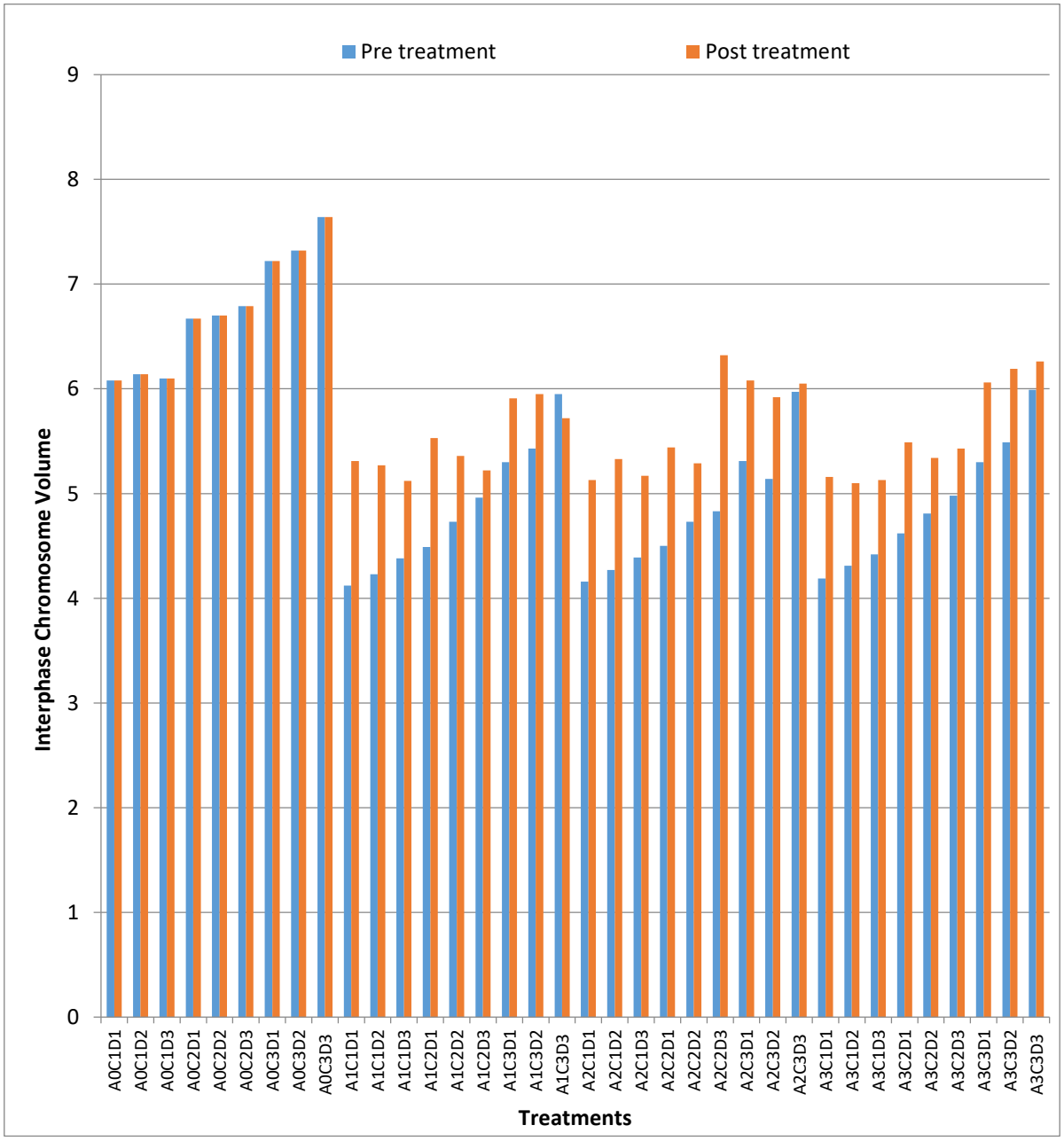
Fig. 2(b): Different types of mitotic abnormalities of wheat induced with different concentration and duration of pesticide in M1 generation e) Precocious movement of anaphase chromosome f) Multi-bridge of telophase chromosome g) Complex form of prophase chromosome h) Chromosome fragment.



Histogram 15: Effect of antioxidant along with control on % of mitotic abnormality of wheat induced with different concentration and duration of pesticide in M1 generation.



Histogram 16: Effect of antioxidant along with control on mitotic index of wheat induced with different concentration and duration of pesticide in M1 generation.



Histogram 17: Effect of antioxidant along with control on interphase chromosome volume of wheat induced with different concentration and duration of pesticide in M1 generation.

Table 8: Effect of antioxidants along with control on % of mitotic abnormality, mitotic index and interphase chromosome volume (ICV of wheat induced with different concentration and duration of pesticide in M1 generation.

Treatment	Pre-treatment			Post-treatment		
	% of mitotic abnormality	Mitotic index	ICV(µ3)	% of mitotic abnormality	Mitotic index	ICV(µ3)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	22.00 ± 1.00	21.00 ± 1.00	6.08 ± 0.07	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	22.00 ± 1.00	20.67 ± 1.15	6.14 ± 0.05	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	23.67 ± 0.58	20.33 ± 0.58	6.10 ± 0.17	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	21.33 ± 2.08	18.67 ± 0.58	6.67 ± 0.14	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	21.00 ± 1.00	19.00 ± 0.00	6.70 ± 0.17	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	22.67 ± 1.15	18.67 ± 0.58	6.79 ± 0.04	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	21.00 ± 1.00	16.00 ± 1.00	7.22 ± 0.11	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	21.67 ± 0.58	14.67 ± 1.53	7.32 ± 0.08	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	24.00 ± 1.00	11.67 ± 0.58	7.64 ± 0.12	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	9.67 ± 2.08	50.67 ± 3.06	4.12 ± 0.03	16.67 ± 1.15	45.00 ± 3.61	5.31 ± 0.04
A1C1D2	10.00 ± 2.00	50.67 ± 1.15	4.23 ± 0.03	16.00 ± 0.00	46.00 ± 2.65	5.27 ± 0.11
A1C1D3	11.33 ± 1.15	50.67 ± 3.06	4.38 ± 0.03	16.00 ± 3.46	43.33 ± 3.06	5.12 ± 0.11
A1C2D1	11.33 ± 1.15	50.67 ± 2.31	4.49 ± 0.01	15.00 ± 2.65	43.00 ± 2.65	5.53 ± 0.06
A1C2D2	9.33 ± 1.15	48.67 ± 2.31	4.73 ± 0.03	16.00 ± 0.00	42.33 ± 2.52	5.36 ± 0.10
A1C2D3	12.00 ± 0.00	51.00 ± 5.57	4.96 ± 0.06	17.00 ± 1.00	43.67 ± 2.52	5.22 ± 0.03
A1C3D1	13.67 ± 2.08	42.00 ± 2.00	5.30 ± 0.02	18.33 ± 0.58	29.67 ± 1.53	5.91 ± 0.06
A1C3D2	12.67 ± 1.15	42.00 ± 2.65	5.43 ± 0.03	17.00 ± 1.00	29.67 ± 0.58	5.95 ± 0.07
A1C3D3	12.67 ± 1.15	40.00 ± 1.00	5.95 ± 0.04	17.33 ± 0.58	27.67 ± 2.08	5.72 ± 0.19
A2C1D1	10.33 ± 1.53	51.00 ± 6.08	4.16 ± 0.02	15.33 ± 3.06	40.67 ± 3.06	5.13 ± 0.14
A2C1D2	8.67 ± 1.15	49.67 ± 2.52	4.27 ± 0.01	17.33 ± 1.15	43.00 ± 3.61	5.33 ± 0.42
A2C1D3	9.33 ± 2.31	51.00 ± 1.73	4.39 ± 0.01	18.00 ± 0.00	41.67 ± 4.51	5.17 ± 0.14
A2C2D1	9.67 ± 2.08	49.00 ± 1.73	4.50 ± 0.02	15.33 ± 2.31	42.00 ± 2.00	5.44 ± 0.08
A2C2D2	8.67 ± 1.15	50.67 ± 2.31	4.73 ± 0.02	14.67 ± 3.06	43.00 ± 2.65	5.29 ± 0.02
A2C2D3	12.00 ± 1.00	52.33 ± 2.89	4.83 ± 0.04	14.67 ± 3.06	44.33 ± 1.53	6.32 ± 0.75
A2C3D1	14.00 ± 2.00	43.00 ± 2.65	5.31 ± 0.02	14.00 ± 2.00	28.33 ± 2.52	6.08 ± 0.14
A2C3D2	12.33 ± 0.58	41.00 ± 1.73	5.14 ± 0.58	16.00 ± 3.46	26.67 ± 2.89	5.92 ± 0.07
A2C3D3	12.33 ± 0.58	41.67 ± 2.08	5.97 ± 0.02	17.00 ± 2.65	27.67 ± 2.52	6.05 ± 0.09
A3C1D1	8.00 ± 4.00	51.33 ± 3.79	4.19 ± 0.01	13.33 ± 3.06	41.00 ± 3.61	5.16 ± 0.08
A3C1D2	7.33 ± 3.06	50.00 ± 2.00	4.31 ± 0.02	12.33 ± 0.58	43.67 ± 5.13	5.10 ± 0.09
A3C1D3	10.67 ± 2.31	49.67 ± 3.21	4.42 ± 0.01	14.33 ± 1.53	40.00 ± 2.00	5.13 ± 0.06
A3C2D1	7.33 ± 3.06	50.67 ± 4.16	4.62 ± 0.03	12.67 ± 1.15	44.00 ± 4.58	5.49 ± 0.11
A3C2D2	11.33 ± 1.15	49.00 ± 1.73	4.81 ± 0.01	12.67 ± 1.15	41.00 ± 1.00	5.34 ± 0.08
A3C2D3	10.67 ± 1.15	47.33 ± 8.33	4.98 ± 0.01	14.00 ± 2.00	37.33 ± 8.33	5.43 ± 0.05
A3C3D1	13.33 ± 2.31	40.67 ± 3.06	5.30 ± 0.01	14.67 ± 1.15	26.67 ± 4.16	6.06 ± 0.12
A3C3D2	12.67 ± 1.15	40.33 ± 2.08	5.49 ± 0.01	17.33 ± 1.15	29.33 ± 2.31	6.19 ± 0.07
A3C3D3	12.67 ± 2.31	39.67 ± 0.58	5.99 ± 0.02	15.33 ± 3.06	27.00 ± 2.65	6.26 ± 0.05
Level of significance	*	*	*	*	*	**
CV(%)	12.58	16.06	2.15	9.51	6.23	3.06

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.1.2.3 Interphase chromosome volume (ICV)

Pre-treatment: Interphase Chromosome Volume (ICV) in the root tip cells emerged from seeds the maximum value (7.64%) was observed due to treatment $A_0C_3D_3$ which was different from that of all other treatments. On the other hand minimum value for interphase chromosome volume (4.12%) was found due to $A_1C_1D_1$, which was also significantly different from that of all other treatments (**Histogram 17 and Table 8)**.

Post-treatment: Highest value for interphase chromosome volume (7.64%) was observed due to treatment $A_0C_3D_3$ and lowest value for interphase chromosome volume (5.10%) was observed at $A_3C_1D_2$ which were both significantly different from that of all other treatments (**Histogram 17 and Table 8)**.

4.1.2.4 % of meiotic abnormality

Meiotic study in the pollen mother cells (PMCs) of wheat plants grown from the seeds of different treatments showed various types of chromosomal aberrations. The different abnormalities found in the PMCs were fragments, bridges and lagging chromosomes. Mean percentage of abnormalities at different treatments are shown in **Fig. 3, Histogram 18 and Table 9.**

Pre-treatment: The highest percentage of meiotic abnormality (14.33%) was observed at $A_0C_3D_3$ which was statistically different from that of all other treatments. On the other hands the lowest percentage of meiotic abnormality (2.00%) was observed at $A_1C_1D_1$, $A_1C_1D_2$, $A_1C_2D_2$ and $A_3C_1D_1$ which were statistically different from that of all the treatments.

Post-treatment: Treatment $A_0C_3D_3$ showed highest percentage of meiotic abnormality (14.33%) and $A_1C_1D_2$ showed lowest percentage of meiotic abnormality (3.33%) which were statistically different from that of all other treatments.

4.1.2.5 Pollen sterility

Pre-treatment: $A_0C_3D_3$ exhibited highest percentage of pollen sterility (22.33%) which was statistically different from that of all other treatments. On the other hand treatment $A_1C_2D_2$ and $A_1C_3D_3$ showed lowest percentage of pollen sterility (4.00%) which were statistically different from all other treatments (**Histogram 19 and Table 9)**.

Post-treatment: The maximum value for percentage of pollen sterility (22.33%) was observed due to treatment $A_0C_3D_3$ (Table 8) and the minimum value for pollen sterility (6.00%) was due to treatment $A_1C_1D_2$ which were statistically different from that of all other treatments (**Histogram 19 and Table 8)**.

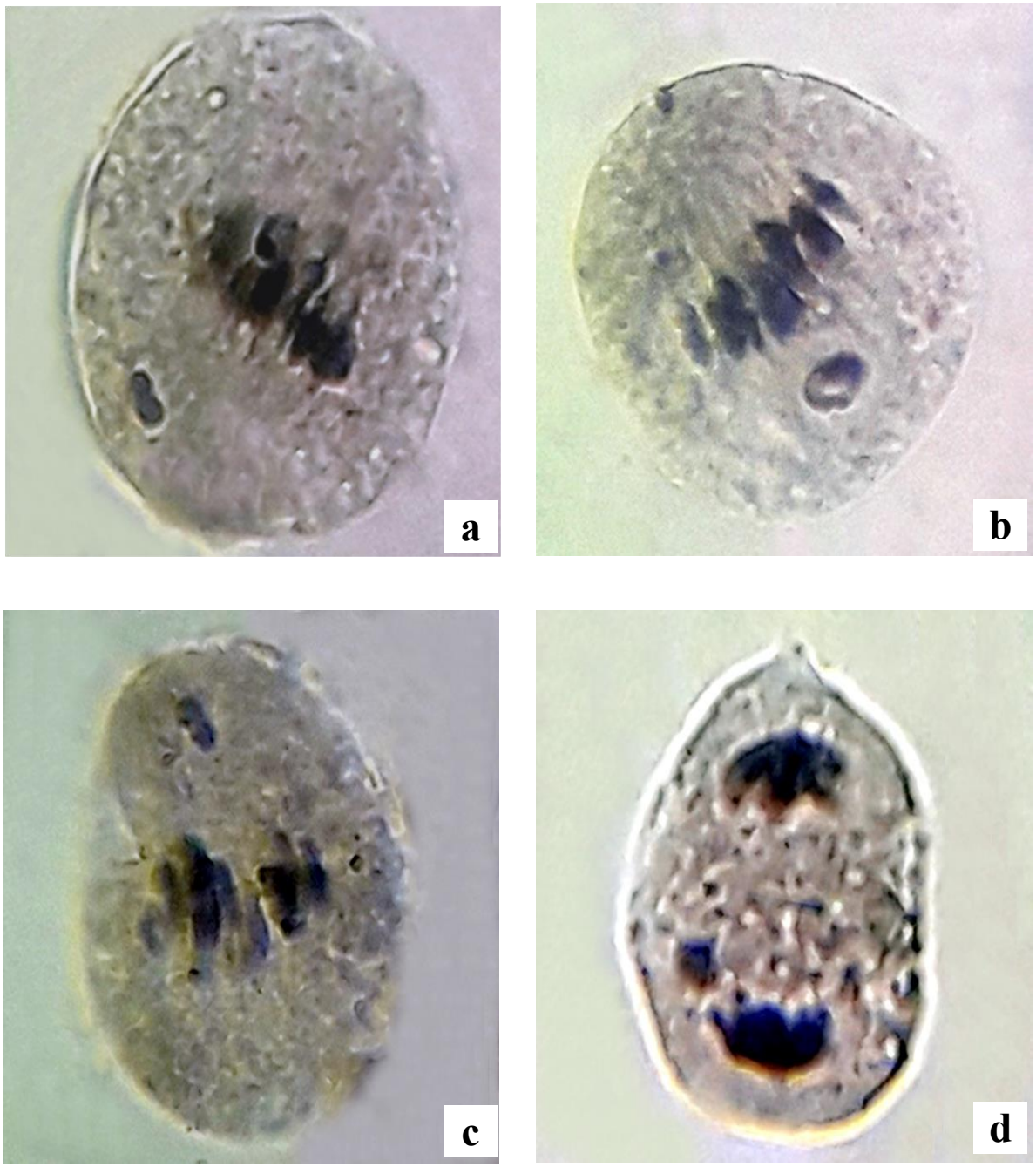


Fig. 3(a): Photomicrographs showing different types of meiotic abnormalities in wheat induced with different concentration and duration of pesticide in M1 generation. a) Metaphase 1 with a displayed bivalent b) Metaphase 1 with ring bivalent c) Disoriented metaphase 1 with a fragment and a bivalent d) One of the dyad of telophase 2 with a large fragment of chromosome.

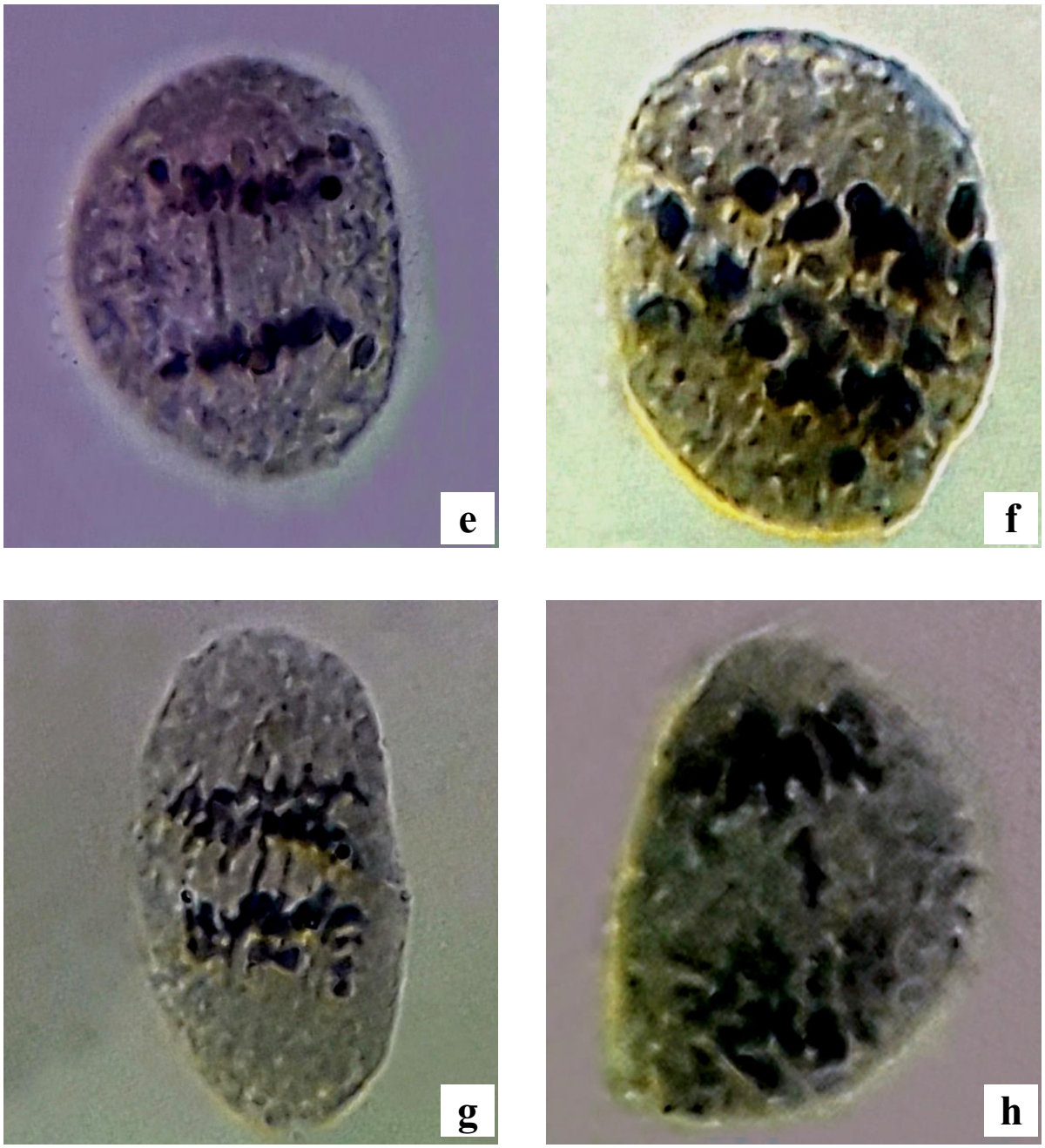
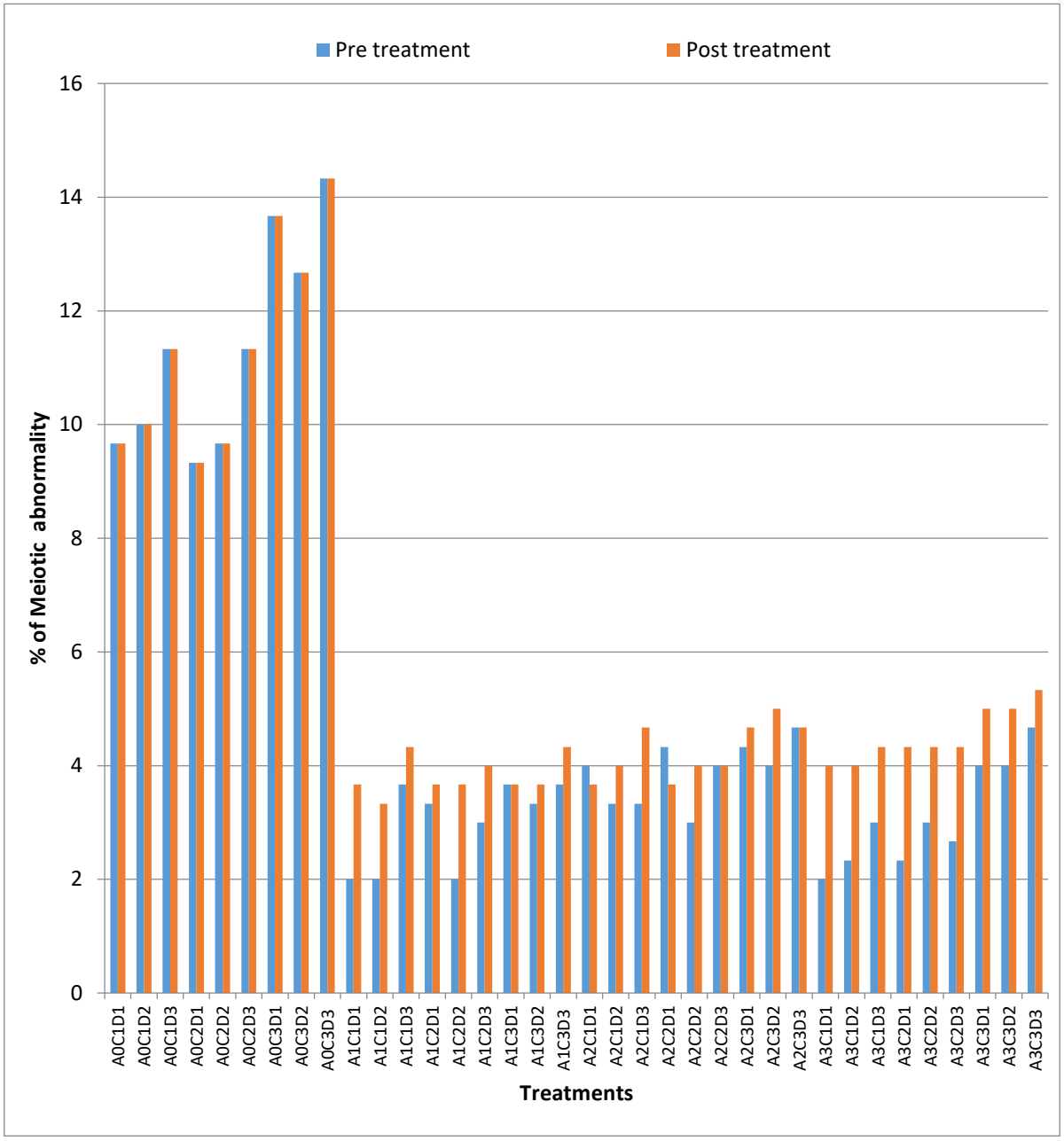
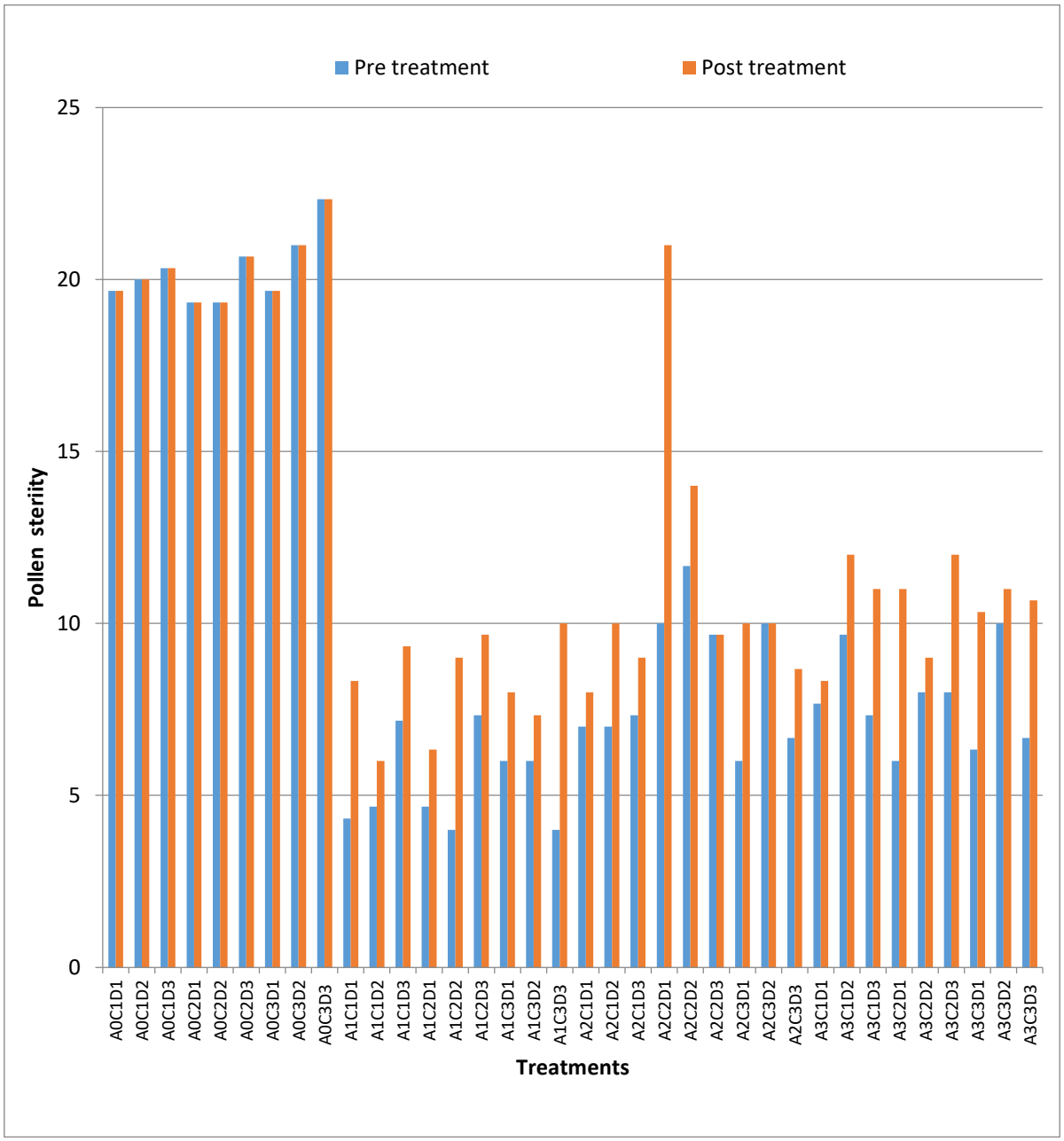


Fig. 3(b): Photomicrographs showing different types of meiotic abnormalities in wheat induced with different concentration and duration of pesticide in M1 generation. e) Bridge, fragment and laggard at Anaphase 1 f) Dislocated metaphase 1 chromosome g) Anaphase 2 with multi bridge h) Anaphase 2 with fragment.



Histogram 18: Effect of antioxidant along with control on % of meiotic abnormality of wheat induced with different concentration and duration of pesticide in M1 generation.



Histogram 19: Effect of antioxidant along with control on pollen sterility of wheat induced with different concentration and duration of pesticide in M1 generation.

4.1.2.6 Pollen abnormality

For studying pollen abnormality of hexaploid wheat *Wheat*ripe anther were collected at anthesis time from different treatments. Photomicrographs and collected data are represented in **Fig. 4, Histogram 20 and Table 9.**

Pre-treatment: The highest percentage of pollen abnormality (26.67%) was observed at $A_0C_3D_3$ and on the other hand lowest percentage of pollen abnormality (9.00%) was found at $A_3C_2D_2$ which were both statistically different from that of all other treatments.

Post-treatment: The maximum value for pollen abnormality (26.67%) was observed due to treatment $A_0C_3D_3$ and on the other hand minimum value for pollen abnormality (19.00%) was due to $A_3C_1D_3$ treatment which were both statistically different from that of all other treatments.

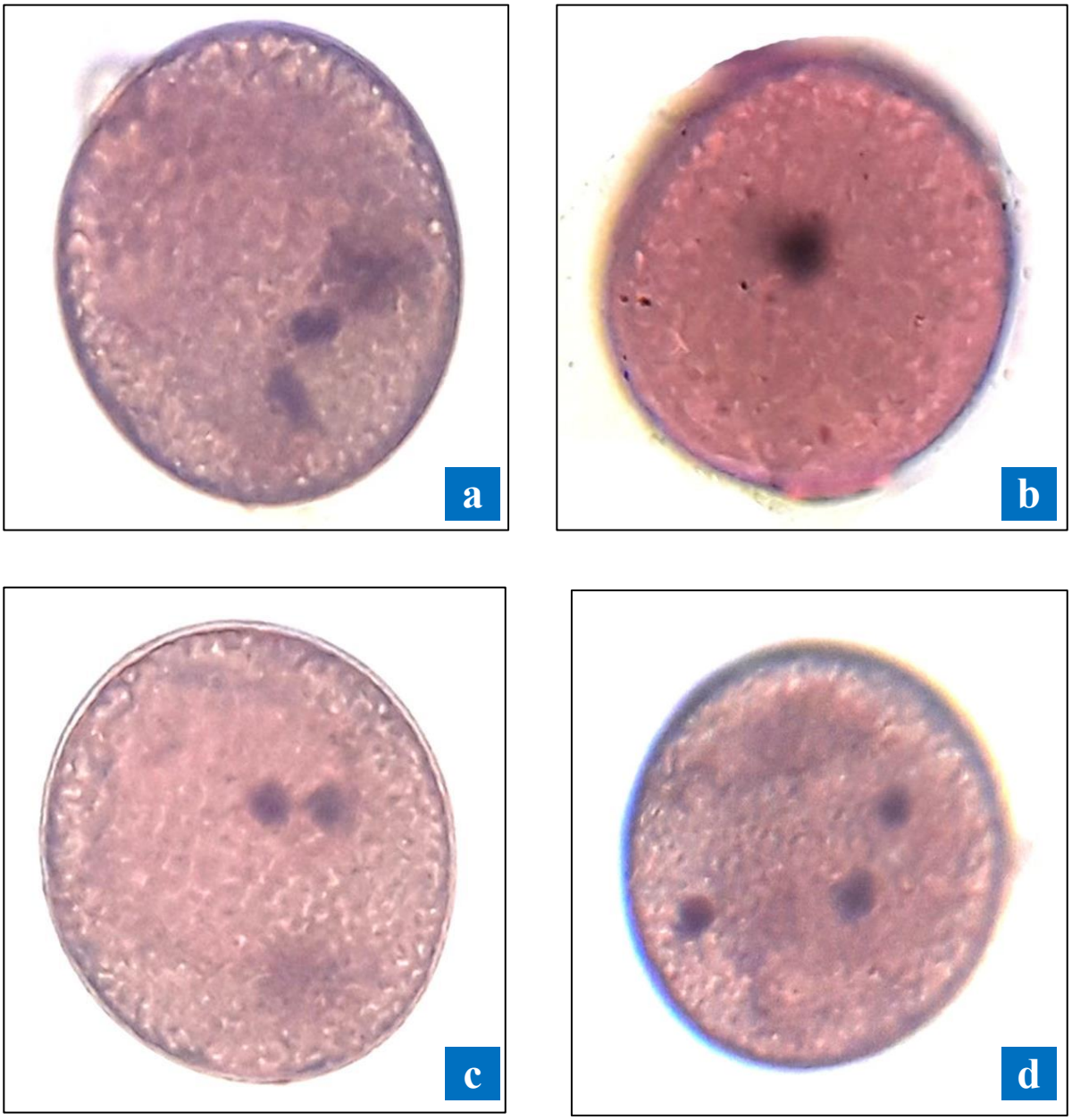
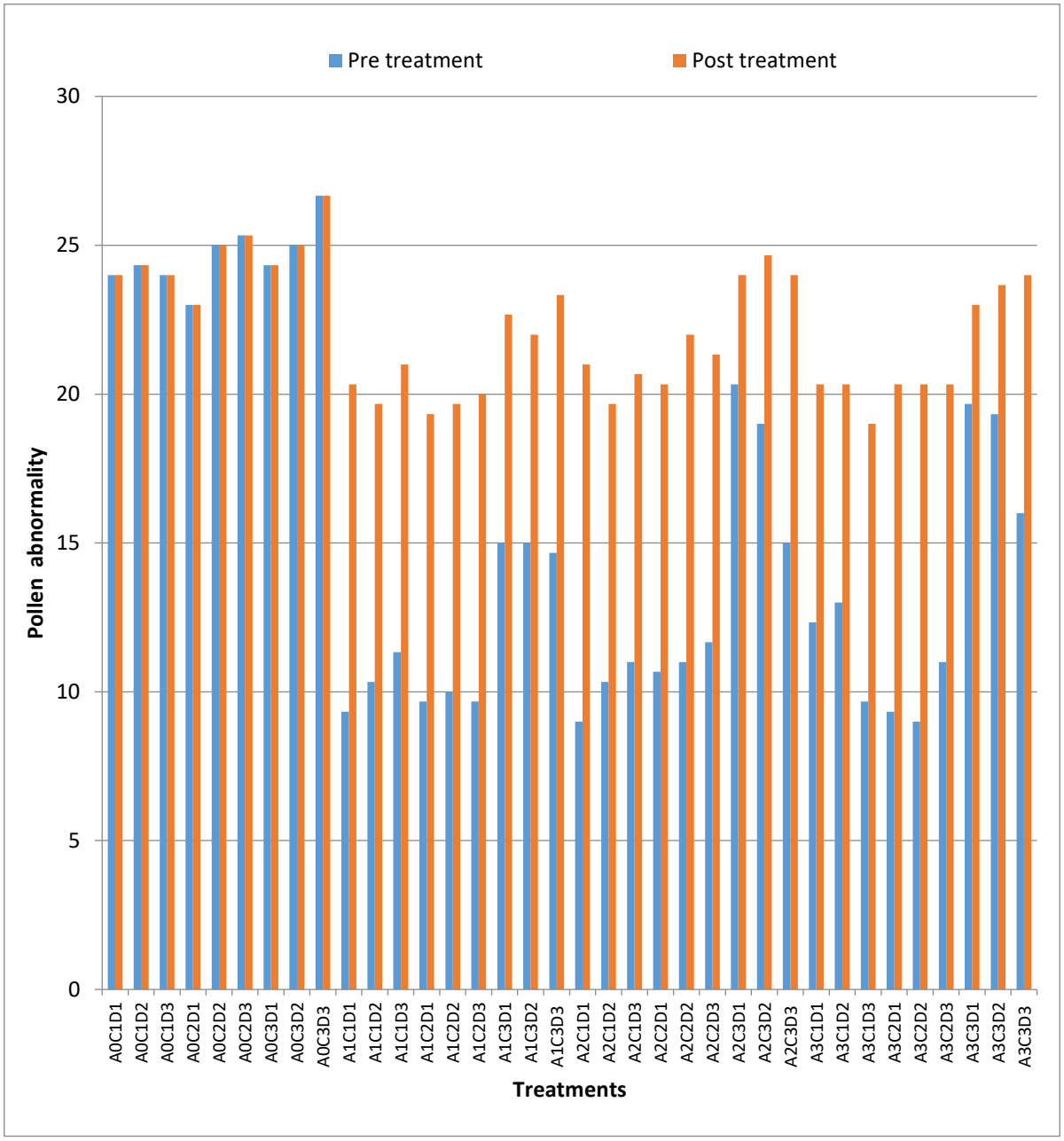


Fig. 4: Photomicrographs showing different types of pollen abnormalities of wheat induced with different concentration and duration of pesticide in M1 generation. a) Normal Binucleate b) Mononucleate c) Binucleate (abnormal) d) Trinucleate.



Histogram 20: Effect of antioxidant along with control on pollen abnormality of wheat induced with different concentration and duration of pesticide in M1 generation.

Table 9: Effect of antioxidants along with control on % of meiotic abnormality, pollen sterility and pollen abnormality of wheat induced with different concentration and duration of pesticide in M1 generation.

Treatment	Pre-treatment			Post-treatment		
	% of Meiotic abnormality	% of Pollen sterility	% of Pollen abnormality	% of Meiotic abnormality	% of Pollen sterility	% of Pollen abnormality
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	9.67 ± 0.58	19.67 ± 0.58	24.00 ± 1.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	10.00 ± 1.00	20.00 ± 1.00	24.33 ± 0.58	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	11.33 ± 1.15	20.33 ± 1.15	24.00 ± 1.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	9.33 ± 0.58	19.33 ± 0.58	23.00 ± 1.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	9.67 ± 0.58	19.33 ± 1.15	25.00 ± 0.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	11.33 ± 1.53	20.67 ± 0.58	25.33 ± 1.53	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	13.67 ± 0.58	19.67 ± 0.58	24.33 ± 1.53	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	12.67 ± 1.15	21.00 ± 1.00	25.00 ± 0.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	14.33 ± 0.58	22.33 ± 0.58	26.67 ± 0.58	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	2.00 ± 0.00	4.33 ± 1.15	9.33 ± 1.53	3.67 ± 0.58	8.33 ± 0.58	20.33 ± 0.58
A1C1D2	2.00 ± 1.00	4.67 ± 0.58	10.33 ± 0.58	3.33 ± 0.58	6.00 ± 0.00	19.67 ± 0.58
A1C1D3	3.67 ± 0.58	7.17 ± 0.76	11.33 ± 0.58	4.33 ± 0.58	9.33 ± 0.58	21.00 ± 1.73
A1C2D1	3.33 ± 1.15	4.67 ± 0.58	9.67 ± 1.53	3.67 ± 1.15	6.33 ± 0.58	19.33 ± 0.58
A1C2D2	2.00 ± 1.00	4.00 ± 0.00	10.00 ± 1.00	3.67 ± 0.58	9.00 ± 1.73	19.67 ± 0.58
A1C2D3	3.00 ± 1.00	7.33 ± 1.15	9.67 ± 1.53	4.00 ± 1.00	9.67 ± 1.53	20.00 ± 0.00
A1C3D1	3.67 ± 0.58	6.00 ± 1.00	15.00 ± 1.73	3.67 ± 1.15	8.00 ± 1.00	22.67 ± 1.53
A1C3D2	3.33 ± 0.58	6.00 ± 2.00	15.00 ± 2.00	3.67 ± 0.58	7.33 ± 1.53	22.00 ± 1.00
A1C3D3	3.67 ± 0.58	4.00 ± 1.00	14.67 ± 2.08	4.33 ± 0.58	10.00 ± 1.00	23.33 ± 0.58
A2C1D1	4.00 ± 0.00	7.00 ± 1.00	9.00 ± 1.00	3.67 ± 0.58	8.00 ± 1.00	21.00 ± 0.00
A2C1D2	3.33 ± 0.58	7.00 ± 0.00	10.33 ± 2.52	4.00 ± 1.00	10.00 ± 0.00	19.67 ± 0.58
A2C1D3	3.33 ± 1.53	7.33 ± 0.58	11.00 ± 1.00	4.67 ± 0.58	9.00 ± 1.00	20.67 ± 1.53
A2C2D1	4.33 ± 0.58	10.00 ± 1.00	10.67 ± 1.15	3.67 ± 0.58	21.00 ± 1.00	20.33 ± 0.58
A2C2D2	3.00 ± 1.00	11.67 ± 0.58	11.00 ± 1.73	4.00 ± 0.00	14.00 ± 1.00	22.00 ± 1.00
A2C2D3	4.00 ± 1.00	9.67 ± 0.58	11.67 ± 1.53	4.00 ± 1.00	9.67 ± 1.53	21.33 ± 3.21
A2C3D1	4.33 ± 1.15	6.00 ± 1.00	20.33 ± 0.58	4.67 ± 0.58	10.00 ± 1.00	24.00 ± 1.00
A2C3D2	4.00 ± 1.00	10.00 ± 0.00	19.00 ± 1.00	5.00 ± 0.00	10.00 ± 1.00	24.67 ± 0.58
A2C3D3	4.67 ± 0.58	6.67 ± 0.58	15.00 ± 1.73	4.67 ± 1.15	8.67 ± 1.53	24.00 ± 1.00
A3C1D1	2.00 ± 1.00	7.67 ± 1.15	12.33 ± 4.93	4.00 ± 1.00	8.33 ± 0.58	20.33 ± 0.58
A3C1D2	2.33 ± 0.58	9.67 ± 0.58	13.00 ± 5.29	4.00 ± 1.00	12.00 ± 1.00	20.33 ± 0.58
A3C1D3	3.00 ± 1.00	7.33 ± 1.53	9.67 ± 1.53	4.33 ± 1.15	11.00 ± 1.00	19.00 ± 1.73
A3C2D1	2.33 ± 0.58	6.00 ± 1.00	9.33 ± 1.15	4.33 ± 0.58	11.00 ± 1.00	20.33 ± 1.53
A3C2D2	3.00 ± 1.00	8.00 ± 1.00	9.00 ± 1.73	4.33 ± 0.58	9.00 ± 0.00	20.33 ± 0.58
A3C2D3	2.67 ± 1.15	8.00 ± 0.00	11.00 ± 1.00	4.33 ± 1.53	12.00 ± 1.00	20.33 ± 1.15
A3C3D1	4.00 ± 1.00	6.33 ± 1.15	19.67 ± 0.58	5.00 ± 1.00	10.33 ± 0.58	23.00 ± 1.00
A3C3D2	4.00 ± 1.00	10.00 ± 1.00	19.33 ± 1.53	5.00 ± 1.00	11.00 ± 1.00	23.67 ± 4.16
A3C3D3	4.67 ± 0.58	6.67 ± 0.58	16.00 ± 2.65	5.33 ± 1.53	10.67 ± 0.58	24.00 ± 3.00
Level of significance	*	**	*	*	**	*
CV(%)	16.31	8.34	11.45	14.31	7.69	6.15

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.2 Result of M2 generation

4.2.1 Morphological feature

In this investigation, various morphological characters as mentioned earlier were considered for morphological study.

4.2.1.1 Germination percentage in laboratory

Seeds collected from M1 generation were allowed to germinate on moist filter paper in petri dishes in the laboratory at room temperature (21-23ºC) during the same time when they were also shown in the pots. Germination percentage of M2 seeds both in laboratory and in pots are shown in **Histogram 21 and Table 10.**

Pre-treatment: Highest value in laboratory (91.33%) for germination percentage was observed at $A_3C_1D_3$ which was statistically different from that of all other treatments. The lowest value (39.33%) for germination percentage was found at $A_0C_3D_3$ which was also significantly different from that of all other treatments. In pot highest value (86.00%) and lowest value (37.00%) were found at $A_3C_1D_2$ and $A_0C_3D_3$ respectively which were statistically different from that of all the treatments.

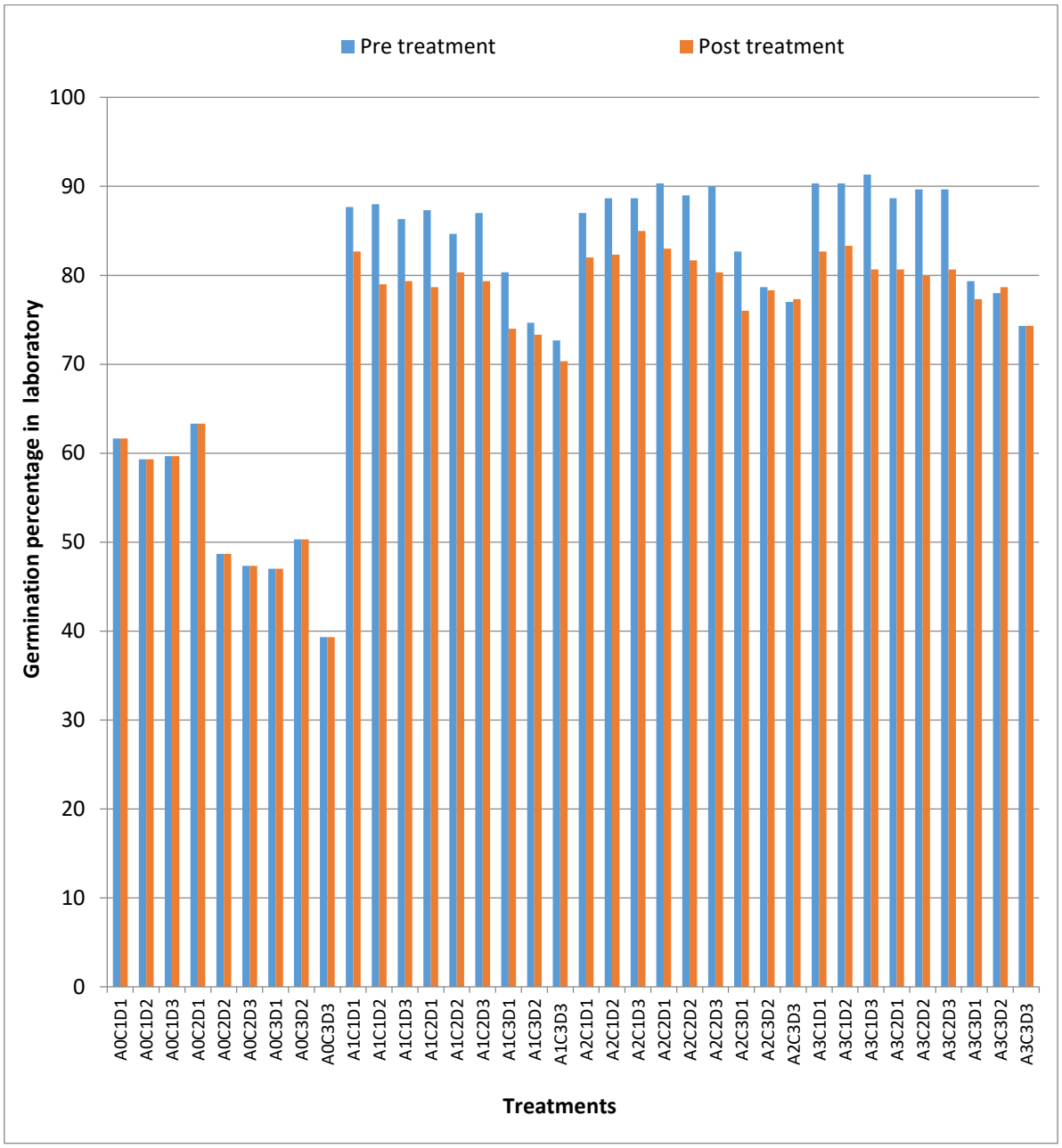
Post-treatment: Maximum value (85.00%) for germination percentage in laboratory was shown at $A_2C_1D_3$ which was significantly different from that all other treatments and on the other hand minimum value (39.33%) was found at $A_0C_3D_3$ which was also different from that of all other treatments. But in pot highest value (76.67%) and lowest value (37.00%) were found at $A_1C_1D_2$ and $A_0C_3D_3$ respectively which were statistically different from with that of all the treatments.

4.2.1.2 Coleoptiles length

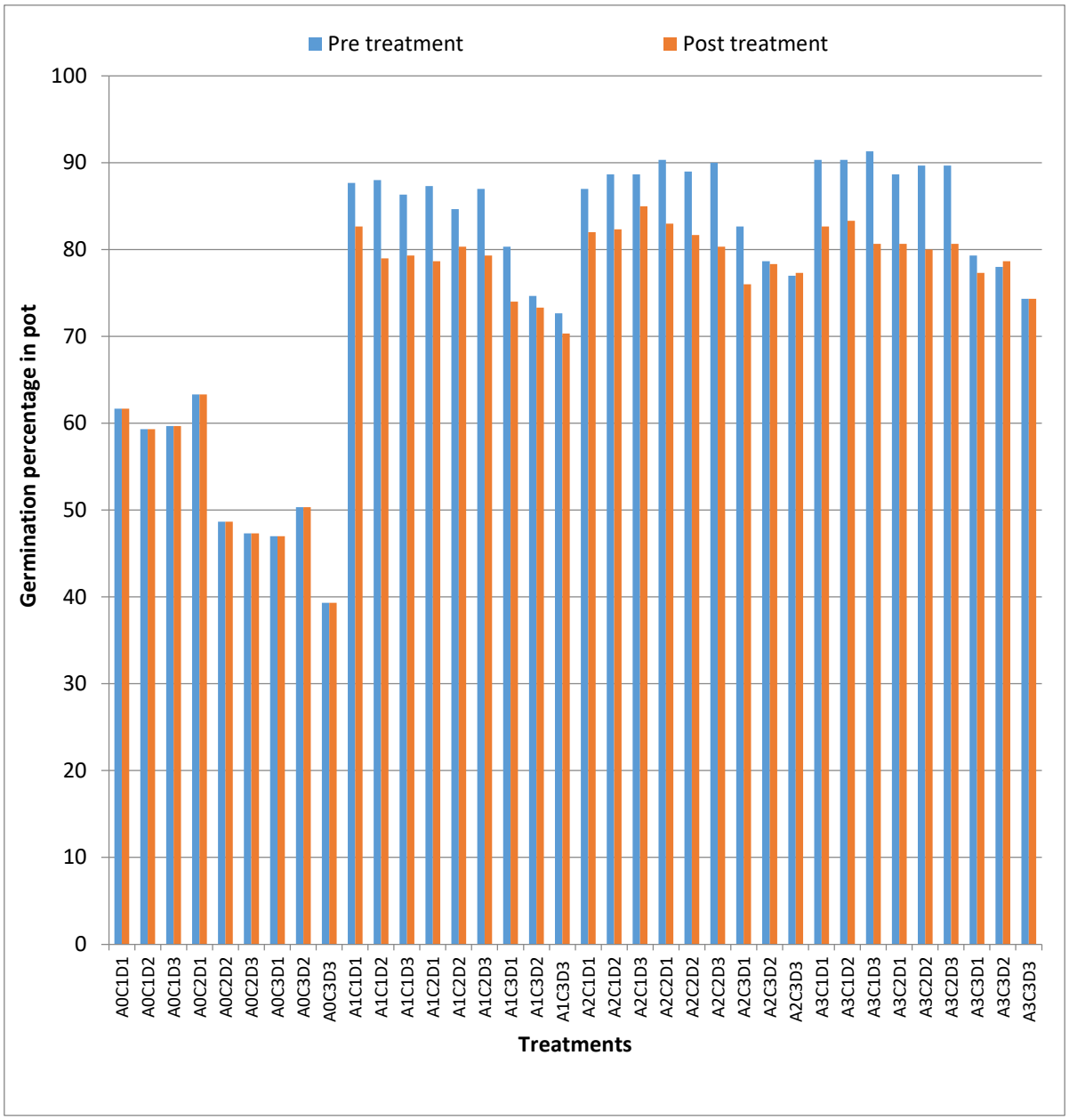
Coleoptiles length at 7 days after germination was recorded and presented in **Histogram 22 and Table 10.**

Pre-treatment: Highest coleoptile length (2.23cm) and lowest coleoptiles length (0.72cm) were observed at $A_2C_1D_1$ and $A_0C_3D_3$ respectively both were statistically different from that of all other treatments.

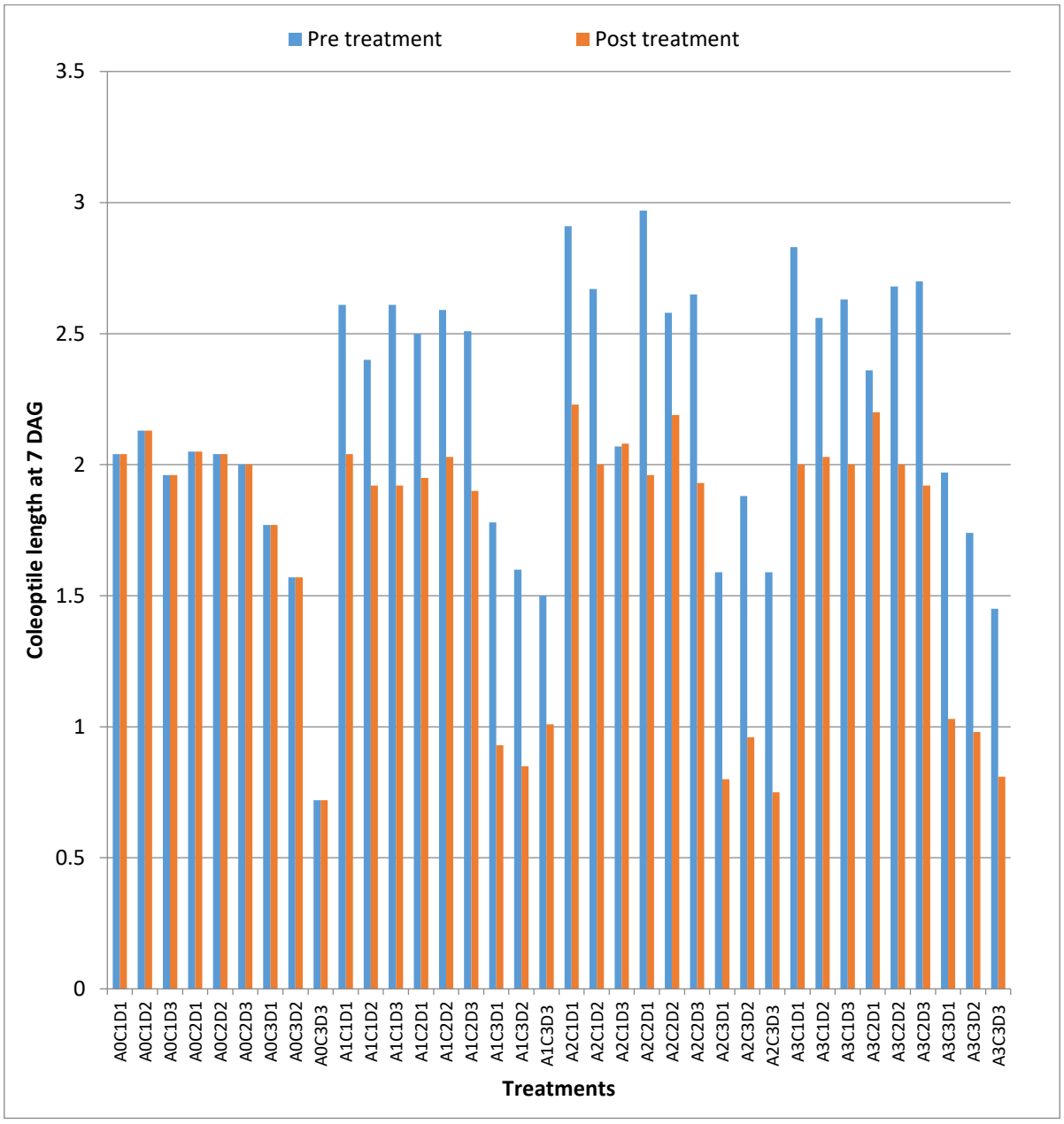
Post-treatment: Highest value for coleoptiles length (2.97cm) and lowest value for coleoptiles length (0.72cm) were observed at $A_2C_2D_1$ and $A_0C_3D_3$ respectively which were statistically nonsignificant.



Histogram 21: Effect of antioxidant along with control on germination percentage of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 22: Effect of antioxidant along with control on germination percentage of wheat induced with different concentration and duration of pesticide in pot in M2 generation.



Histogram 23: Effect of antioxidant along with control on coleoptile length at 7 days after germination of wheat induced with different concentration and duration of pesticide in M2 generation.

Table 10: Effect of antioxidants along with control on germination percentage (in laboratory and in pot) of wheat induced with different concentration and duration of pesticide in M2 generation.

Treatment	Pre-treatment			Post-treatment		
	Germination %		Coleoptile length (cm)	Germination %		Coleoptile length (cm)
	in laboratory	in pot	in laboratory	in laboratory	in pot	in laboratory
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
A0C1D1	61.67 ± 0.58	56.33 ± 2.31	2.04 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	59.33 ± 1.53	58.67 ± 0.58	2.13 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	59.67 ± 1.53	48.33 ± 2.89	1.96 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	63.33 ± 1.53	56.33 ± 1.53	2.05 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	48.67 ± 2.31	55.67 ± 1.53	2.04 ± 0.05	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	47.33 ± 2.08	49.00 ± 3.61	2.00 ± 0.01	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	47.00 ± 2.65	43.33 ± 2.89	1.77 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	50.33 ± 1.53	41.00 ± 1.00	1.57 ± 0.12	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	39.33 ± 1.15	37.00 ± 1.73	0.72 ± 0.32	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	87.67 ± 2.31	84.00 ± 2.65	2.61 ± 0.19	82.67 ± 1.53	75.00 ± 1.00	2.04 ± 0.06
A1C1D2	88.00 ± 1.00	84.67 ± 3.21	2.40 ± 0.39	79.00 ± 1.73	76.67 ± 2.08	1.92 ± 0.07
A1C1D3	86.33 ± 2.31	84.33 ± 3.79	2.61 ± 0.53	79.33 ± 0.58	74.33 ± 1.15	1.92 ± 0.07
A1C2D1	87.33 ± 0.58	84.67 ± 0.58	2.50 ± 0.27	78.67 ± 0.58	76.33 ± 1.15	1.95 ± 0.07
A1C2D2	84.67 ± 0.58	84.33 ± 2.08	2.59 ± 0.44	80.33 ± 1.53	74.67 ± 0.58	2.03 ± 0.06
A1C2D3	87.00 ± 1.00	83.33 ± 1.15	2.51 ± 0.47	79.33 ± 1.15	73.67 ± 0.58	1.90 ± 0.10
A1C3D1	80.33 ± 0.58	69.67 ± 1.53	1.78 ± 0.07	74.00 ± 3.46	70.00 ± 1.00	0.93 ± 0.08
A1C3D2	74.67 ± 0.58	70.00 ± 2.65	1.60 ± 0.52	73.33 ± 2.89	71.00 ± 1.73	0.85 ± 0.08
A1C3D3	72.67 ± 2.52	67.67 ± 0.58	1.50 ± 0.30	70.33 ± 0.58	69.33 ± 2.08	1.01 ± 0.09
A2C1D1	87.00 ± 1.73	84.33 ± 3.06	2.91 ± 0.03	82.00 ± 2.00	74.00 ± 2.65	2.23 ± 0.24
A2C1D2	88.67 ± 1.15	84.33 ± 2.08	2.67 ± 0.15	82.33 ± 2.08	75.67 ± 1.15	2.00 ± 0.11
A2C1D3	88.67 ± 0.58	85.00 ± 3.46	2.07 ± 0.06	85.00 ± 0.00	74.33 ± 1.15	2.08 ± 0.07
A2C2D1	90.33 ± 0.58	84.00 ± 3.61	2.97 ± 0.12	83.00 ± 1.00	76.00 ± 1.00	1.96 ± 0.19
A2C2D2	89.00 ± 1.73	84.33 ± 2.08	2.58 ± 0.12	81.67 ± 1.53	75.00 ± 0.00	2.19 ± 0.24
A2C2D3	90.00 ± 0.00	84.33 ± 1.15	2.65 ± 0.45	80.33 ± 1.53	75.00 ± 1.73	1.93 ± 0.06
A2C3D1	82.67 ± 2.52	71.33 ± 3.21	1.59 ± 0.53	76.00 ± 1.73	70.33 ± 1.53	0.80 ± 0.17
A2C3D2	78.67 ± 1.15	68.67 ± 2.08	1.88 ± 0.07	78.33 ± 2.08	70.00 ± 2.65	0.96 ± 0.04
A2C3D3	77.00 ± 1.00	65.33 ± 1.53	1.59 ± 0.51	77.33 ± 2.08	69.67 ± 1.53	0.75 ± 0.25
A3C1D1	90.33 ± 0.58	85.00 ± 0.00	2.83 ± 0.06	82.67 ± 2.52	75.33 ± 1.15	2.00 ± 0.10
A3C1D2	90.33 ± 0.58	86.00 ± 0.00	2.56 ± 0.49	83.33 ± 3.21	76.00 ± 1.00	2.03 ± 0.06
A3C1D3	91.33 ± 1.15	84.67 ± 0.58	2.63 ± 0.25	80.67 ± 1.15	74.67 ± 0.58	2.00 ± 0.11
A3C2D1	88.67 ± 0.58	83.33 ± 1.15	2.36 ± 0.48	80.67 ± 0.58	75.67 ± 1.53	2.20 ± 0.10
A3C2D2	89.67 ± 1.53	81.67 ± 1.53	2.68 ± 0.23	80.00 ± 0.00	76.33 ± 1.15	2.00 ± 0.10
A3C2D3	89.67 ± 0.58	81.67 ± 1.15	2.70 ± 0.61	80.67 ± 0.58	73.67 ± 1.53	1.92 ± 0.07
A3C3D1	79.33 ± 1.15	74.00 ± 10.15	1.97 ± 0.06	77.33 ± 2.08	69.33 ± 1.15	1.03 ± 0.06
A3C3D2	78.00 ± 2.65	65.67 ± 2.89	1.74 ± 0.10	78.67 ± 0.58	69.33 ± 2.08	0.98 ± 0.12
A3C3D3	74.33 ± 2.08	63.00 ± 4.36	1.45 ± 0.42	74.33 ± 4.04	68.00 ± 3.46	0.81 ± 0.17
Level of significance	**	*	**	**	*	NS
CV(%)	1.98	3.28	13.99	5.53	2.12	6.69

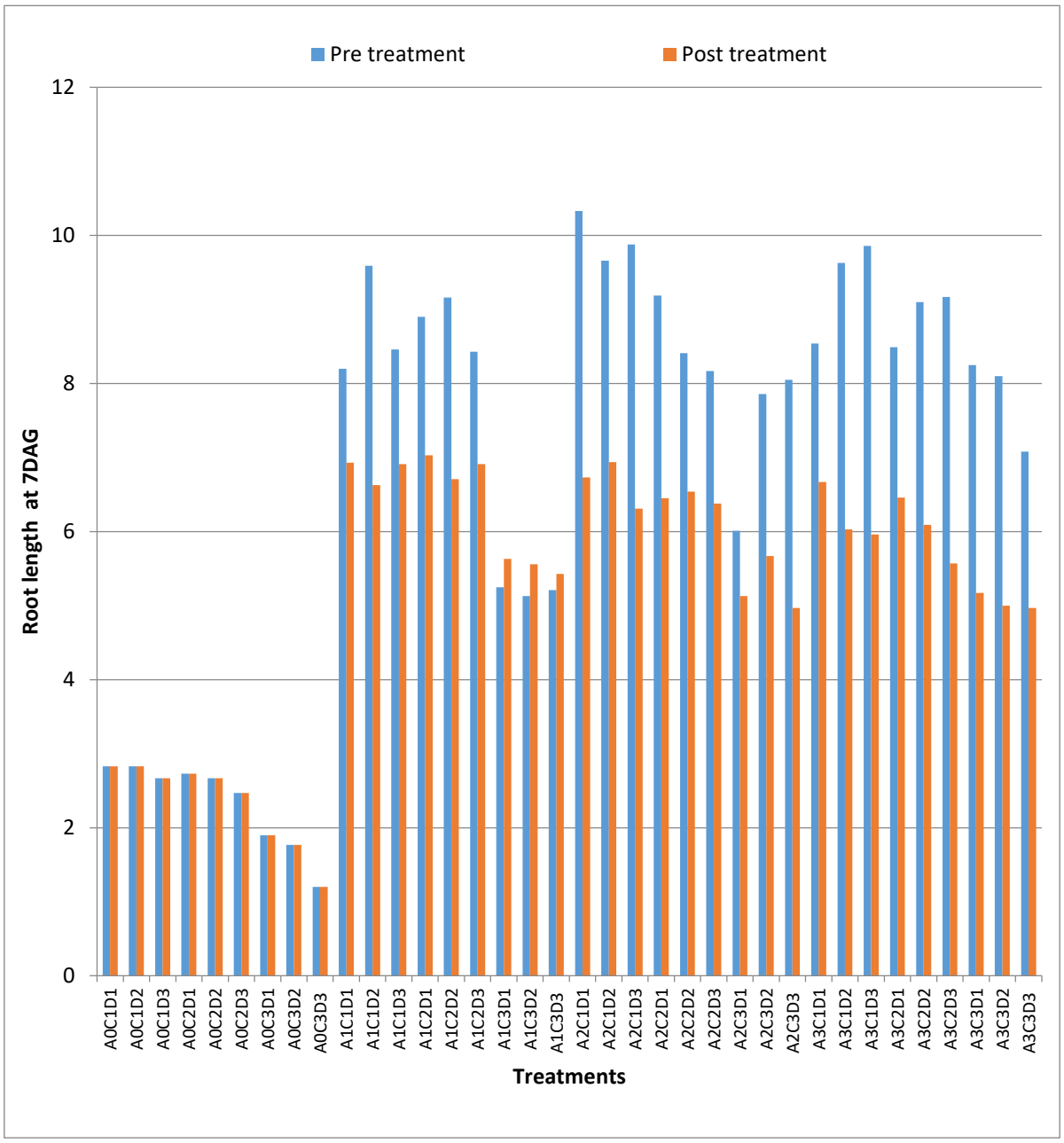
Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.2.1.3 Root length

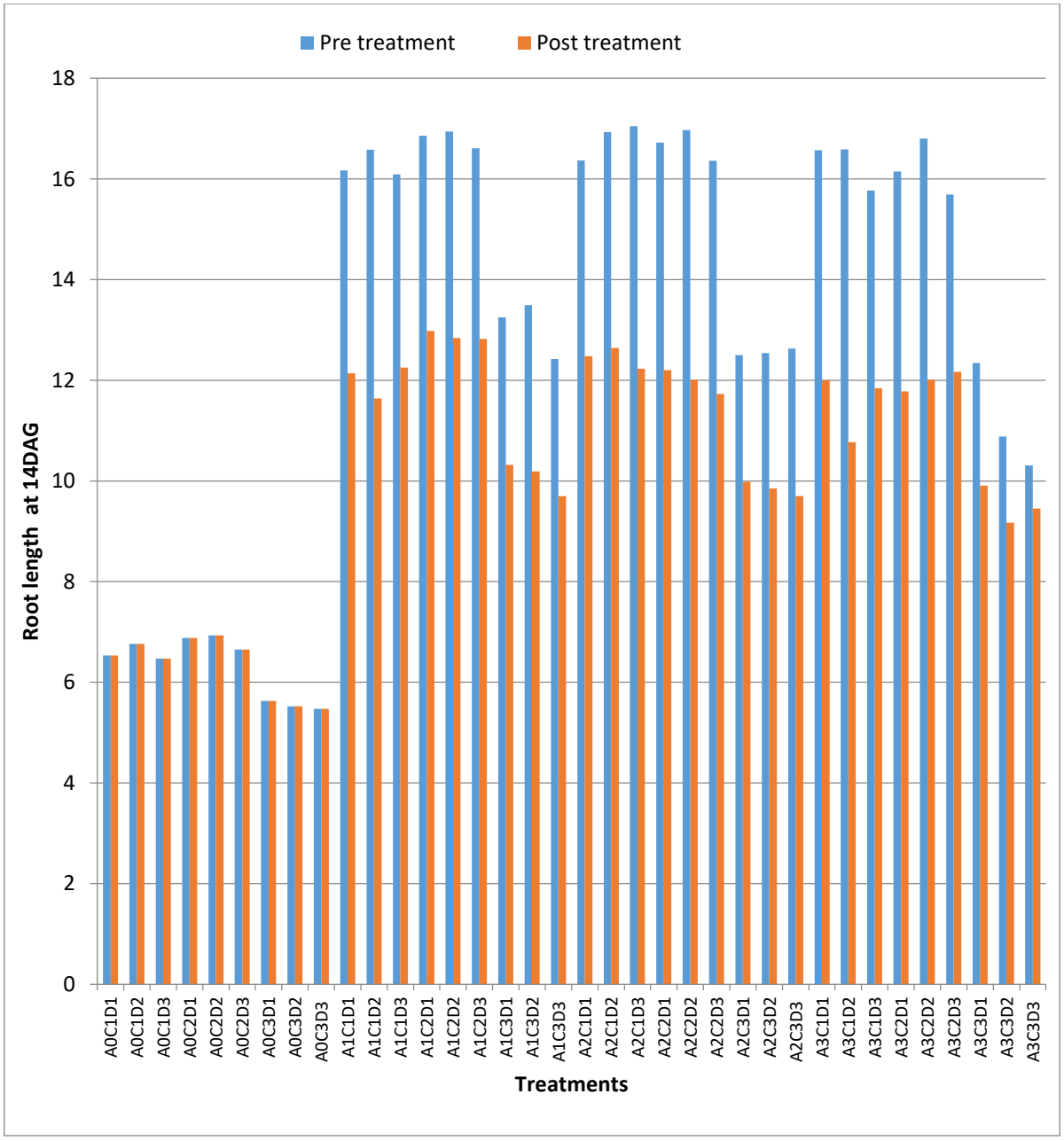
The root length at different days after germination are recorded which are shown in **Histogram 24(a), 24(b), 24(c) and Table 11.**

Pre-treatment: At 7 days after germination (DAG) longest root (10.33cm) was found at $A_2C_1D_1$ and shortest root (1.20cm) was observed at $A_0C_3D_3$ which were both statistically different from that of all other treatments. At 14 days after germination (DAG) longest root (17.05cm) was observed at $A_2C_1D_3$ and shortest root (5.47cm) was observed at $A_0C_3D_3$ which were both statistically similar with that of all other treatments. At 25 days after germination (DAG) longest root (23.49cm) was observed at $A_1C_1D_3$ which was statistically different from that of all other treatments. On the other hand shortest root (11.43cm) was observed at $A_0C_3D_3$ which was statistically different from that of all other treatments.

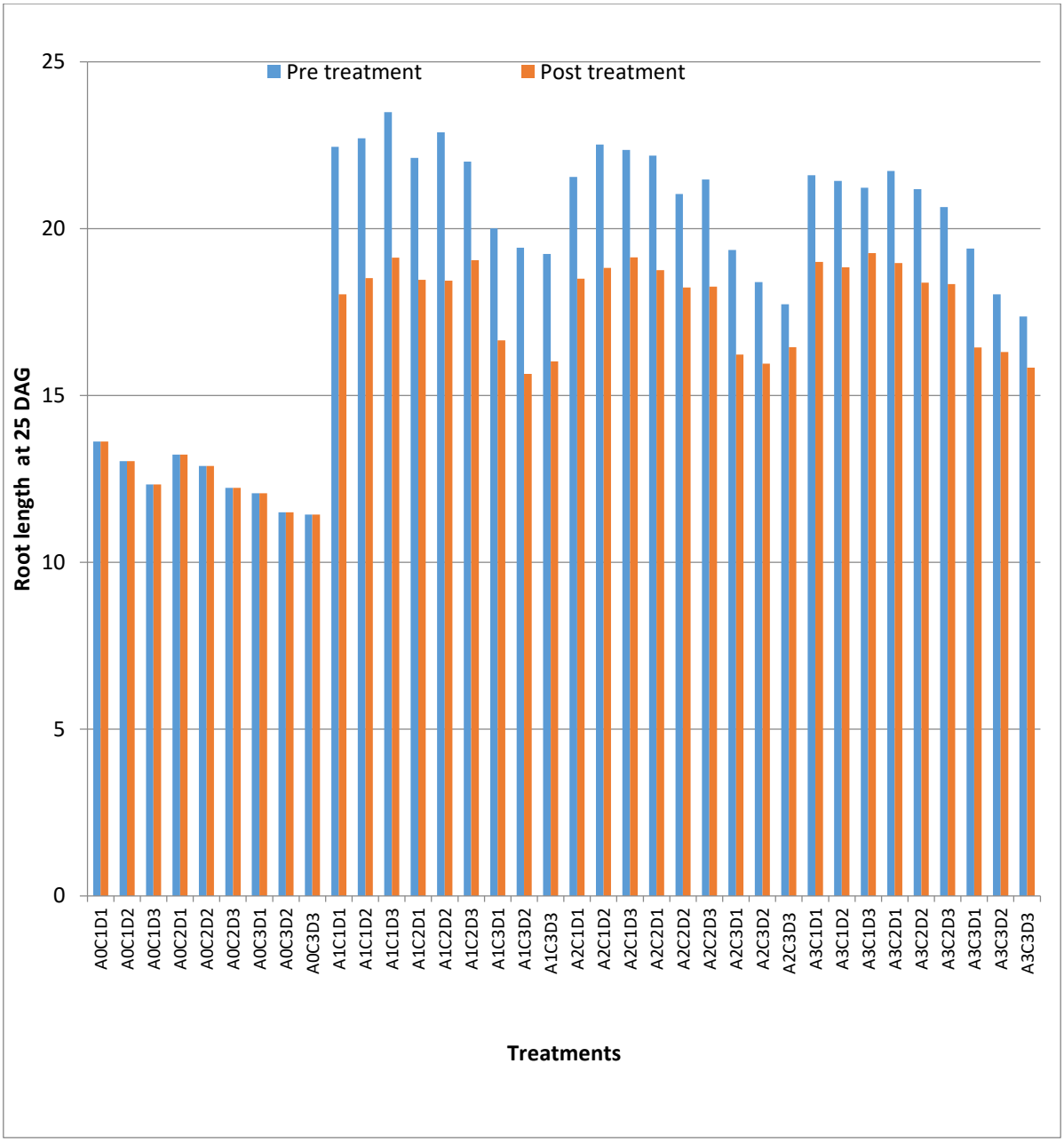
Post-treatment: At 7 days after germination (DAG) longest root (7.03cm) was observed at $A_1C_2D_1$ and shortest root (1.20cm) was observed at $A_0C_3D_3$ which were both statistically different from that of all other treatments. At 14 days after germination (DAG) longest root (12.98cm) was observed at $A_1C_2D_1$ and shortest root (5.47cm) was observed at $A_0C_3D_3$ which were both statistically similar with that of all other treatments. At 25 days after germination (DAG) highest value for root (19.27cm) was found at $A_3C_1D_3$ and lowest value for root (11.43cm) was found at $A_0C_3D_3$ which were statistically different from that of all other treatments.



Histogram 24(a): Effect of antioxidant along with control on root length at 7 DAG of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 24(b): Effect of antioxidant along with control on root length at 25 DAG of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 24(c): Effect of antioxidant along with control on root length at 25 DAG of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.

Table 11: Effect of antioxidants along with control on root length (cm) at 7, 14 & 25 DAG of wheat induced with different concentration and duration of pesticide in M2 generation.

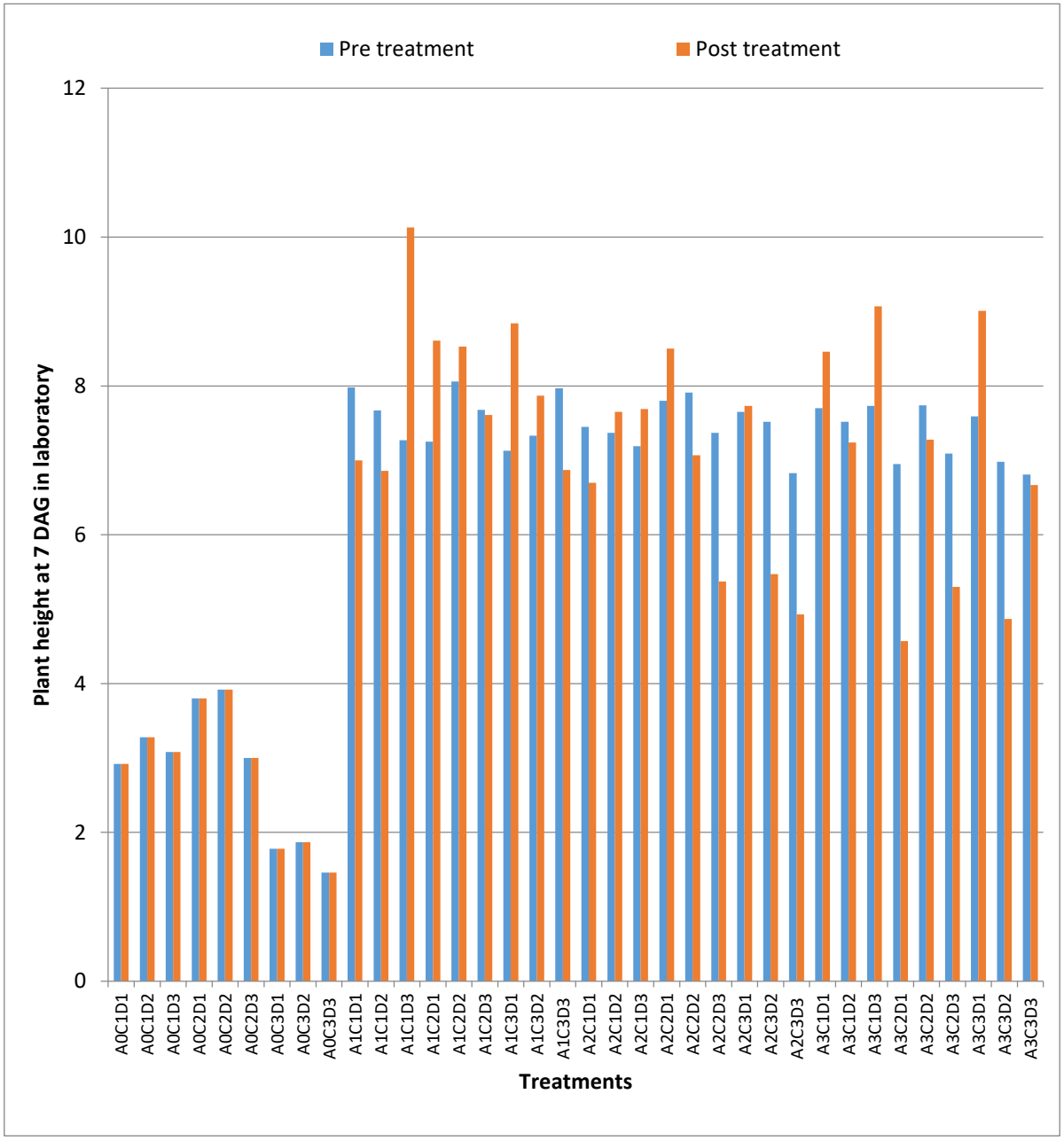
Treatment	Pre treatment			Post treatment		
	Root length (cm) 7 DAG	Root length (cm) 14 DAG	Root length (cm) 25 DAG	Root length (cm) 7 DAG	Root length (cm) 14 DAG	Root length (cm) 25 DAG
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	2.83 ± 0.70	6.53 ± 0.29	13.62 ± 0.33	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	2.83 ± 0.06	6.76 ± 0.23	13.03 ± 0.40	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	2.67 ± 0.42	6.47 ± 0.38	12.33 ± 0.14	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	2.73 ± 0.32	6.88 ± 0.07	13.23 ± 0.49	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	2.67 ± 0.25	6.93 ± 0.08	12.89 ± 0.09	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	2.47 ± 0.06	6.65 ± 0.10	12.23 ± 0.25	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	1.90 ± 0.10	5.63 ± 0.32	12.07 ± 0.55	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	1.77 ± 0.12	5.52 ± 0.51	11.50 ± 0.61	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	1.20 ± 0.26	5.47 ± 0.55	11.43 ± 0.49	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	8.20 ± 0.10	16.17 ± 0.19	22.45 ± 0.83	6.93 ± 0.11	12.14 ± 0.15	18.03 ± 0.95
A1C1D2	9.59 ± 0.44	16.58 ± 0.51	22.71 ± 0.71	6.63 ± 0.55	11.64 ± 0.56	18.52 ± 0.53
A1C1D3	8.46 ± 0.49	16.09 ± 0.13	23.49 ± 0.55	6.91 ± 0.08	12.25 ± 0.39	19.13 ± 0.35
A1C2D1	8.90 ± 0.44	16.86 ± 0.13	22.12 ± 0.98	7.03 ± 0.06	12.98 ± 0.03	18.47 ± 0.56
A1C2D2	9.16 ± 0.29	16.94 ± 0.21	22.89 ± 0.93	6.71 ± 0.49	12.84 ± 0.21	18.44 ± 0.88
A1C2D3	8.43 ± 0.49	16.61 ± 0.34	22.01 ± 0.71	6.91 ± 0.09	12.82 ± 0.31	19.05 ± 0.64
A1C3D1	5.25 ± 0.24	13.25 ± 0.83	20.01 ± 0.11	5.63 ± 0.32	10.32 ± 0.25	16.65 ± 2.08
A1C3D2	5.13 ± 0.24	13.49 ± 0.22	19.43 ± 0.20	5.56 ± 0.29	10.19 ± 0.04	15.65 ± 2.17
A1C3D3	5.21 ± 0.08	12.42 ± 0.11	19.24 ± 0.49	5.43 ± 0.45	9.70 ± 0.39	16.02 ± 0.98
A2C1D1	10.33 ± 0.16	16.37 ± 0.38	21.55 ± 0.48	6.73 ± 0.29	12.48 ± 0.44	18.50 ± 0.40
A2C1D2	9.66 ± 0.29	16.93 ± 0.11	22.52 ± 0.55	6.94 ± 0.15	12.64 ± 0.57	18.82 ± 1.01
A2C1D3	9.88 ± 0.39	17.05 ± 0.23	22.36 ± 0.25	6.31 ± 0.53	12.23 ± 0.36	19.14 ± 0.50
A2C2D1	9.19 ± 0.05	16.72 ± 0.40	22.19 ± 0.45	6.45 ± 0.31	12.20 ± 0.26	18.76 ± 0.91
A2C2D2	8.41 ± 0.42	16.97 ± 0.45	21.04 ± 0.13	6.54 ± 0.29	12.01 ± 0.03	18.24 ± 0.44
A2C2D3	8.17 ± 0.19	16.36 ± 0.37	21.47 ± 0.25	6.38 ± 0.21	11.73 ± 0.46	18.26 ± 0.63
A2C3D1	6.01 ± 0.98	12.50 ± 0.50	19.36 ± 0.54	5.13 ± 0.12	9.98 ± 0.48	16.23 ± 1.80
A2C3D2	7.86 ± 0.06	12.54 ± 0.20	18.40 ± 0.40	5.67 ± 0.21	9.85 ± 0.15	15.95 ± 1.11
A2C3D3	8.05 ± 0.08	12.63 ± 0.55	17.73 ± 0.63	4.97 ± 0.06	9.70 ± 0.46	16.45 ± 1.51
A3C1D1	8.54 ± 0.33	16.57 ± 0.61	21.60 ± 0.87	6.67 ± 0.49	12.00 ± 0.70	19.00 ± 0.80
A3C1D2	9.63 ± 0.55	16.59 ± 0.42	21.43 ± 0.45	6.03 ± 0.06	10.77 ± 0.56	18.84 ± 0.16
A3C1D3	9.86 ± 0.33	15.77 ± 0.67	21.23 ± 0.72	5.96 ± 0.05	11.84 ± 0.16	19.27 ± 0.46
A3C2D1	8.49 ± 0.49	16.15 ± 0.04	21.73 ± 0.31	6.46 ± 0.45	11.78 ± 0.37	18.97 ± 0.64
A3C2D2	9.10 ± 0.11	16.80 ± 0.26	21.18 ± 0.20	6.09 ± 0.08	12.01 ± 0.02	18.38 ± 0.75
A3C2D3	9.17 ± 0.32	15.69 ± 1.00	20.65 ± 0.19	5.57 ± 0.49	12.17 ± 0.55	18.34 ± 0.82
A3C3D1	8.25 ± 0.14	12.34 ± 0.49	19.40 ± 0.53	5.17 ± 0.21	9.91 ± 0.13	16.44 ± 1.57
A3C3D2	8.10 ± 0.11	10.88 ± 0.78	18.03 ± 0.06	5.00 ± 0.10	9.17 ± 1.04	16.30 ± 2.06
A3C3D3	7.08 ± 0.13	10.31 ± 0.14	17.37 ± 0.55	4.97 ± 0.06	9.45 ± 0.57	15.83 ± 2.23
Level of significance	**	NS	*	*	NS	*
CV(%)	5.19	3.17	2.78	5.76	3.97	6.16

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

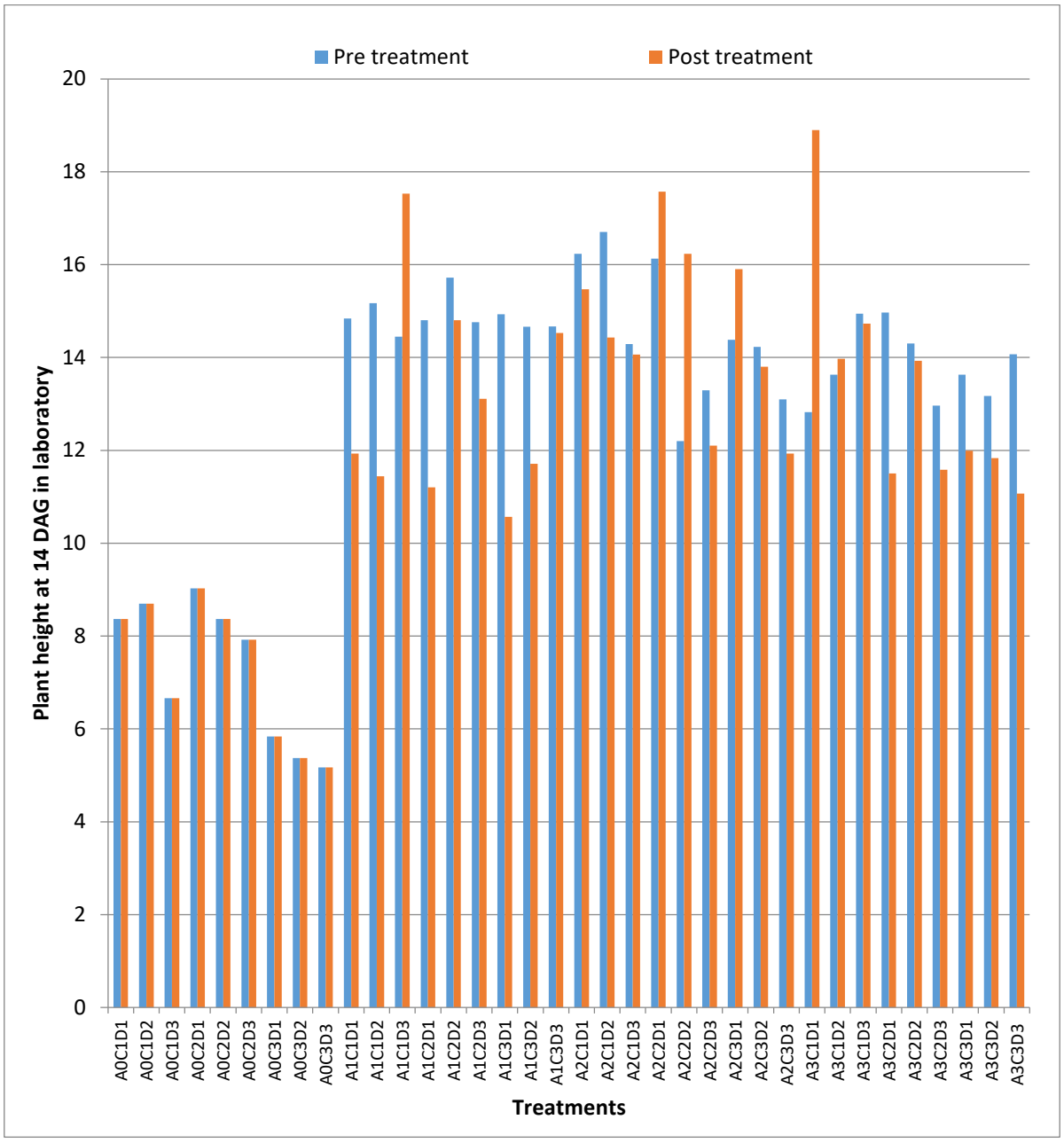
4.2.1.4 Plant height in laboratory

Pre-treatment: Histogram 25(a), 25(b), 25(c) and Table 12 reveals that at 7 days after germination maximum plant height (8.06cm) was observed at $A_1C_2D_2$ which was similar with that of all other treatments. On the other hand minimum plant height (1.46cm) was recorded at $A_0C_3D_3$ which was also similar with that of all other treatments. At 14 days after germination highest value for plant height (16.70cm) was observed at $A_2C_1D_2$ and lowest value for plant height (5.17cm) was observed at $A_0C_3D_3$ which were both statistically different from that of all other treatments. Plant height 25 days after germination highest value (24.32cm) and lowest value (10.48cm) were shown at $A_3C_2D_2$ and $A_0C_3D_3$ respectively which were significantly different from that of all other treatments.

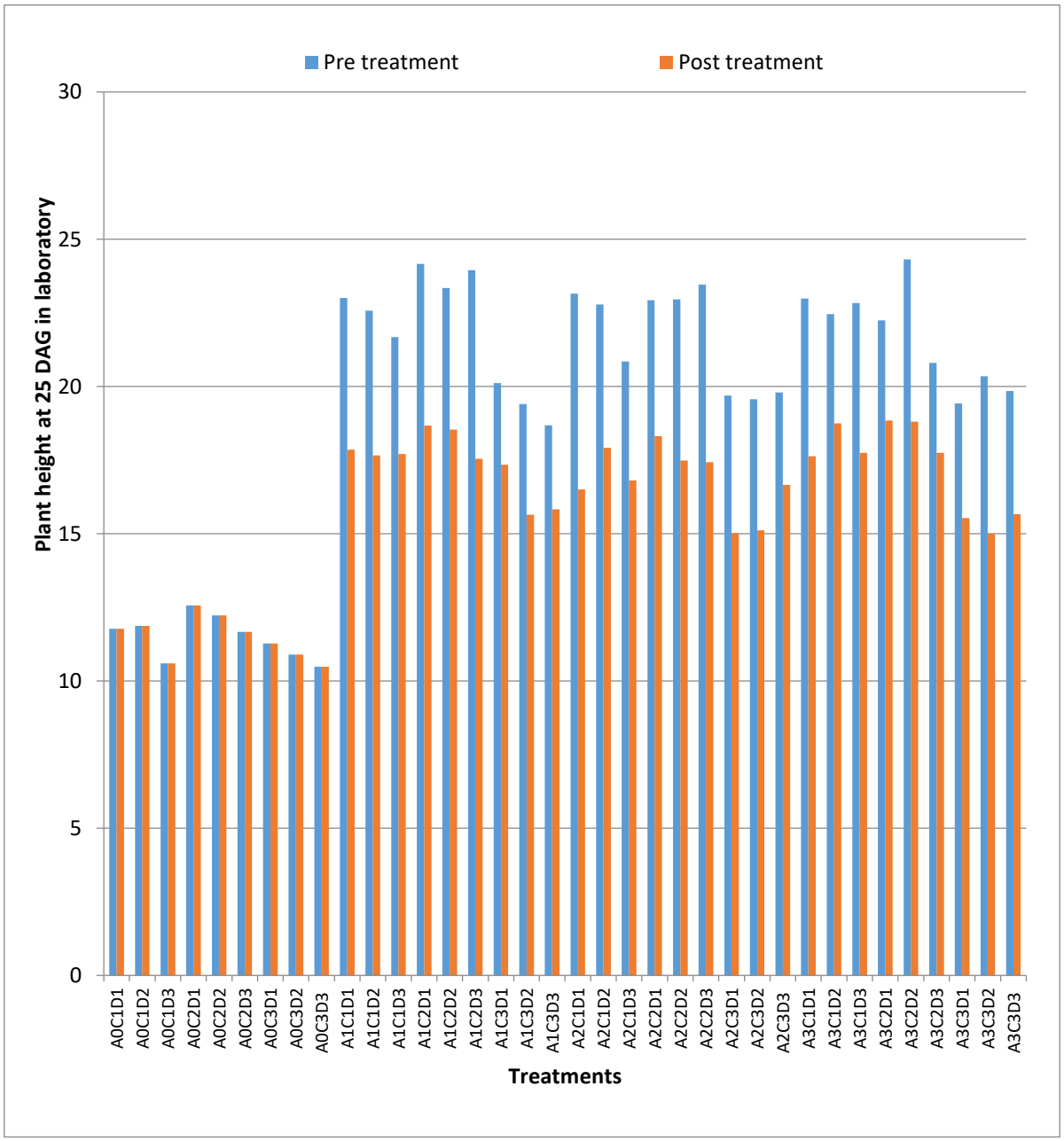
Post-treatment: Histogram 25(a), 25(b), 25(c) and Table 12 showed that at 7 days after germination maximum value for plant height (10.13cm) was found at $A_1C_1D_3$ which was significantly different from that of all other treatments. On the other hand minimum value for plant height (1.46cm) was observed at $A_0C_3D_3$ which was significantly different from that of all other treatments. At 14 days after germination highest value for plant height (18.90cm) was observed at $A_3C_1D_1$ and lowest value for plant height (5.17cm) was observed at $A_0C_3D_3$ which were both significantly different from that of all other treatments. At 25 days after germination highest value for plant height (18.84cm) was observed at $A_3C_2D_1$ and lowest value for plant height (10.60cm) found at $A_0C_1D_3$ which were both significantly similar with that of all other treatments.



Histogram 25(a): Effect of antioxidant along with control on plant height (cm) at 7 DAG of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 25(b): Effect of antioxidant along with control on plant height (cm) at 14 DAG of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 25(c): Effect of antioxidant along with control on plant height (cm) at 25 DAG of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.

Table 12: Effect of antioxidants along with control on plant height (cm) at 7, 14 & 25 DAG in laboratory of wheat induced with different concentration and duration of pesticide in M2 generation

Treatment	Pre treatment			Post treatment		
	Plant height (cm) in Laboratory			Plant height (cm) in Laboratory		
	7 days	14 days	25 days	7 days	14 days	25 days
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	2.92 ± 0.14	8.37 ± 1.03	11.77 ± 0.58	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	3.28 ± 0.20	8.70 ± 0.72	11.87 ± 0.32	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	3.08 ± 0.38	6.66 ± 0.67	10.60 ± 0.17	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	3.80 ± 0.36	9.03 ± 1.05	12.56 ± 0.39	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	3.92 ± 0.14	8.37 ± 0.96	12.23 ± 0.90	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	3.00 ± 0.50	7.92 ± 0.83	11.67 ± 0.42	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	1.78 ± 0.30	5.84 ± 0.12	11.27 ± 0.38	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	1.87 ± 0.32	5.37 ± 0.53	10.90 ± 0.36	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	1.46 ± 0.46	5.17 ± 0.70	10.48 ± 0.42	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	7.98 ± 0.02	14.84 ± 0.71	23.01 ± 1.63	7.00 ± 0.02	11.93 ± 0.55	17.85 ± 0.69
A1C1D2	7.67 ± 0.12	15.17 ± 1.42	22.57 ± 0.86	6.86 ± 0.25	11.44 ± 0.57	17.66 ± 0.96
A1C1D3	7.27 ± 0.03	14.45 ± 1.24	21.68 ± 0.95	10.13 ± 0.74	17.53 ± 0.42	17.71 ± 0.45
A1C2D1	7.25 ± 0.09	14.80 ± 1.22	24.16 ± 0.23	8.61 ± 0.11	11.20 ± 0.68	18.67 ± 0.41
A1C2D2	8.06 ± 0.04	15.72 ± 1.38	23.34 ± 1.41	8.53 ± 0.51	14.80 ± 0.81	18.54 ± 1.34
A1C2D3	7.68 ± 0.02	14.76 ± 0.70	23.95 ± 0.98	7.61 ± 0.51	13.11 ± 0.50	17.54 ± 0.58
A1C3D1	7.13 ± 0.51	14.93 ± 0.06	20.12 ± 0.97	8.84 ± 0.37	10.57 ± 1.08	17.34 ± 1.18
A1C3D2	7.33 ± 0.50	14.66 ± 0.28	19.40 ± 0.53	7.87 ± 0.02	11.71 ± 1.89	15.65 ± 0.82
A1C3D3	7.97 ± 0.01	14.67 ± 0.59	18.68 ± 0.60	6.87 ± 0.16	14.53 ± 2.16	15.83 ± 1.98
A2C1D1	7.45 ± 0.30	16.23 ± 0.15	23.15 ± 0.84	6.70 ± 1.05	15.47 ± 0.81	16.50 ± 0.65
A2C1D2	7.37 ± 0.64	16.70 ± 0.26	22.78 ± 0.77	7.65 ± 0.63	14.43 ± 0.31	17.92 ± 0.87
A2C1D3	7.19 ± 0.19	14.29 ± 0.08	20.85 ± 0.98	7.69 ± 1.39	14.06 ± 1.56	16.81 ± 0.46
A2C2D1	7.80 ± 1.01	16.13 ± 0.06	22.93 ± 0.79	8.50 ± 0.10	17.57 ± 0.83	18.31 ± 2.06
A2C2D2	7.91 ± 1.27	12.20 ± 0.26	22.96 ± 0.94	7.07 ± 0.47	16.23 ± 1.86	17.49 ± 0.78
A2C2D3	7.37 ± 0.99	13.29 ± 0.21	23.46 ± 0.85	5.37 ± 0.31	12.10 ± 1.13	17.43 ± 1.53
A2C3D1	7.65 ± 0.41	14.38 ± 0.30	19.69 ± 0.91	7.73 ± 0.42	15.90 ± 1.24	15.02 ± 0.75
A2C3D2	7.52 ± 1.46	14.23 ± 0.23	19.57 ± 0.24	5.47 ± 0.32	13.80 ± 0.50	15.12 ± 0.77
A2C3D3	6.83 ± 1.10	13.10 ± 0.10	19.80 ± 1.03	4.93 ± 0.21	11.93 ± 0.71	16.66 ± 2.03
A3C1D1	7.70 ± 0.26	12.82 ± 1.27	22.99 ± 0.13	8.46 ± 0.46	18.90 ± 0.43	17.63 ± 0.72
A3C1D2	7.52 ± 0.34	13.63 ± 1.31	22.46 ± 2.01	7.24 ± 0.20	13.97 ± 0.69	18.75 ± 0.83
A3C1D3	7.73 ± 0.38	14.94 ± 0.38	22.83 ± 0.98	9.07 ± 0.41	14.73 ± 1.12	17.75 ± 0.48
A3C2D1	6.95 ± 0.06	14.97 ± 0.15	22.24 ± 1.08	4.57 ± 0.64	11.50 ± 0.41	18.84 ± 0.71
A3C2D2	7.74 ± 0.43	14.30 ± 0.76	24.32 ± 0.34	7.28 ± 0.17	13.93 ± 0.74	18.81 ± 0.21
A3C2D3	7.09 ± 0.08	12.96 ± 1.72	20.80 ± 1.06	5.30 ± 0.10	11.58 ± 0.49	17.75 ± 0.30
A3C3D1	7.59 ± 0.21	13.63 ± 1.10	19.42 ± 0.45	9.01 ± 0.34	11.99 ± 0.51	15.53 ± 2.18
A3C3D2	6.98 ± 0.28	13.17 ± 1.26	20.35 ± 0.38	4.87 ± 0.15	11.83 ± 0.46	15.00 ± 0.71
A3C3D3	6.81 ± 0.56	14.07 ± 0.70	19.85 ± 0.51	6.67 ± 0.59	11.07 ± 0.36	15.67 ± 0.21
Level of significance	NS	**	*	**	**	NS
CV(%)	2.96	6.10	2.20	7.40	8.65	5.89

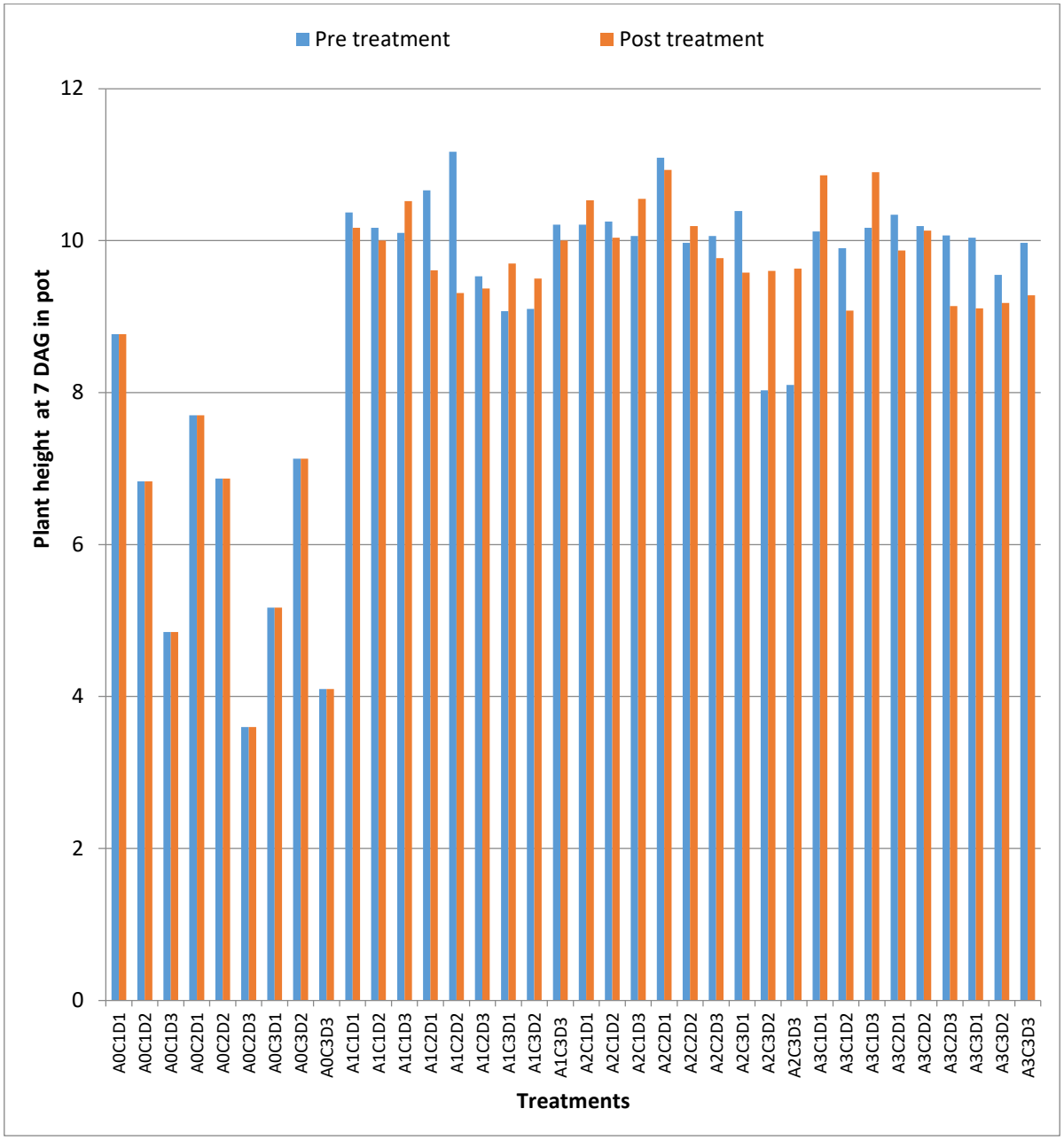
Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.2.1.5 Plant height in pot

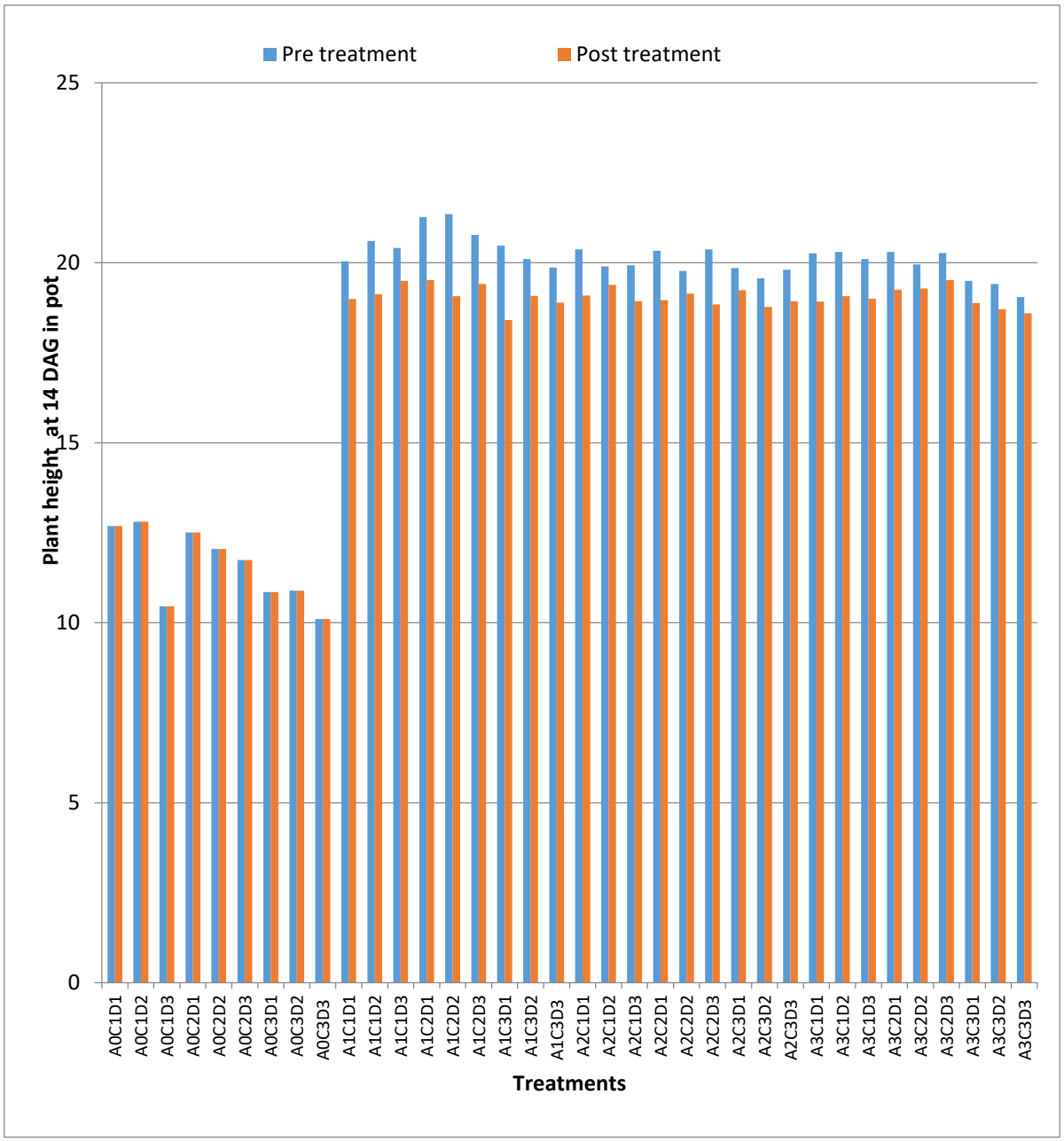
Plant height at 7, 14 and 25 DAG (in pot) are presented at **Histogram 26(a), 26(b), 26(c) and Table 13.**

Pre-treatment: At 7 days after germination (DAG) maximum plant height (11.17cm) was shown at $A_1C_2D_2$ which was significantly different from that of all other treatments. On the other hand minimum plant height (3.60cm) was observed at $A_0C_2D_3$ which was also significantly different from that of all other treatments. At 14 days after germination highest plant height (21.35cm) was recorded at $A_1C_2D_2$ and lowest plant height (10.10cm) was showed at $A_0C_3D_3$. Both were statistically similar with that of all other treatments. At 25 days after germination maximum plant height (34.87cm) was found at $A_1C_1D_3$ & $A_1C_3D_1$ which were significantly different from that of all other treatments. On the other hand minimum plant height (21.50cm) was found at $A_0C_3D_3$ which was significantly different from that of all other treatments.

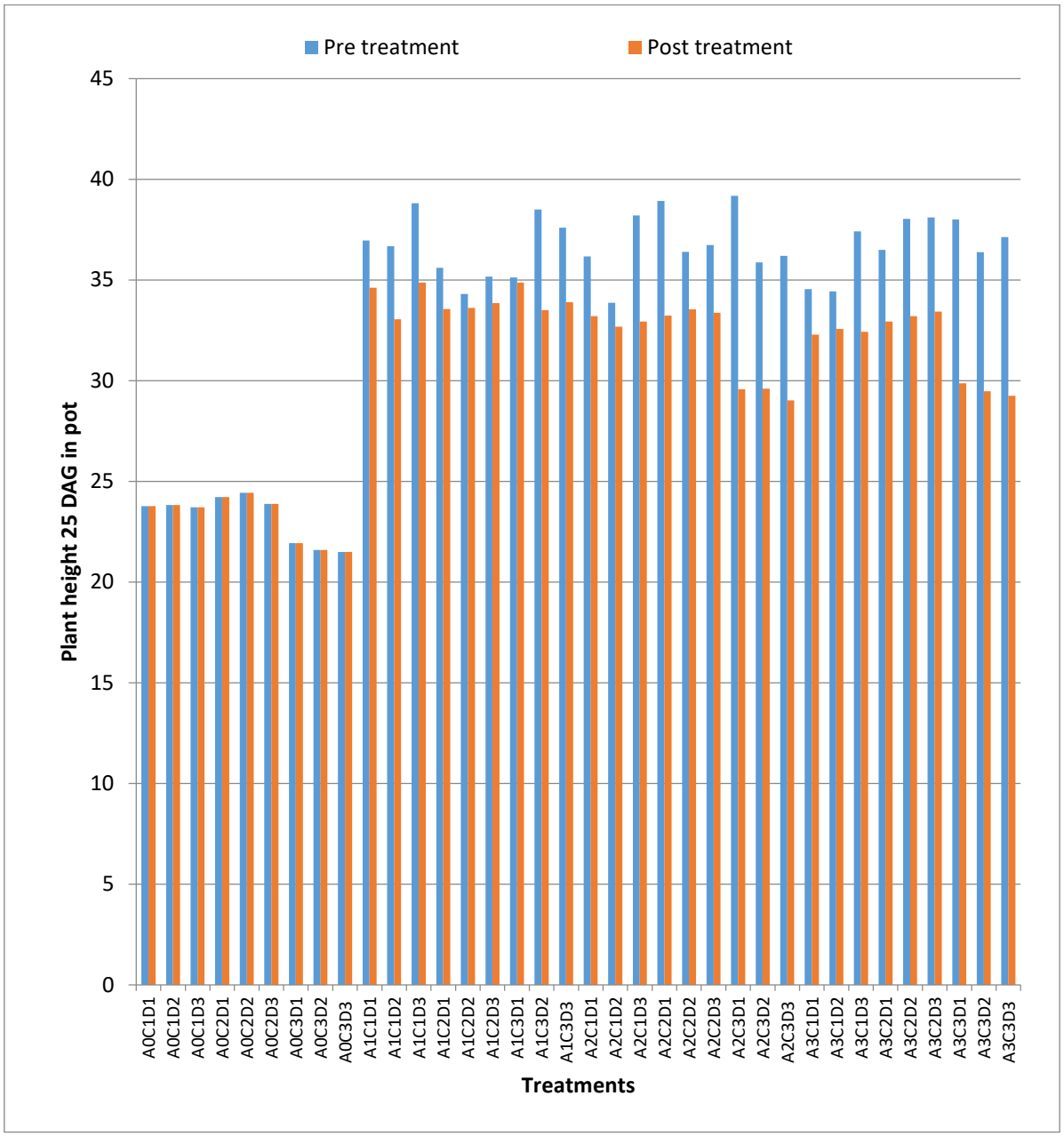
Post-treatment: At 7 days after germination maximum plant height (10.93cm) was observed at $A_2C_2D_1$ & minimum plant height (3.60cm) was observed at $A_0C_2D_3$ both were significantly different from that of all other treatments. At 14 days after germination highest plant height (19.52cm) was showed at $A_1C_2D_1$ and $A_3C_2D_3$ and lowest plant height (10.10cm) was observed at $A_0C_3D_3$ which were significantly similar with that of all other treatments. At 25 days after germination highest value for plant height (39.18cm) was observed at $A_2C_3D_1$ which was significantly different from that of all other treatments. On the other hand lowest value of plant height (21.50cm) was found at $A_0C_3D_3$ which was also significantly different from that of all other treatments.



Histogram 26(a): Effect of antioxidant along with control on plant height (cm) at 7 DAG of wheat induced with different concentration and duration of pesticide in pot in M2 generation.



Histogram 26(b): Effect of antioxidant along with control on plant height (cm) at 14 DAG of wheat induced with different concentration and duration of pesticide in pot in M2 generation.



Histogram 26(c): Effect of antioxidant along with control on plant height (cm) at 25 DAG of wheat induced with different concentration and duration of pesticide in pot in M2 generation.

Table 13: Effect of antioxidants along with control on plant height (cm) at 7, 14 & 25 DAG in pot of wheat induced with different concentration and duration of pesticide in M2 generation.

Treatment	Pre-treatment			Post-treatment		
	Plant height (cm) in pot			Plant height (cm) in pot		
	7 DAG	14 DAG	25 DAG	7 DAG	14 DAG	25 DAG
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	8.77 ± 0.32	12.69 ± 1.02	23.77 ± 0.40	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	6.83 ± 0.76	12.81 ± 0.12	23.82 ± 0.71	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	4.85 ± 0.25	10.46 ± 0.47	23.71 ± 0.20	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	7.70 ± 0.48	12.51 ± 0.45	24.22 ± 0.46	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	6.87 ± 0.15	12.05 ± 0.18	24.43 ± 0.40	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	3.60 ± 0.26	11.74 ± 0.42	23.88 ± 0.13	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	5.17 ± 0.31	10.85 ± 0.12	21.93 ± 0.12	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	7.13 ± 0.25	10.89 ± 0.19	21.59 ± 0.96	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	4.10 ± 0.70	10.10 ± 0.13	21.50 ± 0.50	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	10.37 ± 0.21	20.04 ± 0.06	34.61 ± 0.11	10.17 ± 0.28	18.99 ± 0.43	36.96 ± 0.06
A1C1D2	10.17 ± 0.29	20.60 ± 0.26	33.05 ± 0.43	10.00 ± 0.11	19.13 ± 0.51	36.68 ± 0.13
A1C1D3	10.10 ± 0.10	20.41 ± 0.59	34.87 ± 0.15	10.52 ± 0.47	19.50 ± 0.30	38.81 ± 0.09
A1C2D1	10.66 ± 0.35	21.27 ± 1.08	33.55 ± 0.95	9.61 ± 0.38	19.52 ± 0.36	35.60 ± 0.56
A1C2D2	11.17 ± 0.22	21.35 ± 1.62	33.61 ± 0.88	9.31 ± 1.14	19.07 ± 0.56	34.30 ± 0.19
A1C2D3	9.53 ± 0.12	20.77 ± 1.33	33.85 ± 0.30	9.37 ± 0.33	19.41 ± 0.61	35.17 ± 0.06
A1C3D1	9.07 ± 0.12	20.47 ± 0.49	34.87 ± 0.15	9.70 ± 0.26	18.41 ± 0.63	35.13 ± 0.06
A1C3D2	9.10 ± 0.17	20.10 ± 0.61	33.50 ± 0.40	9.50 ± 0.50	19.08 ± 0.04	38.50 ± 0.40
A1C3D3	10.21 ± 0.29	19.87 ± 0.71	33.89 ± 0.81	10.00 ± 0.10	18.89 ± 0.54	37.60 ± 0.46
A2C1D1	10.21 ± 0.10	20.37 ± 0.61	33.20 ± 0.62	10.53 ± 0.35	19.09 ± 0.07	36.17 ± 0.15
A2C1D2	10.25 ± 0.27	19.90 ± 0.36	32.68 ± 0.50	10.04 ± 0.07	19.39 ± 0.76	33.87 ± 5.87
A2C1D3	10.06 ± 0.10	19.93 ± 0.02	32.94 ± 1.01	10.55 ± 0.32	18.93 ± 0.58	38.21 ± 0.08
A2C2D1	11.09 ± 0.08	20.33 ± 0.06	33.23 ± 1.12	10.93 ± 0.06	18.96 ± 0.05	38.92 ± 0.11
A2C2D2	9.97 ± 0.06	19.77 ± 0.50	33.54 ± 1.36	10.19 ± 0.05	19.14 ± 0.97	36.40 ± 0.40
A2C2D3	10.06 ± 0.12	20.37 ± 0.64	33.37 ± 0.55	9.77 ± 0.23	18.84 ± 0.29	36.73 ± 0.25
A2C3D1	10.39 ± 0.01	19.85 ± 0.33	29.57 ± 1.17	9.58 ± 0.07	19.24 ± 0.54	39.18 ± 0.28
A2C3D2	8.03 ± 0.06	19.57 ± 0.40	29.60 ± 0.56	9.60 ± 0.17	18.77 ± 0.59	35.87 ± 0.78
A2C3D3	8.10 ± 0.11	19.80 ± 0.98	29.03 ± 0.06	9.63 ± 0.38	18.93 ± 0.90	36.20 ± 0.10
A3C1D1	10.12 ± 0.14	20.26 ± 0.24	32.29 ± 0.42	10.86 ± 0.00	18.92 ± 0.68	34.55 ± 0.05
A3C1D2	9.90 ± 0.10	20.30 ± 0.52	32.57 ± 1.37	9.08 ± 0.14	19.07 ± 0.11	34.43 ± 0.21
A3C1D3	10.17 ± 0.06	20.10 ± 0.17	32.43 ± 0.49	10.90 ± 0.36	19.00 ± 0.70	37.41 ± 0.10
A3C2D1	10.34 ± 0.07	20.30 ± 0.26	32.93 ± 0.79	9.87 ± 0.32	19.25 ± 0.50	36.50 ± 0.50
A3C2D2	10.19 ± 0.21	19.95 ± 0.39	33.20 ± 0.89	10.13 ± 0.06	19.28 ± 0.31	38.03 ± 0.06
A3C2D3	10.07 ± 0.12	20.27 ± 0.47	33.43 ± 0.59	9.14 ± 0.15	19.52 ± 0.24	38.11 ± 0.10
A3C3D1	10.04 ± 0.16	19.50 ± 0.50	29.87 ± 0.71	9.11 ± 0.09	18.88 ± 0.78	38.00 ± 0.10
A3C3D2	9.55 ± 0.26	19.41 ± 0.30	29.47 ± 0.64	9.18 ± 0.00	18.71 ± 0.53	36.38 ± 0.45
A3C3D3	9.97 ± 0.06	19.05 ± 0.08	29.25 ± 0.89	9.28 ± 0.20	18.60 ± 0.56	37.13 ± 0.32
Level of sig-ficance	**	NS	*	**	NS	**
CV(%)	2.96	3.14	2.20	4.06	2.90	3.14

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.2.1.6 Number of tiller/plant

Pre-treatment: Highest no of tiller (2.37) was observed at $A_1C_2D_2$ and lowest no. of tiller (1.03) were observed at $A_0C_3D_3$ **(Histogram 27 and Table 14)** which were statistically different from that of all other treatments.

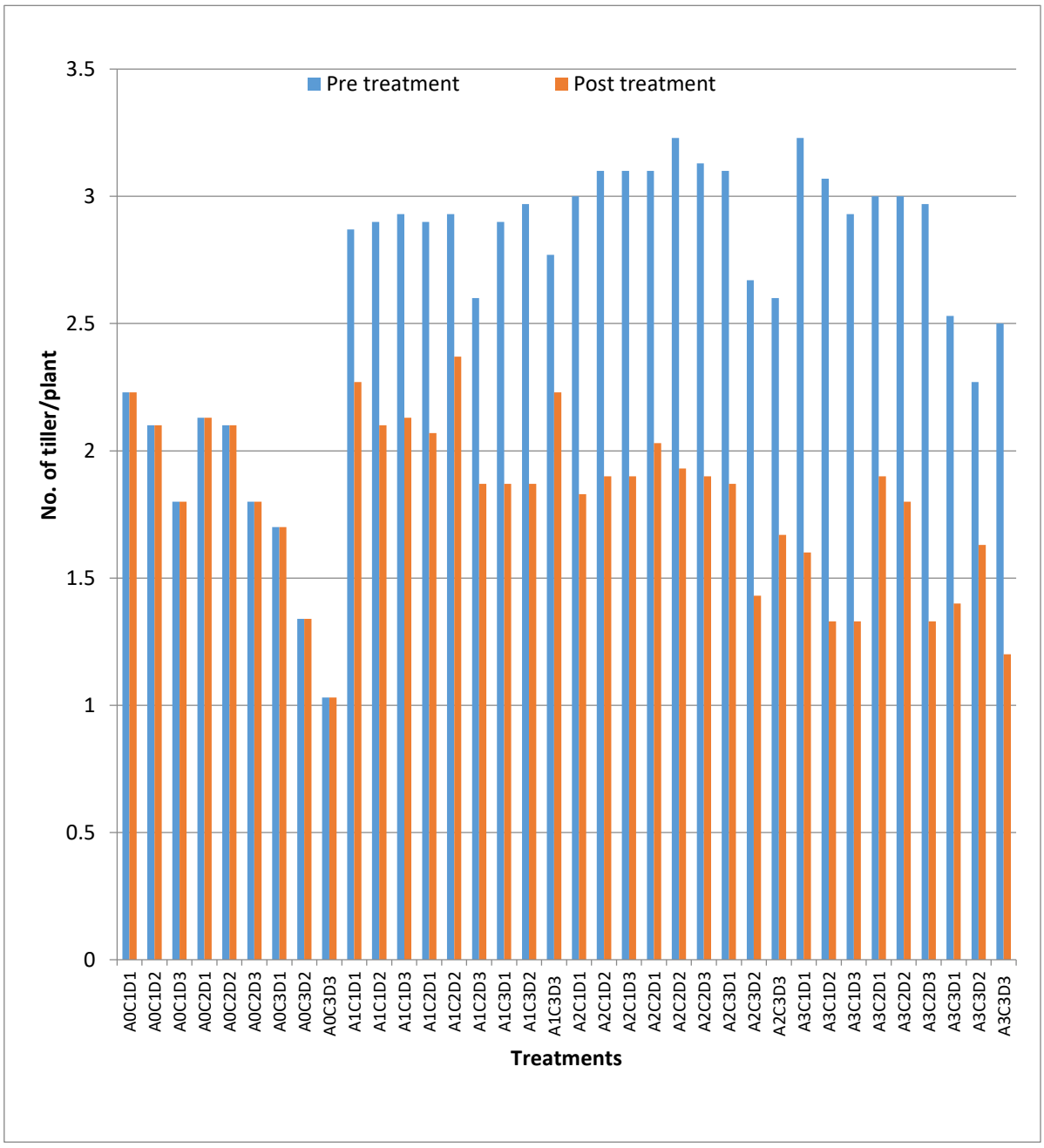
Post-treatment: Highest no of tiller (3.23) was observed at $A_2C_2D_2$ and $A_3C_1D_1$ which were significantly nonsignificant. Lowest no of tiller (1.03) was found at $A_0C_3D_3$ **(Histogram 27 and Table 14)** which was also significantly identical with that of all other treatments.

4.2.1.7 Number of spikelet/spike and Number of seeds/spike

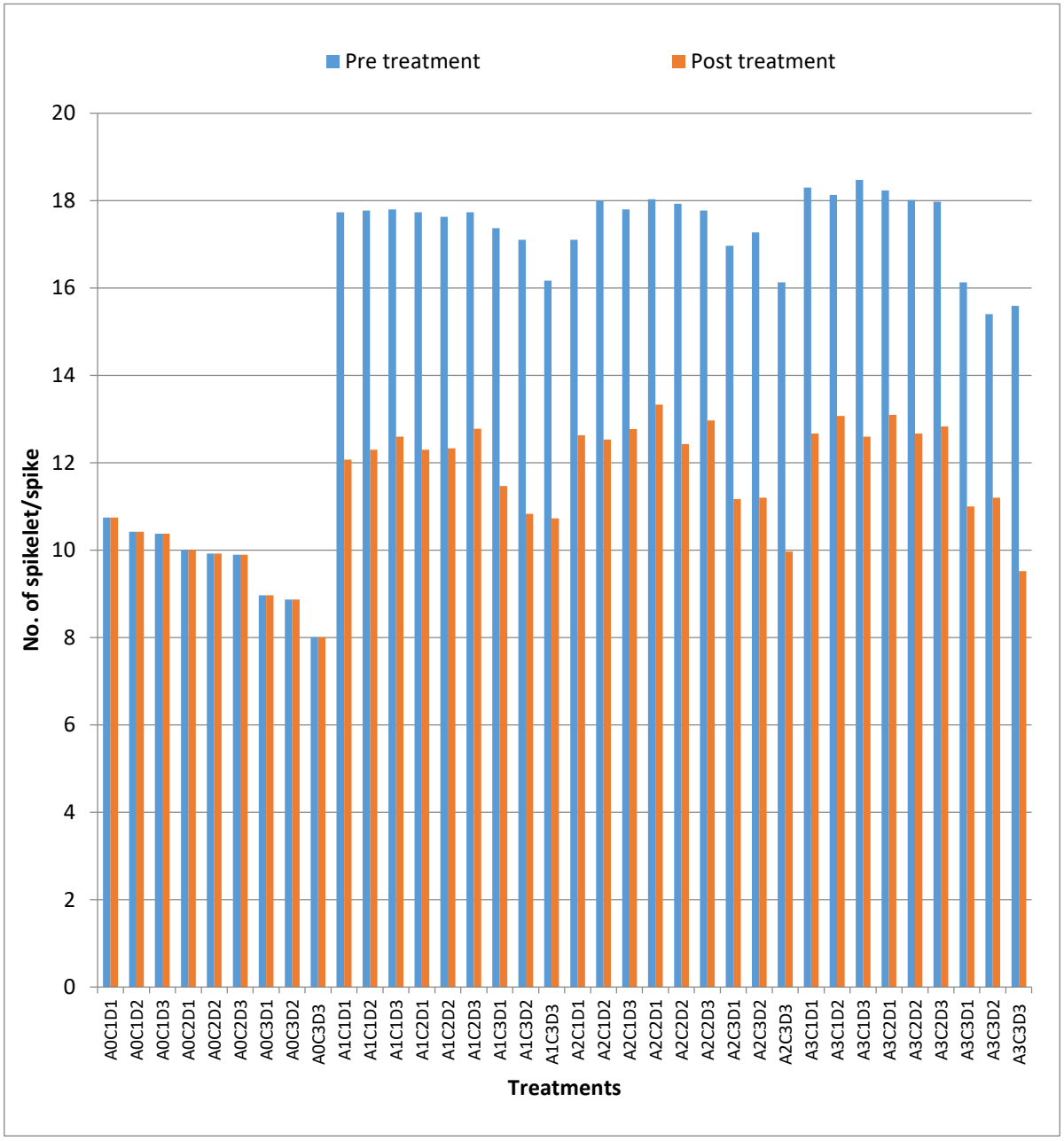
Histogram 28 and Table 14 showing the result of number of spikelet/spike and number of seeds/spike.

Pre-treatment: Maximum no. of spikelet / spike (18.47) and maximum no. of seeds/spike (41.00) both were recorded at $A_3C_1D_3$ which were statistically different from that of all other treatments. On the other hand minimum no. of spikelet/spike (8.01) and minimum no. of seeds/spike (15.00) both were observed at $A_oC_3D_3$ which were also significantly different from that of all other treatments.

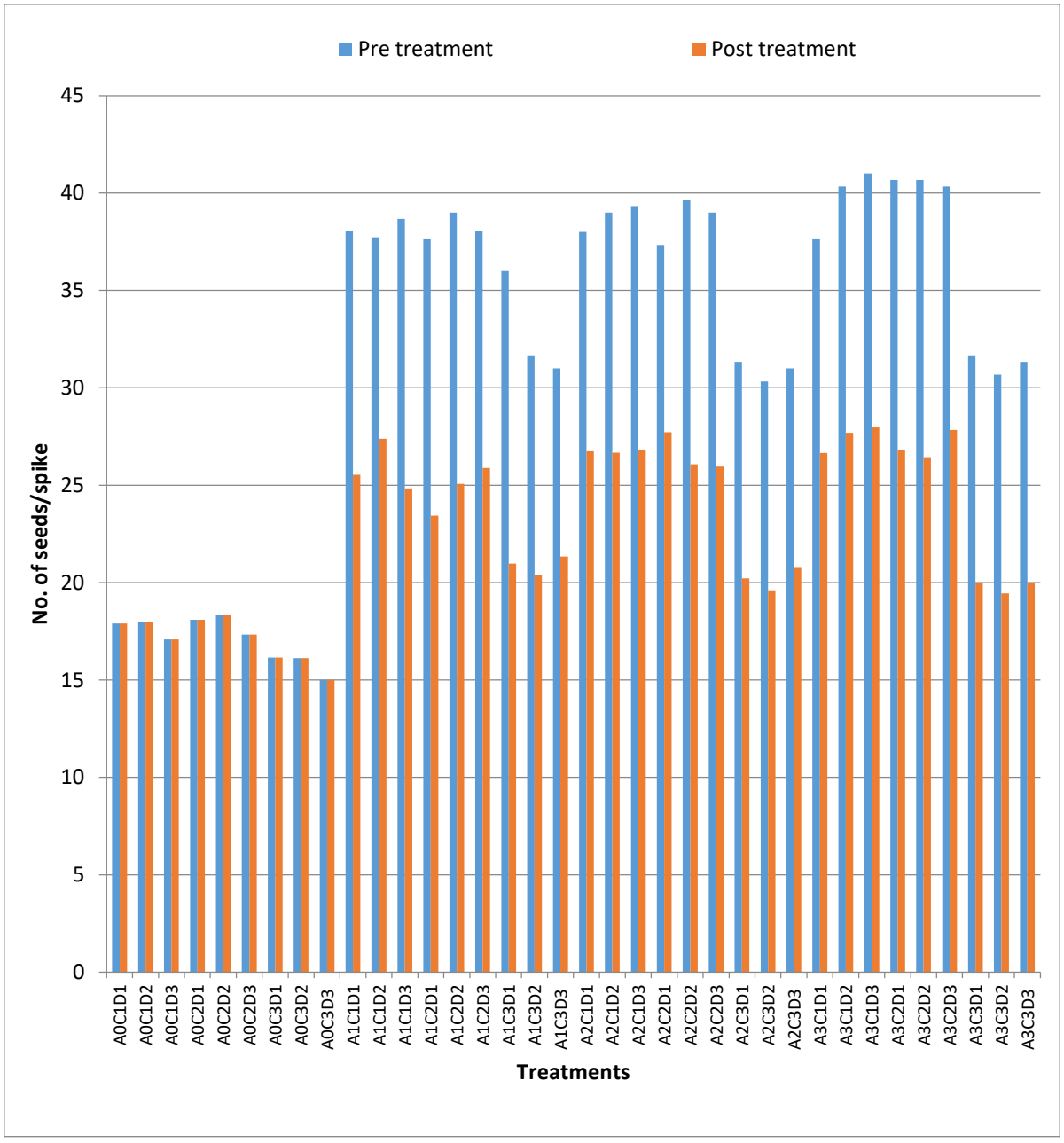
Post-treatment: Highest value for no. of spikelet/spike (13.33) and no. of seeds/spike (27.97) were recorded at $A_2C_2D_1$ & $A_3C_1D_3$ respectively which were significantly different from that of all other treatments. On the other hands lowest value for no. of spikelet/spike (8.01) and no. of seeds/spike (15.00) were observed at $A_0C_3D_3$ which were significantly different from that of all other treatments.



Histogram 27: Effect of antioxidant along with control on no. of tiller/plant of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 28: Effect of antioxidant along with control on no. spikelet/spike of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 29: Effect of antioxidant along with control on no. seeds/spike of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.

Table 14: Effect of antioxidants along with control on no. of tiller/plant, no. of spikelet/spike and no. of seeds/spike of wheat induced with different concentration and duration of pesticide in M2 generation.

Treatment	Pre treatment			Post treatment		
	No. of tiller	No. of spikelet/spike	No. of seeds/spike	No. of tiller	No. of spikelet/spike	No. of seeds/spike
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	2.23 ± 0.38	10.75 ± 0.25	17.90 ± 0.98	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	2.10 ± 0.26	10.42 ± 0.07	17.98 ± 0.48	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	1.80 ± 0.10	10.38 ± 0.10	17.08 ± 0.71	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	2.13 ± 0.06	10.00 ± 0.02	18.09 ± 0.14	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	2.10 ± 0.17	9.92 ± 0.08	18.32 ± 1.15	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	1.80 ± 0.10	9.90 ± 0.04	17.33 ± 0.87	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	1.70 ± 0.20	8.97 ± 0.06	16.15 ± 1.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	1.34 ± 0.15	8.87 ± 0.03	16.13 ± 0.59	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	1.03 ± 0.12	8.01 ± 0.01	15.00 ± 0.11	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	2.27 ± 0.12	17.73 ± 0.21	38.03 ± 1.05	2.87 ± 0.12	12.07 ± 0.06	25.54 ± 1.73
A1C1D2	2.10 ± 0.26	17.77 ± 0.32	37.73 ± 1.55	2.90 ± 0.10	12.30 ± 0.26	27.39 ± 2.01
A1C1D3	2.13 ± 0.23	17.80 ± 0.35	38.67 ± 1.53	2.93 ± 0.12	12.60 ± 0.26	24.83 ± 1.36
A1C2D1	2.07 ± 0.06	17.73 ± 0.32	37.67 ± 0.58	2.90 ± 0.10	12.30 ± 0.20	23.43 ± 0.75
A1C2D2	2.37 ± 0.15	17.63 ± 0.32	39.00 ± 1.00	2.93 ± 0.06	12.33 ± 0.25	25.07 ± 1.68
A1C2D3	1.87 ± 0.12	17.73 ± 0.46	38.03 ± 2.67	2.60 ± 0.53	12.78 ± 0.30	25.89 ± 3.70
A1C3D1	1.87 ± 0.12	17.37 ± 0.55	36.00 ± 1.73	2.90 ± 0.10	11.47 ± 0.42	20.98 ± 0.95
A1C3D2	1.87 ± 0.12	17.10 ± 0.10	31.67 ± 1.53	2.97 ± 0.06	10.83 ± 0.15	20.41 ± 0.52
A1C3D3	2.23 ± 0.12	16.17 ± 0.31	31.00 ± 1.00	2.77 ± 0.15	10.73 ± 0.31	21.34 ± 0.41
A2C1D1	1.83 ± 0.06	17.10 ± 0.10	38.00 ± 1.00	3.00 ± 0.00	12.63 ± 0.55	26.74 ± 1.58
A2C1D2	1.90 ± 0.10	18.00 ± 0.10	39.00 ± 1.00	3.10 ± 0.10	12.53 ± 0.15	26.67 ± 2.37
A2C1D3	1.90 ± 0.10	17.80 ± 0.26	39.33 ± 1.15	3.10 ± 0.10	12.77 ± 0.25	26.82 ± 2.01
A2C2D1	2.03 ± 0.06	18.03 ± 0.06	37.33 ± 2.52	3.10 ± 0.36	13.33 ± 0.58	27.72 ± 2.29
A2C2D2	1.93 ± 0.12	17.93 ± 0.21	39.67 ± 0.58	3.23 ± 0.32	12.43 ± 0.59	26.07 ± 0.86
A2C2D3	1.90 ± 0.10	17.77 ± 0.51	39.00 ± 1.00	3.13 ± 0.15	12.97 ± 0.15	25.96 ± 2.29
A2C3D1	1.87 ± 0.12	16.97 ± 0.06	31.33 ± 1.53	3.10 ± 0.10	11.17 ± 0.64	20.22 ± 1.06
A2C3D2	1.43 ± 0.06	17.27 ± 0.64	30.33 ± 0.58	2.67 ± 0.15	11.20 ± 0.44	19.61 ± 0.84
A2C3D3	1.67 ± 0.15	16.13 ± 0.32	31.00 ± 1.00	2.60 ± 0.20	9.97 ± 0.06	20.80 ± 1.02
A3C1D1	1.60 ± 0.20	18.30 ± 0.20	37.67 ± 1.53	3.23 ± 0.15	12.67 ± 1.15	26.65 ± 1.45
A3C1D2	1.33 ± 0.12	18.13 ± 0.12	40.33 ± 1.53	3.07 ± 0.12	13.07 ± 0.92	27.69 ± 2.34
A3C1D3	1.33 ± 0.12	18.47 ± 0.55	41.00 ± 1.00	2.93 ± 0.12	12.60 ± 0.26	27.97 ± 1.78
A3C2D1	1.90 ± 0.10	18.23 ± 0.40	40.67 ± 1.15	3.00 ± 0.00	13.10 ± 0.66	26.83 ± 1.84
A3C2D2	1.80 ± 0.20	18.01 ± 0.08	40.67 ± 0.58	3.00 ± 0.00	12.67 ± 0.59	26.44 ± 1.58
A3C2D3	1.33 ± 0.12	17.97 ± 0.06	40.33 ± 2.08	2.97 ± 0.06	12.83 ± 0.25	27.84 ± 2.51
A3C3D1	1.40 ± 0.10	16.13 ± 0.42	31.67 ± 0.58	2.53 ± 0.23	11.00 ± 0.10	19.99 ± 0.58
A3C3D2	1.63 ± 0.21	15.40 ± 0.52	30.67 ± 1.15	2.27 ± 0.23	11.20 ± 0.60	19.44 ± 0.74
A3C3D3	1.20 ± 0.00	15.59 ± 0.51	31.33 ± 1.15	2.50 ± 0.46	9.52 ± 0.50	19.95 ± 0.21
Level of sig-ficance	**	*	*	NS	*	*
CV(%)	8.11	1.99	3.83	7.56	3.52	6.30

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.2.1.8 Flag leaf length

Histogram 30 and Table 15 showing the result of flag leaf length are as follows:

Pre-treatment: It was observed that highest length for flag leaf (17.87cm) was found at $A_3C_2D_2$ and lowest length for flag leaf (13.35cm) was recorded at $A_0C_3D_1$ which were both significantly different from that of all other treatments.

Post-treatment: Longest flag length (16.37) was recorded at $A_1C_1D_2$ which was statistically different from that of all other treatments. On the other hand lowest flag length (12.00cm) was observed at $A_3C_2D_1$ which was also statistically different from that of all other treatments.

4.2.1.9 Length of inflorescence (with awn)

Histogram 31 and Table 15 showing the result of length of inflorescence (with awn).

Pre-treatment: Maximum value for length of inflorescence (with awn) (14.60cm) was found at $A_1C_2D_1$ and minimum value for length of inflorescence (with awn) (8.93cm) was recorded at $A_0C_3D_3$ which were both significantly different from that of all other treatments.

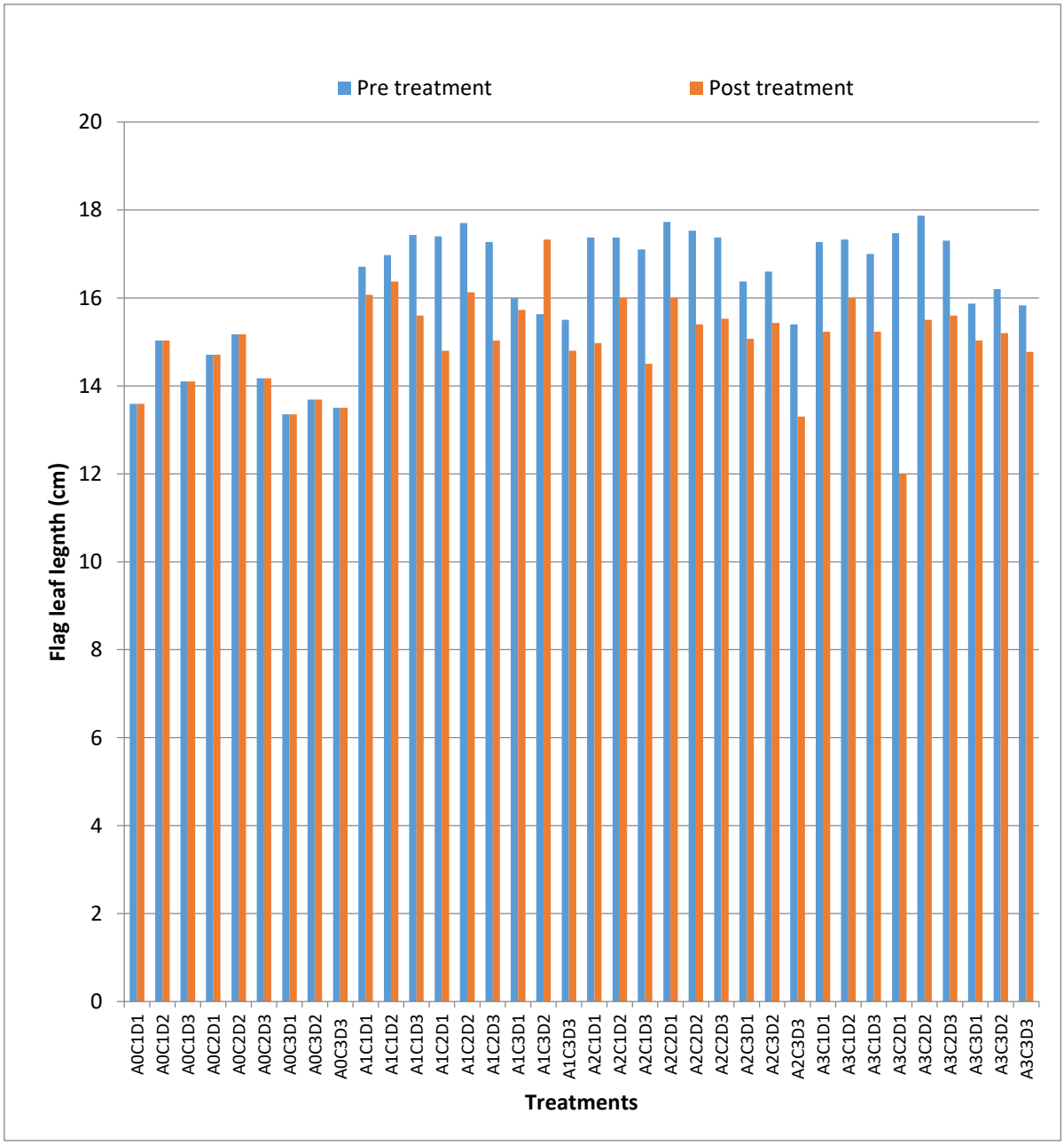
Post-treatment: Maximum value for length of inflorescence (with awn)(12.06cm) and minimum value for length of inflorescence(with awn) (8.93cm) was observed at $A_1C_2D_1$ and $A_0C_3D_3$ respectively were significantly different from that of all other treatments.

4.2.1.10 Length of inflorescence (without awn)

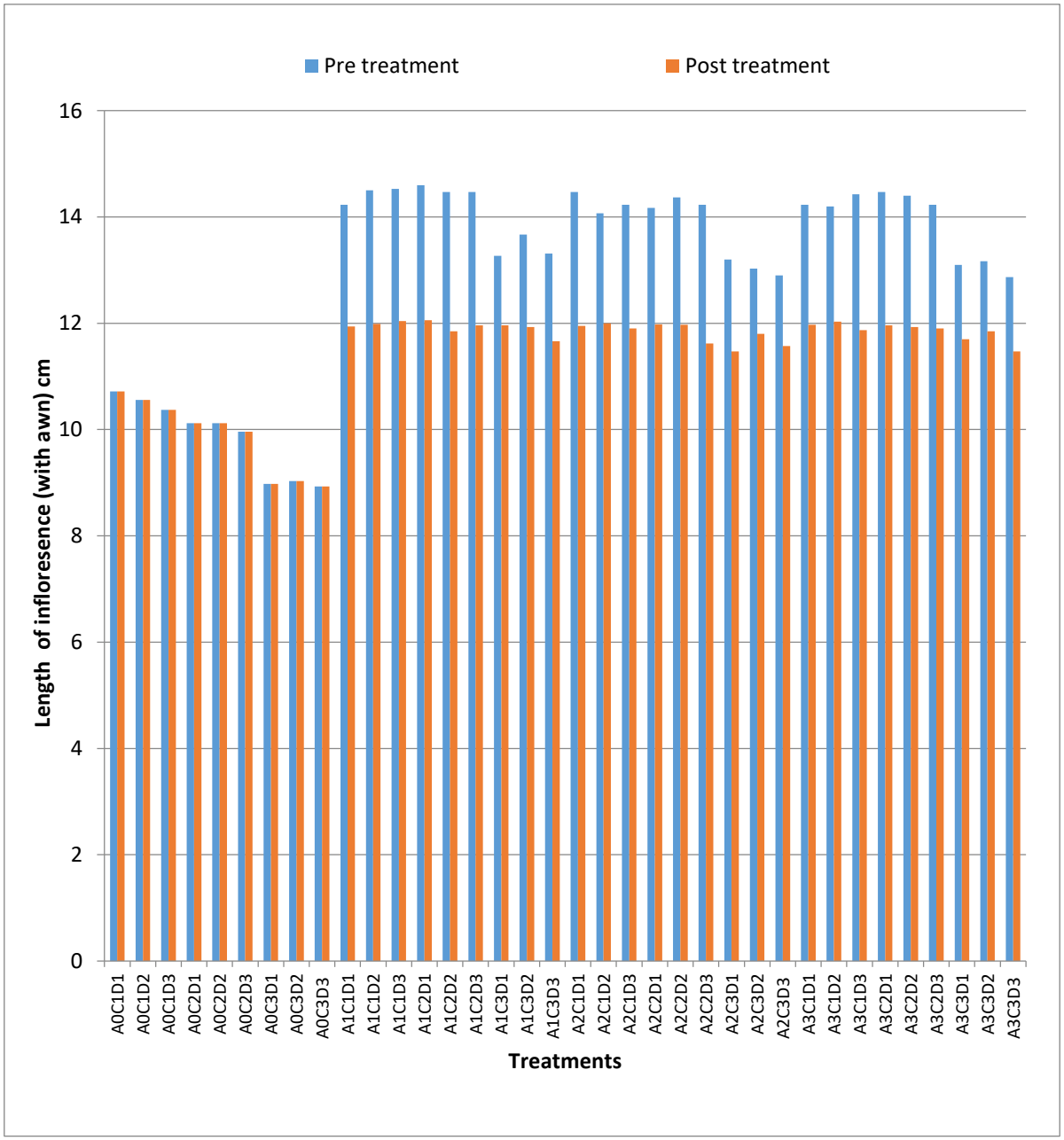
Histogram 32 and Table 15 showing the result of length of inflorescence (without awn).

Pre-treatment: Highest length of inflorescence (without awn) (9.53cm) was recorded at $A_1C_1D_2$ and lowest length of inflorescence (without awn) (4.40cm) was recorded at $A_0C_3D_3$ which were both statistically different from that of all other treatments.

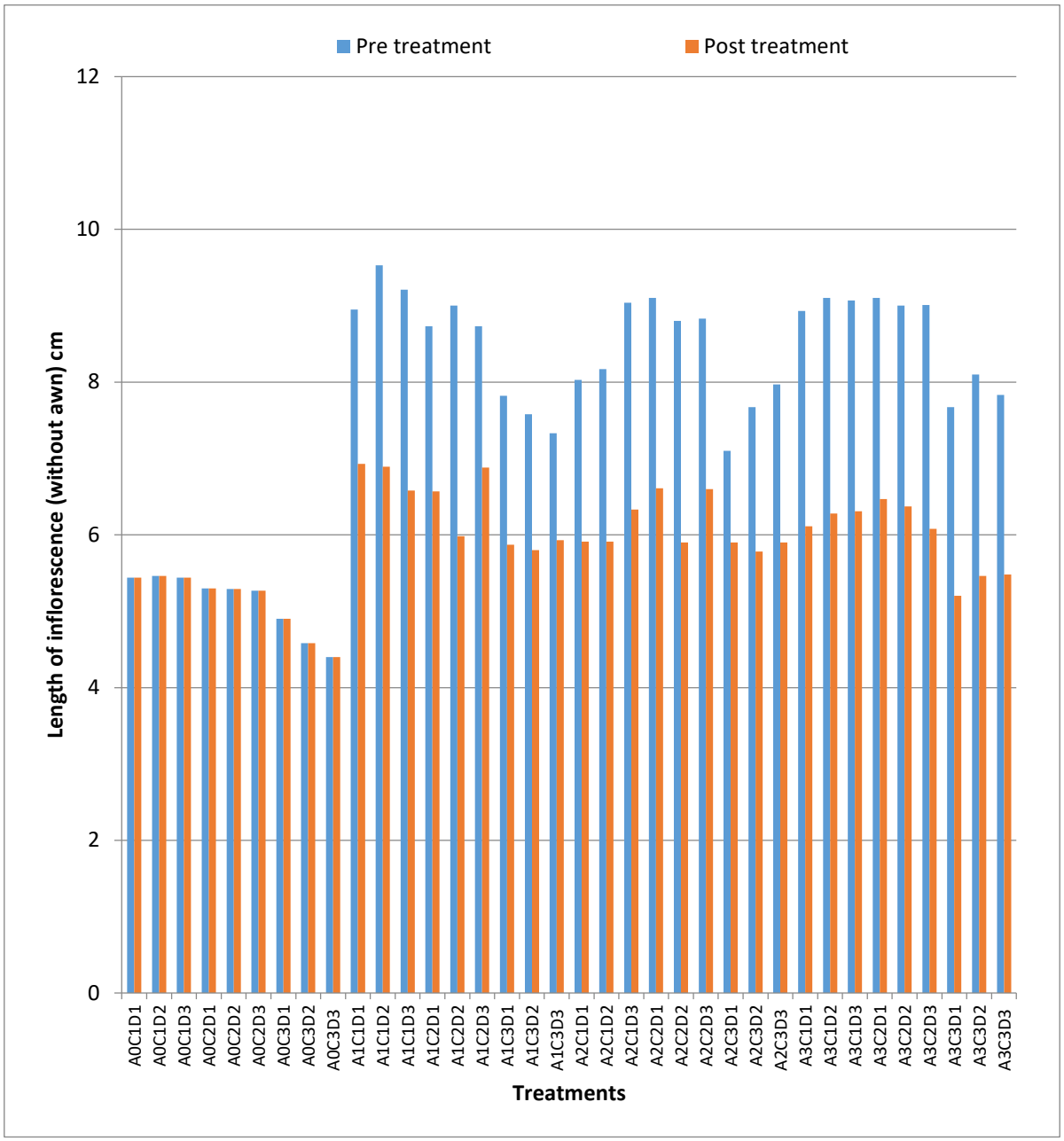
Post-treatment: Highest length of inflorescence (without awn) (6.93cm) was observed at $A_1C_1D_1$ and lowest length of inflorescence (without awn) (4.40cm) was observed at $A_0C_3D_3$ were statistically different from that of all other treatments.



Histogram 30: Effect of antioxidant along with control on flag leaf length of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 31: Effect of antioxidant along with control on length of inflorescence(cm) with awn of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 32: Effect of antioxidant along with control on length of inflorescence(cm) without awn of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.

Table 15: Effect of antioxidants along with control on flag leaf length (cm), length of inflorescence (cm) with & without awn of wheat induced with different concentration and duration of pesticide in M2 generation.

Treatment	Pre treatment			Post treatment		
	Flag leaf length (cm)	Length of inflorescence (cm) (with awn)	Length of inflorescence (cm) without awn	Flag leaf length (cm)	Length of inflorescence (cm) with awn	Length of inflorescence (cm) Without awn
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	13.59 ± 0.60	10.72 ± 0.16	5.44 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	15.03 ± 0.31	10.56 ± 0.05	5.46 ± 0.08	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	14.10 ± 1.15	10.37 ± 0.13	5.44 ± 0.06	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	14.71 ± 0.63	10.12 ± 0.12	5.30 ± 0.03	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	15.17 ± 0.14	10.12 ± 0.12	5.29 ± 0.03	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	14.17 ± 0.14	9.96 ± 0.05	5.27 ± 0.03	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	13.35 ± 0.37	8.98 ± 0.02	4.90 ± 0.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	13.69 ± 0.63	9.03 ± 0.12	4.58 ± 0.02	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	13.50 ± 0.66	8.93 ± 0.06	4.40 ± 0.11	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	16.71 ± 0.61	14.23 ± 0.25	8.95 ± 0.09	16.07 ± 0.11	11.94 ± 0.10	6.93 ± 0.16
A1C1D2	16.97 ± 0.42	14.50 ± 0.17	9.53 ± 0.54	16.37 ± 0.06	11.99 ± 0.01	6.89 ± 0.09
A1C1D3	17.43 ± 0.21	14.53 ± 0.06	9.21 ± 0.17	15.60 ± 0.53	12.04 ± 0.06	6.58 ± 0.50
A1C2D1	17.40 ± 0.17	14.60 ± 0.10	8.73 ± 0.21	14.80 ± 0.72	12.06 ± 0.12	6.57 ± 0.21
A1C2D2	17.70 ± 0.17	14.47 ± 0.06	9.00 ± 0.10	16.13 ± 0.80	11.85 ± 0.24	5.98 ± 0.03
A1C2D3	17.27 ± 0.32	14.47 ± 0.12	8.73 ± 0.25	15.03 ± 0.06	11.96 ± 0.06	6.88 ± 0.30
A1C3D1	16.00 ± 0.46	13.27 ± 0.06	7.82 ± 0.23	15.73 ± 0.67	11.96 ± 0.05	5.87 ± 0.15
A1C3D2	15.63 ± 0.42	13.67 ± 0.25	7.58 ± 0.28	17.33 ± 0.58	11.93 ± 0.13	5.80 ± 0.26
A1C3D3	15.50 ± 0.46	13.31 ± 0.51	7.33 ± 0.29	14.80 ± 0.26	11.66 ± 0.22	5.93 ± 0.15
A2C1D1	17.37 ± 0.15	14.47 ± 0.12	8.03 ± 0.15	14.97 ± 0.84	11.95 ± 0.10	5.91 ± 0.10
A2C1D2	17.37 ± 0.25	14.07 ± 0.12	8.17 ± 0.12	16.00 ± 0.56	12.00 ± 0.01	5.91 ± 0.10
A2C1D3	17.10 ± 0.53	14.23 ± 0.25	9.04 ± 0.15	14.50 ± 0.50	11.90 ± 0.10	6.33 ± 0.99
A2C2D1	17.73 ± 0.21	14.17 ± 0.06	9.10 ± 0.36	16.00 ± 0.50	11.98 ± 0.13	6.61 ± 0.62
A2C2D2	17.53 ± 0.25	14.37 ± 0.15	8.80 ± 0.62	15.40 ± 0.40	11.97 ± 0.06	5.90 ± 0.10
A2C2D3	17.37 ± 0.21	14.23 ± 0.06	8.83 ± 0.35	15.53 ± 0.23	11.62 ± 0.53	6.60 ± 0.70
A2C3D1	16.37 ± 0.06	13.20 ± 0.53	7.10 ± 0.10	15.07 ± 0.21	11.47 ± 0.06	5.90 ± 0.10
A2C3D2	16.60 ± 0.26	13.03 ± 0.06	7.67 ± 0.49	15.43 ± 0.51	11.80 ± 0.10	5.78 ± 0.13
A2C3D3	15.40 ± 0.53	12.90 ± 0.10	7.97 ± 0.15	13.30 ± 0.44	11.57 ± 0.12	5.90 ± 0.10
A3C1D1	17.27 ± 0.32	14.23 ± 0.25	8.93 ± 0.21	15.23 ± 0.80	11.97 ± 0.06	6.11 ± 0.10
A3C1D2	17.33 ± 0.06	14.20 ± 0.10	9.10 ± 0.10	16.00 ± 0.60	12.03 ± 0.07	6.28 ± 0.19
A3C1D3	17.00 ± 0.10	14.43 ± 0.23	9.07 ± 0.25	15.23 ± 1.55	11.87 ± 0.32	6.31 ± 0.46
A3C2D1	17.47 ± 0.06	14.47 ± 0.06	9.10 ± 0.17	12.00 ± 0.58	11.96 ± 0.17	6.47 ± 0.45
A3C2D2	17.87 ± 0.06	14.40 ± 0.26	9.00 ± 0.17	15.50 ± 0.50	11.93 ± 0.12	6.37 ± 0.38
A3C2D3	17.30 ± 0.10	14.23 ± 0.25	9.01 ± 0.08	15.60 ± 0.36	11.90 ± 0.11	6.08 ± 0.13
A3C3D1	15.87 ± 0.35	13.10 ± 0.10	7.67 ± 0.58	15.03 ± 0.06	11.70 ± 0.26	5.20 ± 0.26
A3C3D2	16.20 ± 0.30	13.17 ± 0.06	8.10 ± 0.10	15.20 ± 0.82	11.85 ± 0.05	5.46 ± 0.47
A3C3D3	15.83 ± 0.35	12.87 ± 0.15	7.83 ± 41.71	14.77 ± 0.49	11.47 ± 0.44	5.48 ± 0.40
Level of sig-ficance	*	**	*	*	*	*
CV(%)	2.51	1.35	3.62	6.92	1.49	5.38

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.2.1.11 100 seed weight

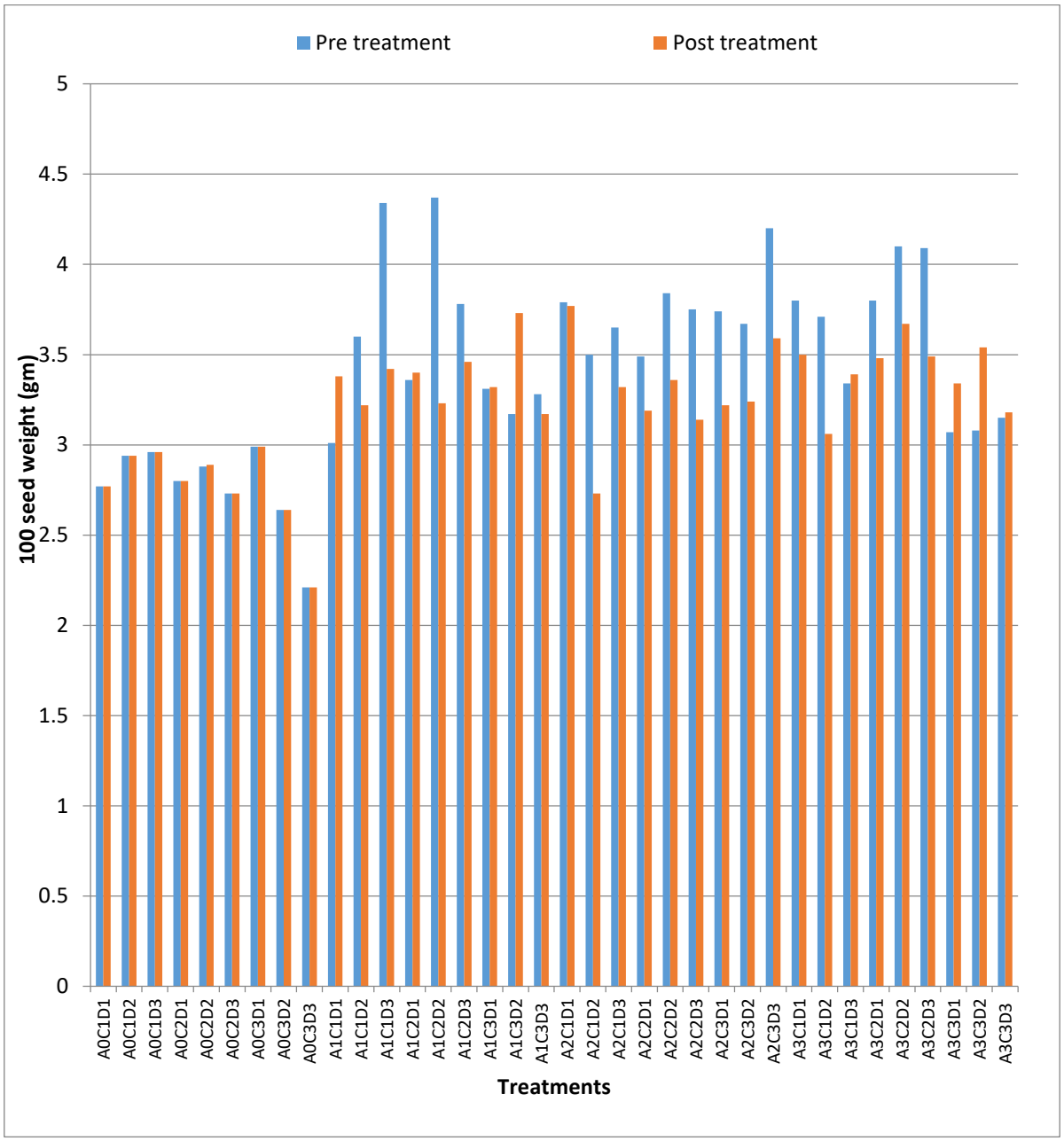
Pre-treatment: Highest 100 seed weight (4.37gm) was recorded at $A_1C_2D_2$ **(Histogram 33 and Table 16)** and lowest 100 seed weight (2.21gm) was recorded at $A_0C_3D_3$ which were significantly different from that of all other treatments.

Post-treatment: Highest 100 seed weight (3.77gm) was found at $A_2C_1D_1$ **(Histogram 33 and Table 16)** and lowest 100 seed weight (2.21gm) was found at $A_0C_3D_3$ which were significantly different from that of all other treatments.

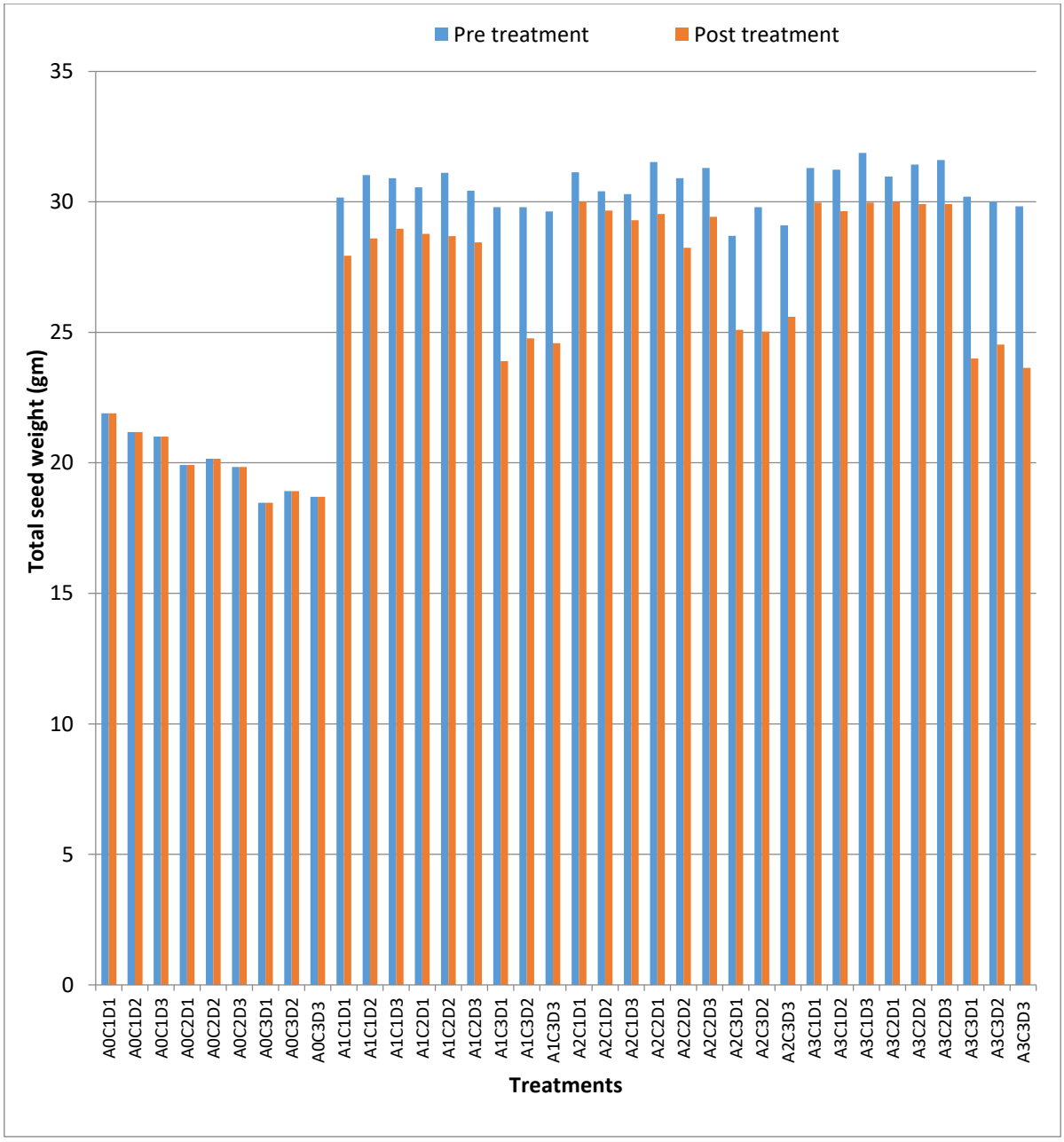
4.2.1.12 Total seed weight

Pre-treatment: Maximum weight of total seed (31.87gm) was observed at $A_3C_1D_3$ which was significantly different from that of all other treatments. On the other hands minimum weight of total seeds (18.47gm) was shown at $A_0C_3D_1$ which was also significantly different from that of all other treatments (**Histogram 34 and Table 16)**.

Post-treatment: Maximum weight of total seed (30.00gm) was recorded at $A_2C_1D_2$ and minimum weight of total seed (18.47gm) was recorded at $A_0C_3D_1$ which were both statistically different from that of all other treatments (**Histogram 34 and Table 16).**



Histogram 33: Effect of antioxidant along with control on 100 seed weight of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 34: Effect of antioxidant along with control on total seed weight of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.

Table 16: Effect of antioxidants along with control on 100 seed weight and total seed weight of wheat induced with different concentration and duration of pesticide in M2 generation.

Treatment	Pre-treatment		Post-treatment	
	100 seed wt (gm)	Total seed weight (gm)	100 seed wt (gm)	Total seed weight (gm)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	2.77 ± 0.39	21.90 ± 0.14	Same as pre-treatment	Same as pre-treatment
A0C1D2	2.94 ± 0.07	21.18 ± 0.58	Same as pre-treatment	Same as pre-treatment
A0C1D3	2.96 ± 0.08	21.01 ± 0.66	Same as pre-treatment	Same as pre-treatment
A0C2D1	2.80 ± 0.30	19.92 ± 0.94	Same as pre-treatment	Same as pre-treatment
A0C2D2	2.88 ± 0.10	20.16 ± 0.81	Same as pre-treatment	Same as pre-treatment
A0C2D3	2.73 ± 0.37	19.84 ± 0.55	Same as pre-treatment	Same as pre-treatment
A0C3D1	2.99 ± 0.00	18.47 ± 0.60	Same as pre-treatment	Same as pre-treatment
A0C3D2	2.64 ± 0.34	18.92 ± 0.80	Same as pre-treatment	Same as pre-treatment
A0C3D3	2.21 ± 0.26	18.70 ± 0.61	Same as pre-treatment	Same as pre-treatment
A1C1D1	3.01 ± 0.01	30.17 ± 0.50	3.38 ± 0.29	27.94 ± 0.90
A1C1D2	3.60 ± 0.53	31.02 ± 0.28	3.22 ± 0.11	28.60 ± 0.69
A1C1D3	4.34 ± 0.14	30.91 ± 0.80	3.42 ± 0.50	28.97 ± 0.85
A1C2D1	3.36 ± 0.47	30.56 ± 0.82	3.40 ± 0.34	28.77 ± 1.10
A1C2D2	4.37 ± 0.46	31.11 ± 0.63	3.23 ± 0.06	28.69 ± 0.75
A1C2D3	3.78 ± 0.68	30.43 ± 1.29	3.46 ± 0.22	28.45 ± 0.91
A1C3D1	3.31 ± 0.36	29.80 ± 0.52	3.32 ± 0.15	23.90 ± 0.90
A1C3D2	3.17 ± 0.04	29.80 ± 0.89	3.73 ± 0.25	24.77 ± 0.32
A1C3D3	3.28 ± 0.24	29.63 ± 0.15	3.17 ± 0.02	24.58 ± 0.11
A2C1D1	3.79 ± 0.51	31.13 ± 0.40	3.77 ± 0.52	30.00 ± 0.44
A2C1D2	3.50 ± 0.17	30.40 ± 0.52	2.73 ± 0.64	29.67 ± 0.91
A2C1D3	3.65 ± 0.32	30.30 ± 0.66	3.32 ± 0.12	29.30 ± 0.62
A2C2D1	3.49 ± 0.32	31.53 ± 1.01	3.19 ± 0.17	29.53 ± 0.57
A2C2D2	3.84 ± 0.08	30.91 ± 0.21	3.36 ± 0.22	28.24 ± 0.81
A2C2D3	3.75 ± 0.11	31.30 ± 0.80	3.14 ± 0.14	29.43 ± 0.50
A2C3D1	3.74 ± 0.24	28.70 ± 0.52	3.22 ± 0.06	25.10 ± 0.52
A2C3D2	3.67 ± 0.35	29.80 ± 0.82	3.24 ± 0.05	25.03 ± 0.32
A2C3D3	4.20 ± 0.60	29.10 ± 0.56	3.59 ± 0.38	25.60 ± 0.79
A3C1D1	3.80 ± 0.18	31.30 ± 0.87	3.50 ± 0.30	29.97 ± 0.06
A3C1D2	3.71 ± 0.08	31.23 ± 0.74	3.06 ± 0.39	29.64 ± 0.81
A3C1D3	3.34 ± 0.21	31.87 ± 0.12	3.39 ± 0.22	29.97 ± 0.81
A3C2D1	3.80 ± 0.09	30.97 ± 0.45	3.48 ± 0.09	29.98 ± 0.03
A3C2D2	4.10 ± 0.10	31.43 ± 0.49	3.67 ± 0.11	29.92 ± 0.19
A3C2D3	4.09 ± 0.22	31.60 ± 0.62	3.49 ± 0.43	29.91 ± 0.89
A3C3D1	3.07 ± 0.10	30.20 ± 0.66	3.34 ± 0.24	24.00 ± 0.10
A3C3D2	3.08 ± 0.12	30.00 ± 0.26	3.54 ± 0.47	24.53 ± 0.55
A3C3D3	3.15 ± 0.06	29.83 ± 0.76	3.18 ± 0.11	23.64 ± 0.50
Level of significance	**	*	*	*
CV(%)	8.80	2.39	8.10	2.51

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.2.2 Cytological features

4.2.2.1 % mitotic abnormality

Pre-treatment: Highest value for mitotic abnormality (24.00%) was observed at $A_0C_3D_3$ which was statistically different from that of all other treatments. On the other hand lowest value for mitotic abnormality (11.33%) was observed at $A_3C_2D_3$ which was also statistically different from that of all other treatments **(Histogram 35 and Table 17).**

Post-treatment: Highest value for mitotic abnormality (24.00%) was found at $A_0C_3D_3$ and lowest value for mitotic abnormality (13.67%) was found at $A_2C_3D_1$ and $A_3C_2D_1$ which were both statistically different from that of all other treatments **(Histogram 35 and Table 17).**

4.2.2.2 Mitotic index

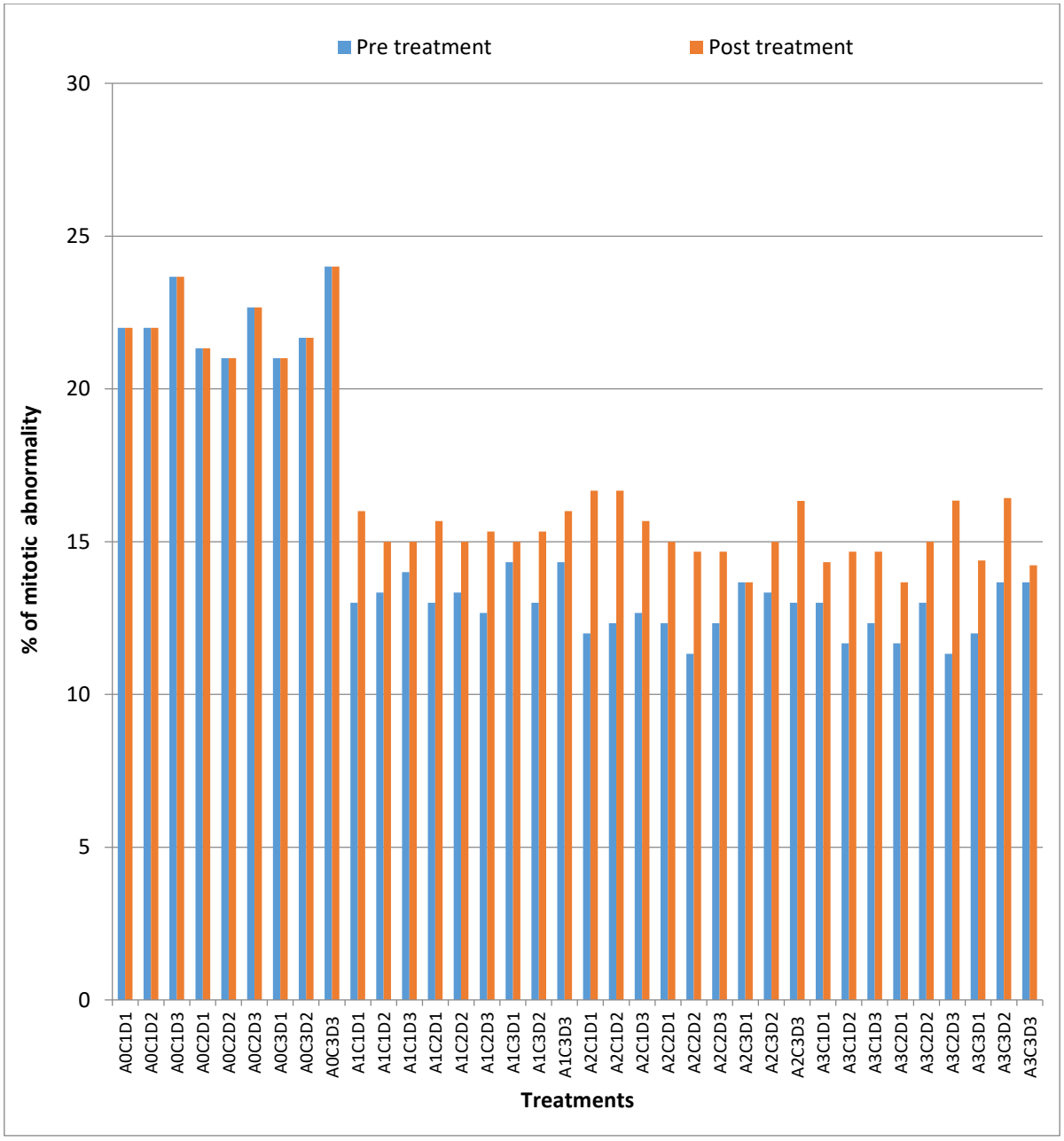
Pre-treatments: Highest mitotic index (50.67) was observed at $A_2C_1D_1$ and lowest mitotic index (11.670) was observed at $A_0C_3D_3$ which were both statistically different from that of all other treatments **((Histogram 36 and Table 17).**

Post-treatment: Highest mitotic index (43.33) was observed at $A_2C_2D_2$ which was statistically different from that of all other treatments. On the other hand lowest mitotic index (11.67) was observed at $A_0C_3D_3$ which were statistically different from that of all other treatments **(Histogram 36 and Table 17).**

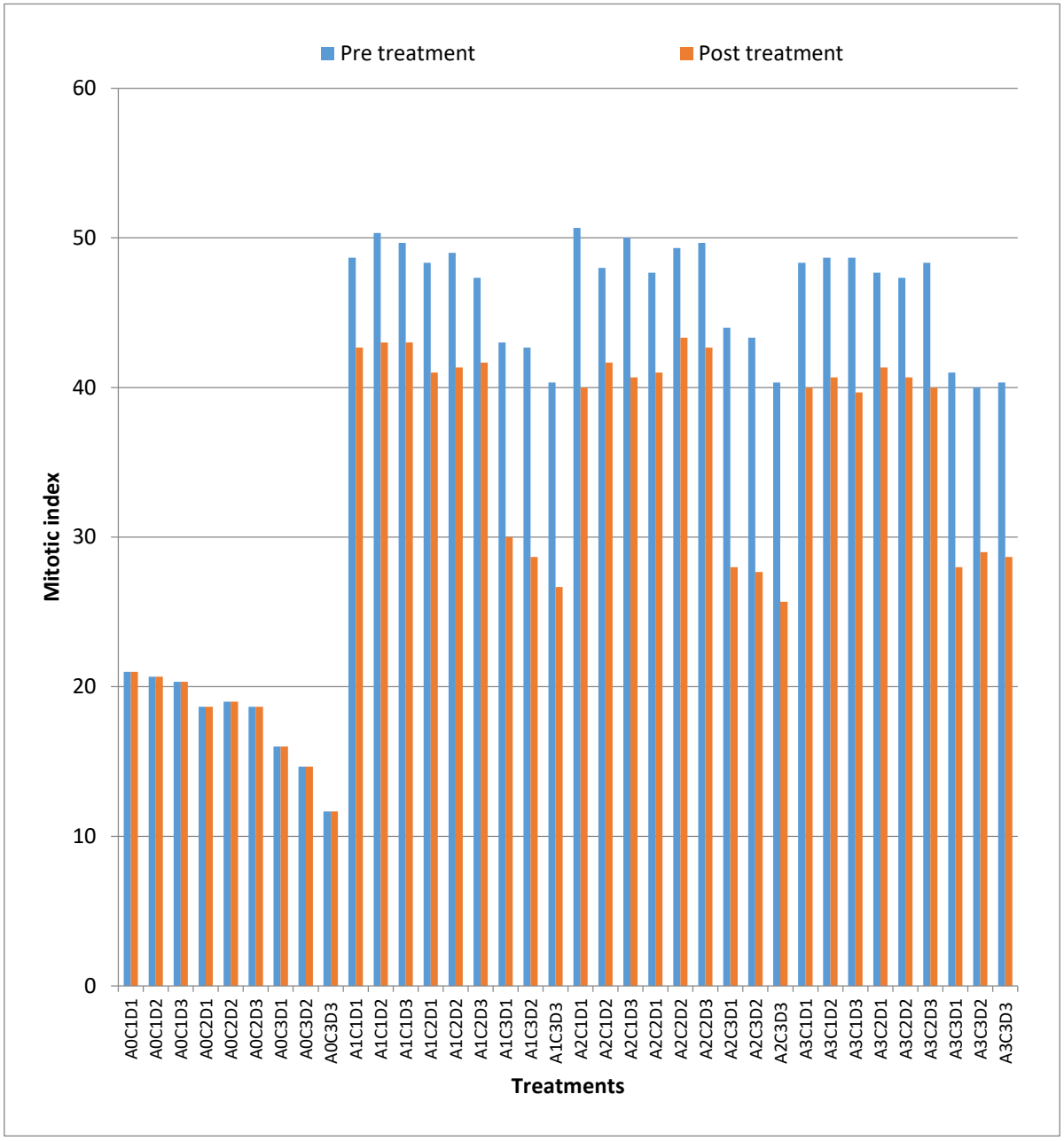
4.2.2.3 Interphase chromosome volume (ICV)

Pre-treatments: Maximum ICV (7.64) was recorded at $A_0C_3D_3$ and minimum ICV (3.43) was recorded at $A_3C_1D_1$ which were both statistically different from that of all other treatments **((Histogram 37 and Table 17).**

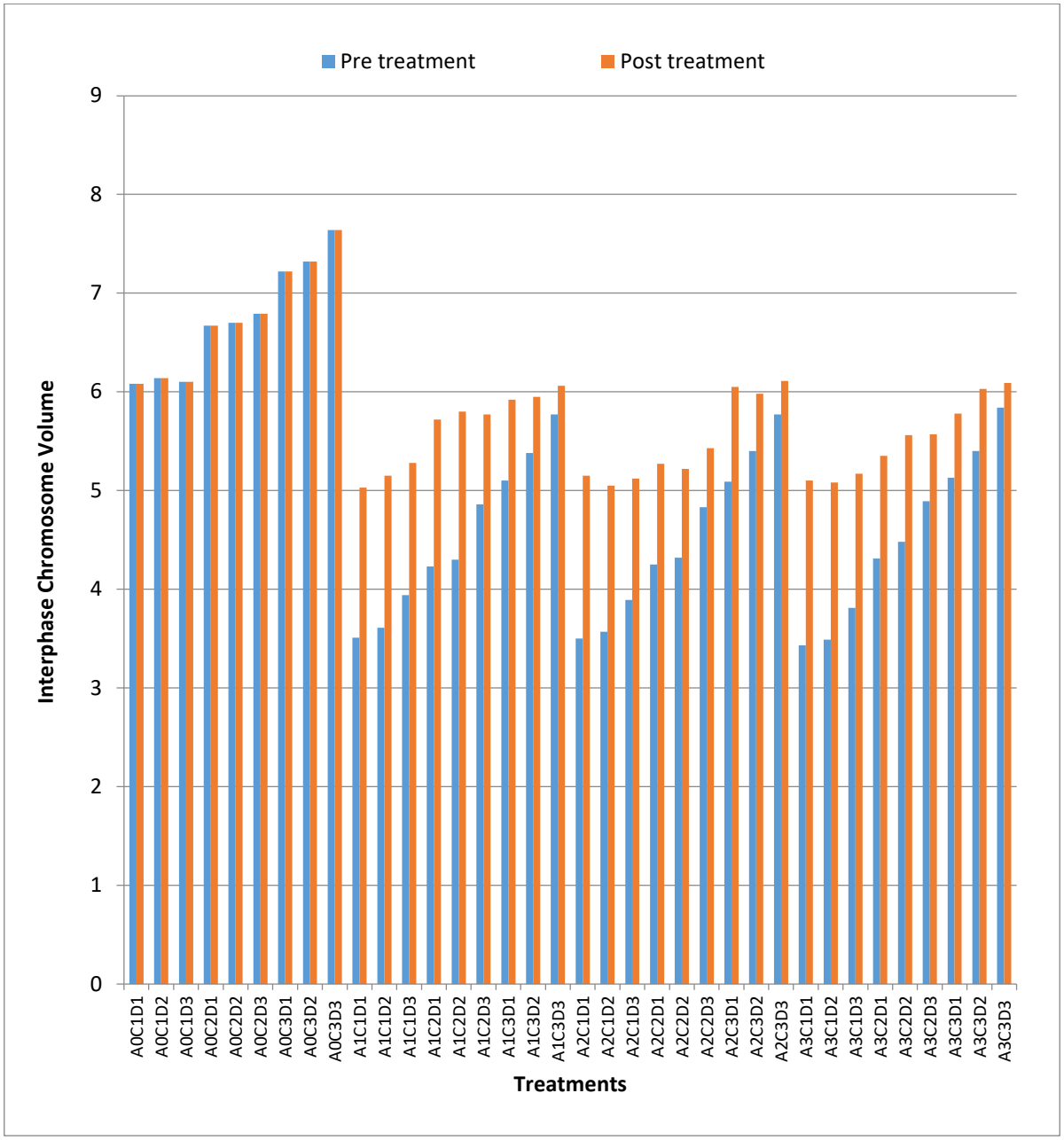
Post-treatments: Maximum ICV (7.64) was recorded at $A_0C_3D_3$ and minimum ICV (5.03) was recorded at $A_1C_1D_1$ which were both statistically different from that of all other treatments **(Histogram 37 and Table 17).**



Histogram 35: Effect of antioxidant along with control on % of mitotic abnormality of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 36: Effect of antioxidant along with control on Mitotic index of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 37: Effect of antioxidant along with control on interphase chromosome volume of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.

Table 17: Effect of antioxidants along with control on % of mitotic abnormality, mitotic index and interphase chromosome volume (ICV) of wheat induced with different concentration and duration of pesticide in M2 generation.

Treatment	Pre-treatment			Post-treatment		
	% of abnormality (Miotic)	Miotic index	ICV(µ3)	% of abnormality (Miotic)	Miotic index	ICV(µ3)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	22.00 ± 1.00	21.00 ± 1.00	6.08 ± 0.07	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	22.00 ± 1.00	20.67 ± 1.15	6.14 ± 0.05	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	23.67 ± 0.58	20.33 ± 0.58	6.10 ± 0.17	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	21.33 ± 2.08	18.67 ± 0.58	6.67 ± 0.14	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	21.00 ± 1.00	19.00 ± 0.00	6.70 ± 0.17	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	22.67 ± 1.15	18.67 ± 0.58	6.79 ± 0.04	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	21.00 ± 1.00	16.00 ± 1.00	7.22 ± 0.11	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	21.67 ± 0.58	14.67 ± 1.53	7.32 ± 0.08	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	24.00 ± 1.00	11.67 ± 0.58	7.64 ± 0.12	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	13.00 ± 1.00	48.67 ± 2.08	3.51 ± 0.09	16.00 ± 0.00	42.67 ± 4.62	5.03 ± 0.02
A1C1D2	13.33 ± 0.58	50.33 ± 4.93	3.61 ± 0.09	15.00 ± 1.00	43.00 ± 1.73	5.15 ± 0.04
A1C1D3	14.00 ± 0.00	49.67 ± 2.08	3.94 ± 0.08	15.00 ± 1.00	43.00 ± 2.65	5.28 ± 0.11
A1C2D1	13.00 ± 1.00	48.33 ± 1.53	4.23 ± 0.05	15.67 ± 1.53	41.00 ± 1.00	5.72 ± 0.06
A1C2D2	13.33 ± 1.15	49.00 ± 2.65	4.30 ± 0.02	15.00 ± 1.00	41.33 ± 2.52	5.80 ± 0.09
A1C2D3	12.67 ± 0.58	47.33 ± 2.31	4.86 ± 0.14	15.33 ± 1.15	41.67 ± 2.89	5.77 ± 0.10
A1C3D1	14.33 ± 0.58	43.00 ± 1.00	5.10 ± 0.08	15.00 ± 1.00	30.00 ± 1.00	5.92 ± 0.04
A1C3D2	13.00 ± 0.00	42.67 ± 1.53	5.38 ± 0.02	15.33 ± 0.58	28.67 ± 0.58	5.95 ± 0.07
A1C3D3	14.33 ± 0.58	40.33 ± 0.58	5.77 ± 0.02	16.00 ± 0.00	26.67 ± 0.58	6.06 ± 0.13
A2C1D1	12.00 ± 0.00	50.67 ± 4.62	3.50 ± 0.02	16.67 ± 0.58	40.00 ± 5.57	5.15 ± 0.05
A2C1D2	12.33 ± 0.58	48.00 ± 2.00	3.57 ± 0.05	16.67 ± 0.58	41.67 ± 5.51	5.05 ± 0.06
A2C1D3	12.67 ± 0.58	50.00 ± 1.73	3.89 ± 0.02	15.67 ± 0.58	40.67 ± 4.62	5.12 ± 0.09
A2C2D1	12.33 ± 0.58	47.67 ± 2.08	4.25 ± 0.05	15.00 ± 1.00	41.00 ± 3.46	5.27 ± 0.07
A2C2D2	11.33 ± 1.15	49.33 ± 4.04	4.32 ± 0.00	14.67 ± 1.15	43.33 ± 3.51	5.22 ± 0.12
A2C2D3	12.33 ± 0.58	49.67 ± 1.53	4.83 ± 0.07	14.67 ± 1.15	42.67 ± 1.15	5.43 ± 0.09
A2C3D1	13.67 ± 0.58	44.00 ± 1.00	5.09 ± 0.01	13.67 ± 0.58	28.00 ± 1.73	6.05 ± 0.06
A2C3D2	13.33 ± 0.58	43.33 ± 1.15	5.40 ± 0.03	15.00 ± 1.00	27.67 ± 0.58	5.98 ± 0.09
A2C3D3	13.00 ± 1.00	40.33 ± 0.58	5.77 ± 0.02	16.33 ± 0.58	25.67 ± 0.58	6.11 ± 0.11
A3C1D1	13.00 ± 1.00	48.33 ± 3.21	3.43 ± 0.10	14.33 ± 0.58	40.00 ± 1.73	5.10 ± 0.06
A3C1D2	11.67 ± 1.53	48.67 ± 1.53	3.49 ± 0.01	14.67 ± 0.58	40.67 ± 2.08	5.08 ± 0.05
A3C1D3	12.33 ± 0.58	48.67 ± 4.73	3.81 ± 0.13	14.67 ± 0.58	39.67 ± 2.52	5.17 ± 0.01
A3C2D1	11.67 ± 1.53	47.67 ± 4.62	4.31 ± 0.02	13.67 ± 0.00	41.33 ± 5.86	5.35 ± 0.13
A3C2D2	13.00 ± 1.00	47.33 ± 4.04	4.48 ± 0.03	15.00 ± 0.58	40.67 ± 3.79	5.56 ± 0.22
A3C2D3	11.33 ± 1.15	48.33 ± 3.21	4.89 ± 0.01	16.34 ± 0.58	40.00 ± 1.00	5.57 ± 0.03
A3C3D1	12.00 ± 1.00	41.00 ± 2.65	5.13 ± 0.06	14.38 ± 0.58	28.00 ± 3.61	5.78 ± 0.38
A3C3D2	13.67 ± 0.58	40.00 ± 3.00	5.40 ± 0.01	16.43 ± 1.00	29.00 ± 1.73	6.03 ± 0.06
A3C3D3	13.67 ± 0.58	40.33 ± 3.51	5.84 ± 0.05	14.23 ± 0.58	28.67 ± 1.15	6.09 ± 0.08
Level of significance	*	*	*	*	*	*
CV(%)	6.11	3.30	12.87	5.15	2.01	5.45

Here, * and ** indicates significant at 5% and 1% of probability respectively. NS = non-significant.

4.2.2.4 % meiotic abnormality

Pre-treatment: Maximum meiotic abnormality (14.33%) was observed at $A_0C_3D_3$ which was significantly different from that of all other treatments. On the other hands minimum meiotic abnormality (4.33%) was observed at $A_1C_1D_1$, $A_1C_1D_2$ & $A_3C_2D_1$ which were also significantly different from that of all other treatments (**Histogram 38 and Table 18)**.

Post-treatment: Highest value for meiotic abnormality (14.33%) was observed at $A_0C_3D_3$ which was significantly different from that of all other treatments. On the other hands lowest value for meiotic abnormality (5.00%) was observed at $A_1C_1D_3$ which was also statistically different from that of all other treatments (**Histogram 38 and Table 18)**.

4.2.2.5 Pollen sterility

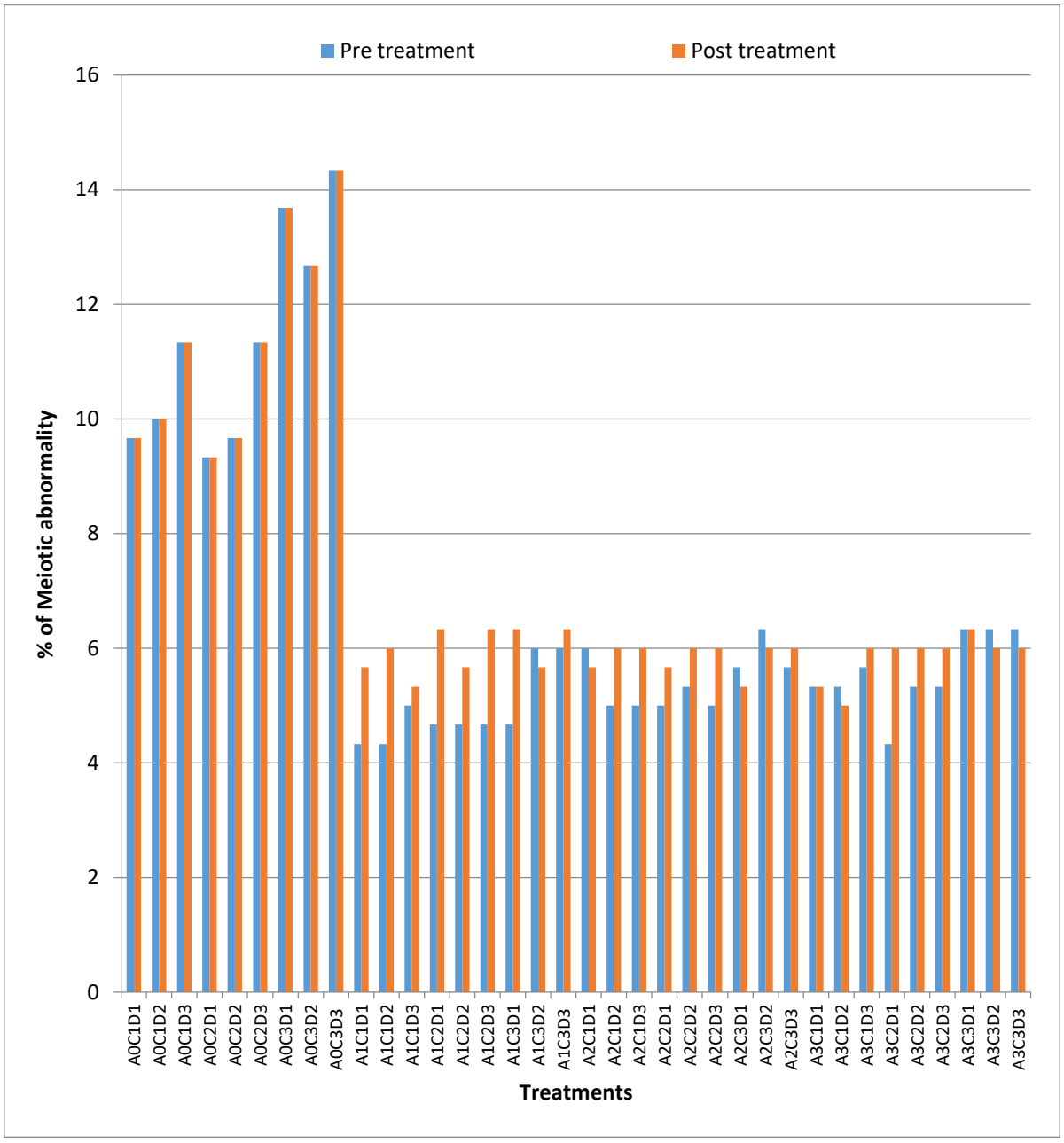
Pre-treatment: Maximum value for pollen sterility (22.33%) was observed at $A_0C_3D_3$ and minimum value for pollen sterility (2.67%) was observed at $A_3C_2D_3$ which were both statistically different from that of all other treatments **(Histogram 39 and Table 18).**

Post-treatment: Highest value for pollen sterility (22.33%) was found at $A_0C_3D_3$ and lowest value for pollen sterility (2.33%) was found at $A_1C_2D_1$ which were both statistically different from that of all other treatments **(Histogram 39 and Table 18).**

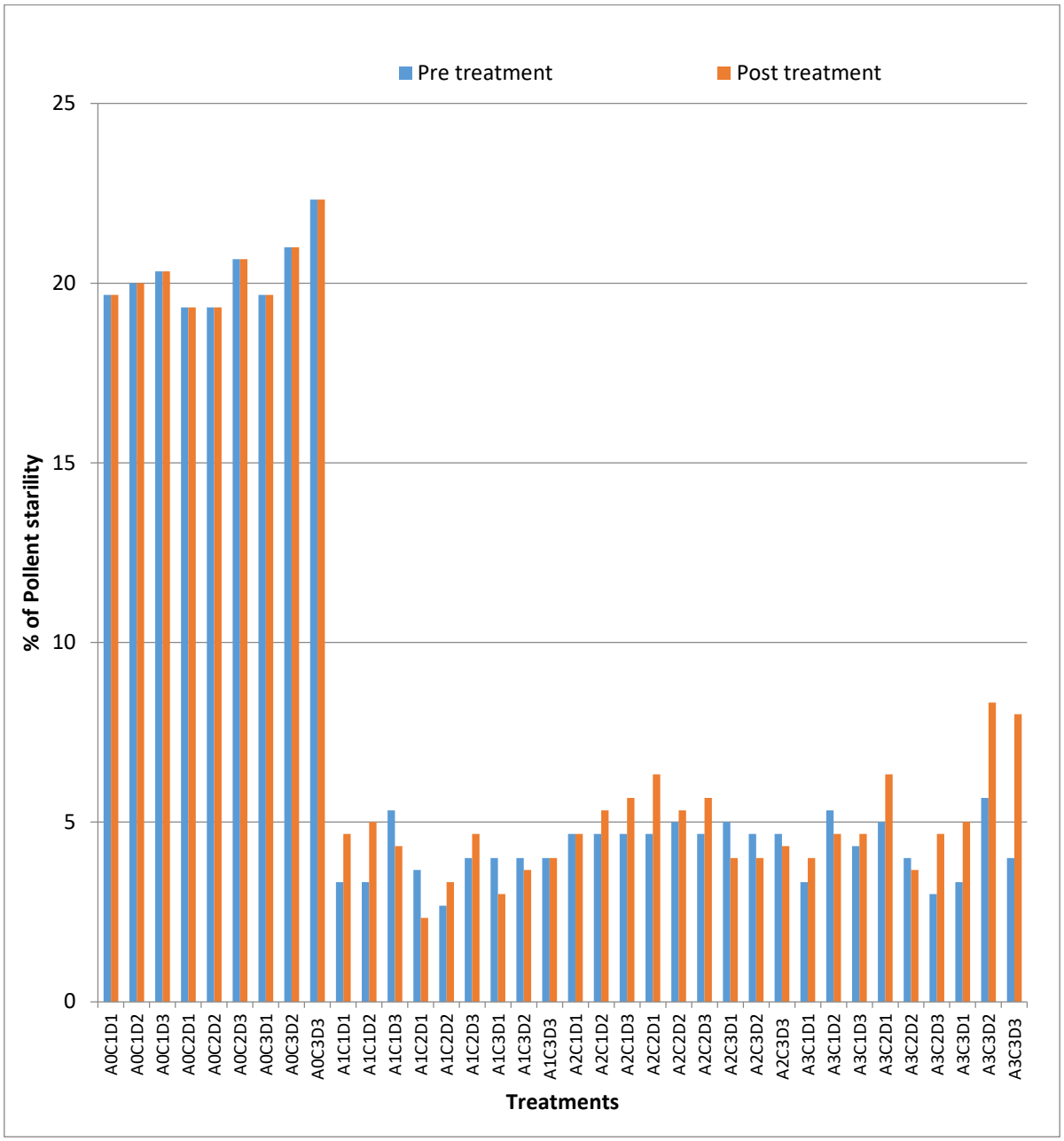
4.2.2.6 Pollen abnormality

Pre-treatment: Highest value for pollen abnormality (26.67%) was found at $A_0C_3D_3$ and lowest value for pollen abnormality (12.67%) was found at $A_2C_1D_2$ which were both statistically different from that of all other treatments (**Histogram 40 and Table 18)**.

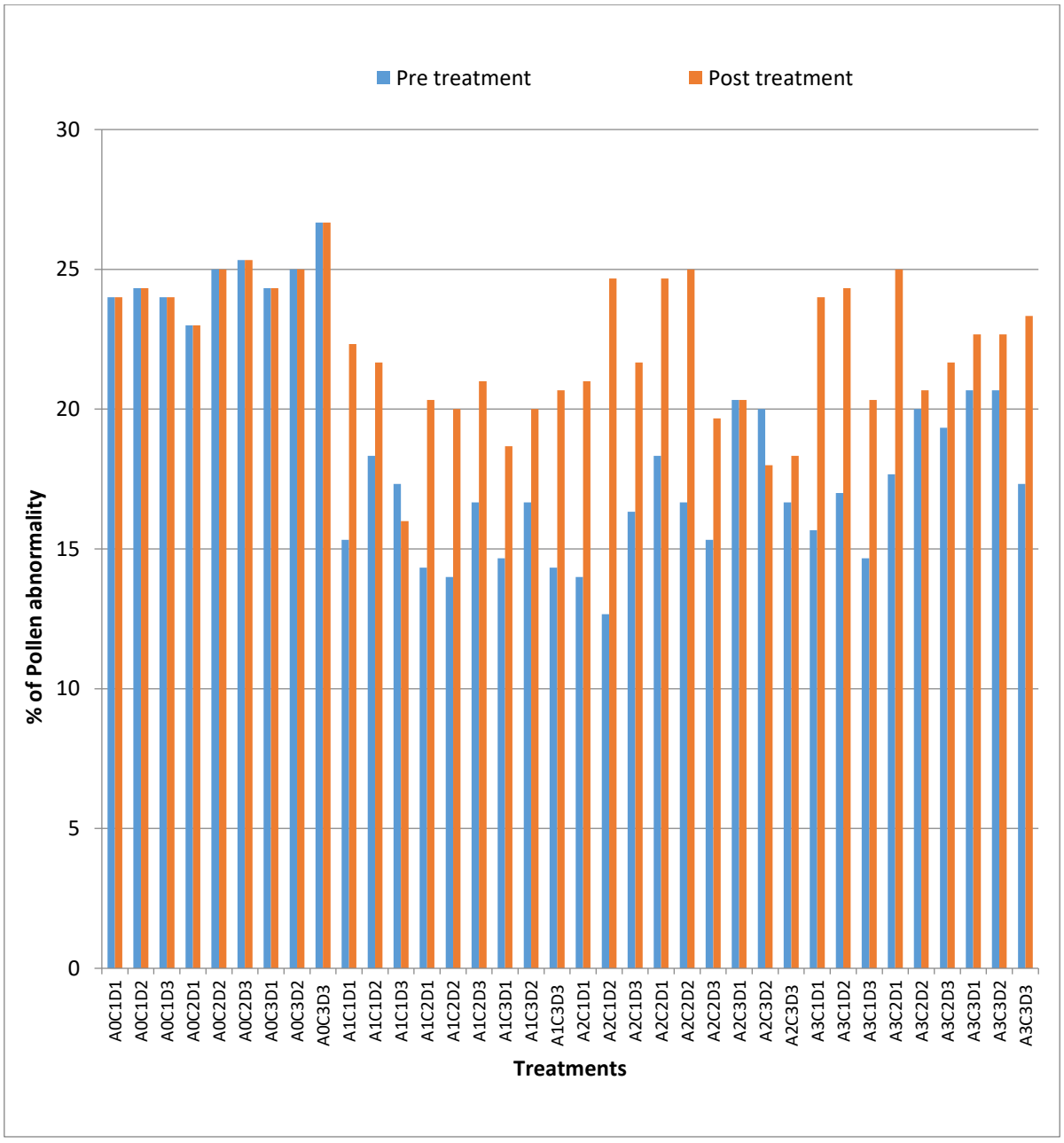
Post-treatment: Highest value for pollen abnormality (26.67%) was found at $A_0C_3D_3$ and lowest value for pollen abnormality (16.00%) was found at $A_1C_1D_3$ which were both statistically different from that of all other treatments (**Histogram 40 and Table 18)**.



Histogram 38: Effect of antioxidant along with control on % of meiotic abnormality of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 39: Effect of antioxidant along with control on % of pollen sterility of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.



Histogram 40: Effect of antioxidant along with control on % of pollen abnormality of wheat induced with different concentration and duration of pesticide in laboratory in M2 generation.

Table 18: Effect of antioxidants along with control on meiotic abnormality, pollen sterility and pollen abnormality of wheat induced with different concentration and duration of pesticide in M2 generation.

Treatment	Pre-treatment			Post-treatment		
	% of Meiotic abnormality	% of Pollen sterility	% of Pollen abnormality	% of Meiotic abnormality	% of Pollen sterility	% of Pollen abnormality
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A0C1D1	9.67 ± 0.58	19.67 ± 0.58	24.00 ± 1.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D2	10.00 ± 1.00	20.00 ± 1.00	24.33 ± 0.58	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C1D3	11.33 ± 1.15	20.33 ± 1.15	24.00 ± 1.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D1	9.33 ± 0.58	19.33 ± 0.58	23.00 ± 1.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D2	9.67 ± 0.58	19.33 ± 1.15	25.00 ± 0.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C2D3	11.33 ± 1.53	20.67 ± 0.58	25.33 ± 1.53	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D1	13.67 ± 0.58	19.67 ± 0.58	24.33 ± 1.53	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D2	12.67 ± 1.15	21.00 ± 1.00	25.00 ± 0.00	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A0C3D3	14.33 ± 0.58	22.33 ± 0.58	26.67 ± 0.58	Same as pre-treatment	Same as pre-treatment	Same as pre-treatment
A1C1D1	4.33 ± 0.58	3.33 ± 1.15	15.33 ± 0.58	5.67 ± 0.58	4.67 ± 0.58	22.33 ± 0.58
A1C1D2	4.33 ± 0.58	3.33 ± 0.58	18.33 ± 1.15	6.00 ± 1.00	5.00 ± 1.00	21.67 ± 0.58
A1C1D3	5.00 ± 1.00	5.33 ± 0.58	17.33 ± 0.58	5.33 ± 0.58	4.33 ± 2.08	16.00 ± 4.36
A1C2D1	4.67 ± 0.58	3.67 ± 0.58	14.33 ± 0.58	6.33 ± 0.58	2.33 ± 0.58	20.33 ± 0.58
A1C2D2	4.67 ± 0.58	2.67 ± 0.58	14.00 ± 2.00	5.67 ± 0.58	3.33 ± 1.53	20.00 ± 0.00
A1C2D3	4.67 ± 1.15	4.00 ± 1.00	16.67 ± 1.53	6.33 ± 1.15	4.67 ± 0.58	21.00 ± 1.00
A1C3D1	4.67 ± 0.58	4.00 ± 1.00	14.67 ± 3.06	6.33 ± 0.58	3.00 ± 1.00	18.67 ± 0.58
A1C3D2	6.00 ± 1.00	4.00 ± 1.00	16.67 ± 0.58	5.67 ± 0.58	3.67 ± 0.58	20.00 ± 1.00
A1C3D3	6.00 ± 0.00	4.00 ± 1.00	14.33 ± 0.58	6.33 ± 0.58	4.00 ± 0.00	20.67 ± 0.58
A2C1D1	6.00 ± 1.00	4.67 ± 0.58	14.00 ± 1.00	5.67 ± 0.58	4.67 ± 0.58	21.00 ± 1.00
A2C1D2	5.00 ± 1.00	4.67 ± 0.58	12.67 ± 1.15	6.00 ± 1.00	5.33 ± 0.58	24.67 ± 0.58
A2C1D3	5.00 ± 1.73	4.67 ± 0.58	16.33 ± 2.31	6.00 ± 1.00	5.67 ± 0.58	21.67 ± 1.53
A2C2D1	5.00 ± 1.00	4.67 ± 0.58	18.33 ± 0.58	5.67 ± 0.58	6.33 ± 1.15	24.67 ± 0.58
A2C2D2	5.33 ± 1.53	5.00 ± 0.00	16.67 ± 2.89	6.00 ± 1.00	5.33 ± 0.58	25.00 ± 1.00
A2C2D3	5.00 ± 1.00	4.67 ± 0.58	15.33 ± 0.58	6.00 ± 1.00	5.67 ± 1.15	19.67 ± 1.53
A2C3D1	5.67 ± 0.58	5.00 ± 0.00	20.33 ± 1.15	5.33 ± 0.58	4.00 ± 1.73	20.33 ± 3.21
A2C3D2	6.33 ± 0.58	4.67 ± 0.58	20.00 ± 0.00	6.00 ± 1.00	4.00 ± 1.00	18.00 ± 5.20
A2C3D3	5.67 ± 1.15	4.67 ± 0.58	16.67 ± 2.08	6.00 ± 1.00	4.33 ± 0.58	18.33 ± 5.77
A3C1D1	5.33 ± 1.53	3.33 ± 0.58	15.67 ± 4.93	5.33 ± 0.58	4.00 ± 1.00	24.00 ± 2.65
A3C1D2	5.33 ± 0.58	5.33 ± 0.58	17.00 ± 7.00	5.00 ± 1.00	4.67 ± 0.58	24.33 ± 2.08
A3C1D3	5.67 ± 1.53	4.33 ± 0.58	14.67 ± 0.58	6.00 ± 1.00	4.67 ± 0.58	20.33 ± 1.53
A3C2D1	4.33 ± 0.58	5.00 ± 1.00	17.67 ± 3.21	6.00 ± 1.00	6.33 ± 1.15	25.00 ± 1.73
A3C2D2	5.33 ± 0.58	4.00 ± 1.00	20.00 ± 1.00	6.00 ± 1.00	3.67 ± 0.58	20.67 ± 0.58
A3C2D3	5.33 ± 0.58	3.00 ± 1.00	19.33 ± 0.58	6.00 ± 1.00	4.67 ± 0.58	21.67 ± 0.58
A3C3D1	6.33 ± 0.58	3.33 ± 1.15	20.67 ± 0.58	6.33 ± 0.58	5.00 ± 0.00	22.67 ± 2.31
A3C3D2	6.33 ± 0.58	5.67 ± 0.58	20.67 ± 0.58	6.00 ± 1.00	8.33 ± 1.53	22.67 ± 0.58
A3C3D3	6.33 ± 0.58	4.00 ± 1.73	17.33 ± 1.53	6.00 ± 1.00	8.00 ± 1.73	23.33 ± 1.15
Level of significance	*	**	*	*	*	**
CV(%)	11.42	9.48	9.83	11.15	11.20	8.65

Here, * and ** indicates significant at 5% and 1% of probability respectively.

CHAPTER-V

DISCUSSION

5. DISCUSSION

5.1 Study of M1 generation

5.1.1 Morphological features

Any kind of genetic mutation directs the morphology of the plants and that is why morphological study is extremely used for analyzing the effect of genetic alteration.

5.1.1.1 Germination percentage

Germination is a critical stage in the life cycle of plants. It starts with the uptake of water by the dry seed (imbibition), continues with biochemical preparative processes and the elongation of the embryonic axis, and usually terminates with the protrusion of the radicle out of the seed (Bewley and Black, 1994). The effects of environmental factors on seed germination are important for plant establishment, which is relevant in both ecology and agriculture. Optimal germination is a critical factor leading to the successful establishment and growth of crops plants. The germination test of a non-dormant seed determines the germination potential or viability of that seed. When the seed germinates, the time between the beginning of imbibition and radicle protrusion out of the seed reflects the rate at which bio-physical and physiological processes occur in the embryo and the seed as a whole (Bewley and Black, 1994; Baskin and Baskin, 2014). On the other hand, the germination test of a seedlot aims to determine the germination capacity (germinability) the rate of germination and the homogeneity of this seedlot .

From agronomic point of view, the germinability of a particular seedlot is central to the calculation of optimum seeding rates, as well as helping to determine whether a particular seedlot has the potential to produce a good crop, or should not be used at all. A germination test determines the percentage of seeds that are alive in any seed lot. The level of germination in association with seed vigor provides a very good estimate of the potential

field performance. While the speed of germination varies slightly across varieties, seeds should absorb moisture within two days and produce a root and the first leaf within four days. At this point, the seed is considered to have germinated. A germination test is often the only test a farmer can conduct on the seed to determine if it is suitable for planting. When seed is stored in traditional open systems, the germination rate of most rice seed begins to deteriorate rapidly after six months. Also, many varieties have a dormancy period immediately after harvest that can last for 1−2 months. By knowing the germination rate, farmers can adjust their planting rates to attain the desired plant population in the field. In this study germination percentage were decreased with the increasing doses of pesticide concentration respectively. Kabir (1981) and Alam *et al.* (1984) found similar result in their study. Germination percentage both in laboratory and pot were significantly low at control than both pre- and post-treatment. Bonea and Bonciu (2017) observed fungicide Royal Flo influenced maize seed germination negatively. The same result has been reported in *Vigna mungu* (Maity, 2014) and *Wheat* (Marini *et al.,* 2011). But Alrajhi (2014) reported a stimulation of germination after treatment with fungicides Dithane M-45 and Amister. Present study showed higher germination percentage at pre- treatment compared to post-treatment and control.

5.1.1.2 Coleoptile length

Successful crop establishment is required for optimal yields and depends upon rapid shoot emergence and leaf area development. The coleoptile plays an important role in the establishment of wheat (*Triticum aestivum* L.), as it transports the young stem and first leaf from the embryo to the soil surface and protects the plumule as it moves through the soil layers. The coleoptile also determines the maximum depth at which wheat seeds can be sown. Approximately 37% of the wheat cropping area in developing countries consists of semi-arid/arid environments (Sinclair, 2011; Sadok, 2017), and compared to regions with higher precipitation levels, wheat is often planted deeper in these arid regions to ensure adequate soil moisture for germination (Schillinger *et al.,* 1998; Mohan *et al.,* 2013). With

deep sowing, however, short coleoptiles might limit the ability of the seedling to push the first leaf through the soil surface, resulting in poor establishment. Therefore, wheat cultivars with long coleoptiles are preferable for deep planting. The present study coleoptile length was found to be decreased with an increased concentration of pesticide in control. Similarly Akhter *et al.* (2009) and Razu *et al.* (2012) observed that herbicide Fielder decreased coleoptile length with the increase of doses compared to seeds treated with water. But present study indicates that antioxidant treatments (pre and post) did not disturb for remaining the actual length of coleoptile than that of control. And in case of pre-treatment longest coleoptile was found to be produced compared to that of post-treatment. It might be due to scavenging role of antioxidant against pesticide induced oxidation.

5.1.1.3 Root length

Unlike aboveground plant parts, most roots are hidden in the soil, and we cannot directly observe their way of life. Scientists have been able to uncover relatively little about root biology compared to other plant parts and processes because examining roots is often time-consuming and labor-intensive (Gewin, 2010). Because of this, roots are known as the "hidden half" of plants (Eshel and Beeckman, 2013), and have been a fascinating object for many scientists (De Kroon and Visser, 2003). Roots are gradually becoming more visible to emerging technologies such as computed tomography (Rogers *et al.*, 2016 ; Teramoto *et al.*, 2020) and computer simulation modeling (Dunbabin *et al.*, 2013) and our understanding of them is improving.

Roots play an important role in crop growth because roots are the plant part that absorbs water and nutrients from the soil. One would expect that roots play a critical role in various environments based on these functions. In fact, deeper roots (higher deep root ratio and rooting depth) seem to uptake more water from the soil, and higher root length densities seem to absorb more nutrients. Depending on individual crop species and environments, the results of some studies have agreed with these expectations (Kawata *et al.*, 1978; Nemoto *et al.*, 1998). Result of the present study reveals that root length at different days after germination in

control was decreased with an increased concentration of pesticide. These findings are in conformity with work reported by Aslam *et al.* (2014) and Akhter *et al.* (2009). Aksoy *et al.* (2013) reported that the root and seedling length were significantly affected by quizalofop-p-ethyl (herbicide) treatment in *Glycine max*. In this study, both pre- and post-treatments produced increased root length compared to that of control and root length of 7 and 14 DAG were significantly different. Suzuki (2005) and Ismail (2008) found that the addition of antioxidant metal $CaCl_2$ to cadmium-stressed common bean plants improved the stem fresh weight, root length, number of flowers and number of pods per plant. It may be due to the protective role of antioxidant against the pesticide toxicity. El-Ashry and Mohamed (2012) reported that Ca^{+2} had more antagonistic effect on root-growth than $Se^{4+}$and Zn^{2+} but these three metals have a protective role against the genotoxicity of Cd^{2+} on root-growth especially at low concentration. But only treatment ($A_3C_3D_3$) of post-treatment with highest concentration of pesticide at 7 days showed exceptionally longest root length.

5.1.1.4 Plant height

Plant height is an important morphological and developmental phenotype that directly indicates overall plant growth and is widely predictive of final grain yield and biomass. Plant height is a central part of plant ecological strategy. It is strongly correlated with life span, seed mass and time to maturity, and is a major determinant of a species' ability to compete for light. Plant height is also related to critical ecosystem variables such as animal diversity and carbon storage capacity. Continuous plant height measurements throughout the season can contribute to identifying different growing stages and consequently select genotypes that have the longer grain filling period to produce more yield. Theoretically, plant height is defined as the shortest distance between the upper boundary (the highest point) of the main photosynthetic tissues (excluding inflorescences) and the ground level (Perez-Harguindeguy, 2013). Result of this research in pot, control showed minimum plant height at 7, 14 and 25 DAG compared to pre and post treatments. In laboratory, control also showed minimum plant height in case of pre-treatment. It may be consequence of the

pesticide induced stressed condition. Similar results were found in water stressed by Shrivastava and Shrivastava (2014) in Stevia plants, Wu *et al.* (2008) in Citrus plants, Zhang *et al.* (2004) in Soyabean. Alavi-Samani *et al.* (2013) also observed reduced plant height in two species of thyme in response to water deficit conditions. Razu *et al.* (2012) found that Fielder (herbicide) decreased the characteristic values with the increase of doses compared to seeds treated with water. Klingman *et.al.* (1982) noticed reduced plant height using herbicide 2, 4-D on wheat due to inhabitance of cell division and growth in the meristematic region. Suggesting antioxidant mediated treatments (pre and post) were managed to produce better plant height compared to the control. But in case of post-treatment, among the treatments $A_3C_3D_3$ showed lowest plant height in laboratory. In which pesticide concentration and duration were high. Result of this study on plant height showing scattered information, indicating antioxidant protective role was missing there. This result is conformational with the report of Bibi *et al.* (2008), Ahmad *et al.* (1993) and Khalil *et al.* (2000) that plant height is strongly under genetic control and not affected by herbicides application. Al-Musa *et al.* (2013) stated that the variation in plant height was found due to its genetic variation.

5.1.1.5 Number of tiller per plant, Number of spikelet/spike, Number of seeds/spike

According Lindsey *et al.* (2013) "Yield potential is reduced if tiller numbers fall below 25 per square foot after green up." A tiller is a shoot that originates at the coleoptilar node. Tillers share the same root mass with the main stem. During tillering, the major management consideration is whether stands are adequate to achieve yield goals. Management inputs will not compensate for skimpy or erratic stands caused by insects, seedling diseases, poor seed quality, herbicide injury etc. Tillers are additional stems that develop of the main shoot of the plant. Primary tillers form in the axils of the first four or more true leaves of the main stem. Secondary tillers may develop from the base of primary tillers if conditions favor tiller development. Grain yield depends on plants per area, tiller per plant, kernels per tiller and weight per kernel therefore, tillering is essential for productivity.

Wheat spikelets are small, usually oblong or ovate reproductive structures found in the inflorescence of wheat plants (genus *Triticum)*. They consist of several components: These are the outermost structures of the spikelet, serving as protective coverings. There are typically two glumes in each spikelet. Inside the glumes, there are these structures play a crucial role in the wheat plant's reproductive process, leading to the production of grains. Wheat spikelets are organized in a specific arrangement within the inflorescence, which is the flowering part of the plant. Florets contain the reproductive organs of the wheat plant. Wheat spikelets are arranged on the central stem of the inflorescence, known as the rachis. The rachis is part of the wheat head, and spikelets are attached to it in a specific pattern. The arrangement can vary based on the wheat species and variety. There are different types of wheat inflorescences, which determine how spikelets are organized. The main type spikelets are directly attached to the rachis, giving a more compact appearance. This is common in durum wheat. Spikelets have short pedicels (stalks) that attach them to the rachis, creating a partially loose structure. Spikelets are attached to secondary branches, creating a more open and branched appearance. This type is common in some bread wheat varieties. Wheat spikelets play a critical role in the reproduction of wheat plants and the eventual formation of grains. They contain the structures necessary for pollination and fertilization to occur, leading to the development of wheat kernels or grains that are harvested for various purposes, including food production. The production of organic matter per spike and spike weight are directly related with a grain number and grain weight per spike. A grain number per spike depends on a spikelet number, a flower number per spike, the success of pollination and the success of the early organogenetic stages of flowers (Kraljevich-Balalich, 1978).

The present result reveals that lowest value of number of tiller/plant, number of spikelet/spike and number of seeds/spike was observed at highest pesticide concentration of control. Bibi *et al.* (2008), Baldha *et al.* (1998) and Sohail (1993) also reported that application of herbicides significantly influenced the number of tillers. Meadly (1964)

reported the scattering of heads in wheat treated with 2,4-D at early tillering stage. Asana *et.al.* (1950) also found less tillers, unfolded leaves, spikelets and shorter stems leads to grain yield using herbicide 2, 4-D in two wheat varieties. Result of this study showed that antioxidant treatments were managed to significantly increased value for number of spikelet/spike and number of seed /spike in case of both pre and post-treatments. But increased value of tiller/plant was not significant.

5.1.1.6 Flag leaf length

The growth stage in wheat begins when the last leaf (flag leaf) begins to emerge from the whorl. This stage is particularly significant because the flag leaf makes up approximately 75 percent of the effective leaf area that contributes to grain fill. It is therefore important to protect and maintain this leaf heathy (free of disease and insect damage) before and during grain development. When the flag leaf emerges, three nodes are visible above the soil surface. To confirm that the leaf emerging is the flag leaf, split the leaf sheath above the highest node, if the head and no additional leaves are found inside. Flag leaf length is the most important for yield potential. Because flag leaf indicating the transition from plant growth to grain production. Photosynthesis in this leaf provides the majority of the carbohydrates needed for grain filling. Results of this research showed that pesticide had obvious toxic effects on flag leaf length. As a result decreased flag leaf length was found in control. Similarly a decrease effect of flag leaf length was noticed for all the treatments of herbicide concentration Akhter *et al.* (2009). But antioxidant (pre and post) treatments increased flag leaf length and pre-treatments showed longest flag leaf length among all the treatments.

5.1.1.7 12 Length of inflorescence with awn and without awn, 100 seed weight and total seed weight

These morphological parameters are directly related with crop yield. Seed yield in grasses is known to be associated with a number of seed yield components, such as spikelets per inflorescence, seeds per inflorescence, seed size and seed weight and seems impossible to

achieve realized seed yields without considering these traits (Abel *et al.,* 2017; Rebetzke *et al.,* 2016; Wang *et al.,* 2010). A number of considerable works have shown awns are positively associated with larger seed size and thereby increased yield in less favorable conditions (Liller *et al.,* 2017 ; Motzo and Giunta, 2002). Under favorable growing conditions, however, long awns compete with florets for assimilates, which exacerbates assimilate partitioning to florets. Number of studies have shown that long and barbed awns assist seed dispersal by adhesion to animal fur (Elbaum *et al.,* 2007 ; Jung *et al.,* 2014 ; Vervelde, 1953).

Awns can be considered an alternative target for the improvement of wheat grain yield through their known functions, including photosynthesis, carbohydrate storage and increased water-use efficiency (Weyhrich *et al.,* 1995). Arguments about their putative role (costs or benefits) for grain yield are ongoing, although some functions have been validated. Considerable work in previous studies suggest that both positive and negative effects on wheat grain yield are possible with different growth conditions (Motzo and Giunta, 2002; Maydup *et al.,* 2010). Use of assimilates allowing awn development seems to be the main negative factor.

The research by Rebetzke *et al.* (2016) is particularly important in explaining the way in which awns affect grain yield. They found that awns are coupled with larger grain size and yield in less favourable environments but reduce grain number in more favourable environments. They also observed that awns do not significantly affect the total number of spikelets and anthesis time, but instead markedly increase the number of sterile spikelets and grain size in some environments. This result reveals that length of inflorescence with and without awn, 100 seed weight and total seed weight was lower in control. Kumer and Singh (2010) found decreased 1000 (thousand) grain weight in different varieties of wheat. Klingman *et al.* (1982) noticed delayed maturity and reduced grain yield using herbicide 2, 4-D on wheat due to inhabitance of cell division and growth in the meristematic region. Pre-

and post-treatment of this study showed increased length of inflorescence with and without awn, 100 seed weight and total seed weight, suggesting that the antioxidants are reducing pesticide induced morphological consequences.

5.1.2 Cytological features

5.1.2.1 Mitotic abnormality

Cytological behavior like mitotic abnormality is considered as one of the most dependable indexes to estimate the potency of mutagen. In the present study, mitotic abnormality was observed in the root tip cells of wheat emerged from different treatment along with controls. Pesticide was capable of inducing varying degrees of mitotic abnormalities and they were characterized by chromosome fragments, lagging chromosomes, bridges. The bridges were either single, double or multiple with or without fragments or lagging chromosome. The result obtained on mitotic abnormality in control indicates that increased pesticide concentrations caused increased mitotic abnormality gradually. This result showed resemblance with the findings of Zaman and Saleh (2005) who obtained increased frequency of chromosomal changes like chromatid bridges, chromosome fragments, laggards, etc. of wheat with the increased doses of chemical mutagen Ethylene Glycol (EG). Similarly Alam *et al.* (1980a, 1980b) found increased mitotic abnormality with an increased doses of Carbicron, Dimecron and Vapona. Khan (1983) also observed same phenomenon due to treatment with different pesticides and insecticides. This might be due to the mutagenic or cytotoxic effect of pesticide.

The result also revealed that both pre-treatments and post-treatments with antioxidants reduced pesticide induced mitotic abnormalities. Aslanturk and Askin Celik (2005, 2006) observed that pre and post-treatment with lycopene reduced total chromosomal aberrations induced by ethyl methane sulfonate in *Allium cepa*. It is due to the function of antioxidant properties used in this experiment such as Vitamin C, Zinc, Copper and Lutein. Because antioxidants like vitamin C (ascorbic acid) has been proven to ameliorate the oxidative stress

and sperm toxicity induced by endosulfan in rats (Takhshid *et al.,* 2012). Vitamin C comprises 65% of the antioxidant capacity of semen and is the most critical seminal antioxidant (Agarwal and Sekhon, 2011). Ozawa *et al.* (2012) suggested that lutein inhibited oxidative stress-induced triggering of inflammatory signaling pathways such as the activated signal transducer and activator of transcription 3 (STAT3) signaling pathway and IL-6 expression in the retina. Xanthophyll carotenoids modulate oxidative stress and regulate redox-sensitive intracellular signaling (Kaulmann and Bohn, 2014). In addition to vitamin C and zinc, low doses of copper improve the yield since they avoid cellular oxidation by increasing the capacity of plants to resist oxidative stress caused by reactive oxygen species under stress conditions (Fatani *et al.*, 2015). Copper acts as catalytic centers for proteins in plant cell metabolism (Shimazu *et al.*, 2019) and regulates the activities of enzymes such as GPX, SOD, CAT, among others, which are important scavengers of reactive oxygen species since they form the first line of defense against oxidative stress (Bohm *et al.*, 2012).

Apart from that pre-treatments showed minimum mitotic abnormality than that of the post-treatments. Similarly Aslanturk and Askin Celik (2016) found decreased cytotoxicity and DNA damage in lymphocytes pre-treated with lycopene before treated with pesticides. This might be due to the role of antioxidant which may be preparing the cell to protect from destructive effect of ROS. Similarly Rao and Agarwal, (1998) found that lycopene has the ability to act as a powerful antioxidant and singlet oxygen quencher thus it can protect the destructive effect of the ROS.

5.1.2.2 Mitotic index (MI)

Mitotic index is an important parameter, reflects the frequency of cell division, so far its value being acknowledged as a measure of cytotoxicity for any living organism. High mitotic index value indicates that the cell division rate is high and low mitotic index value indicates relatively lower divisional rate. In this study it was observed that control value of mitotic index was lower than both of the pre and post treatments. It is because of the different control composition i.e. wheat seeds treated only with the pesticide. Mitotic index

was found to be the highest (14.92%) in case of control while the lowest value (8.80%) was observed in case of 4% Safathrin-10EC which was similar to 4% dose of Morter-48EC (Akhter *et al.,* 2015). Bonea and Bonciu (2017) found in their study, the fungicide (royal flo) reduced the mitotic index compared to the control. The result also revealed that antioxidant activity increased mitotic index (around 22% than control) suggesting that antioxidant minimized the effect of pesticides in both the cases of pre and post treatment. Similarly Taspinar *et al.* (2010) observed ca^{2+} and zn^{2+} ions to reduce the toxic effect of cd^{2+} in *Vicia faba* seedlings. Agar and Taspinar (2003) also found increased mitotic index through a simultaneous treatment of ca^{2+}, zn^{2+}, se^{4+} together with cd^{2+}. However, in this study pre-treatment showed better mitotic index than that of the post treatment.

5.1.2.3 Interphase chromosome volume (ICV)

Interphase chromosome volume (ICV) is a cytological parameter to know about chromosomal morphology. In the cell cycle interphase is an important stage in which all sorts of metabolic reactions take place and many metabolic products are accumulated. Thus, the interphase is sometimes termed as metabolic state of nucleus. At this condition the nuclear size generally increase and if this increased state is induced by any chemical or physical agent, aging of that cells may be caused rapidly and that precede cell deaths. Result obtained from this study revealed that among all the treatments highest value of ICV was observed in highest pesticide concentration of control. Razu *et al.* (2012) showed that with the increase of herbicides concentration there was an increase in interphase chromosome volume (ICV) in case of both the Topstar and Fielder than the control. This result showed resemblance with Akhter *et al.* (2009) who stated that interphase chromosome volume of hexaploid wheat was found to be increased in case of Ronstar with increasing the concentration of herbicides. Whereas minimum ICV was found in lowest concentration of pesticide in both pre and post treatments compared to control. So it is clear that the protective effect of antioxidant played a pivotal role against pesticide to decrease the ICV value. Thus, ICV values were minimum in both pre and post treatment than that of control values.

5.1.2.4 Meiotic abnormality

As mitotic abnormality, meiotic abnormality is also considered as one of the most dependable indexes to estimate the potency of mutagen. Different treatments of Wheat seeds showed various types of chromosomal aberrations such as fragment, bridge, laggard, stickiness etc. in this study. These types of aberrations were found more or less in both Meiosis I and Meiosis II.

The result of meiotic study indicated that percentage of abnormality was increased with an increased pesticide concentration in control. Similar results were observed by Wuu and Grant (1966) after seed treatment of barley plants with Monuron. Alam *et al.* (1980a, 1980b and 1981) also reported similar result after treating the wheat plants with insecticides Dimecron-100, Vapona-50, Carbicron-100 and chemical mutagens hydroxyl ammonium chloride, ethylene glycol and tri-ethanol amine. Zaman *et al.* (1978) reported that different concentrations of herbicide 'Ansar' produced cytological changes in barley plant. Meiotic abnormality was decreased in case of both pre and post treatment compared to that of control. It is because of anti-mutagenic activity of antioxidant against pesticide toxicity. A number of dietary antioxidants have been reported to decrease free radical attack on biomolecules (El-Habit *et al.* 2000). The result also indicates that pre-treatment with antioxidant helps maximum decreased meiotic abnormality compared to post-treatment as well as control. Aslanturk and Askin Celik (2016) observed that pre-treatment with lycopene protects the lymphocytes against cytotoxic and genotoxic effects induced by pesticides and pesticides mixture. Antioxidant properties of vitamin C used in this study has been found to help in preventing cell damage by neutralizing free radicals (Fraga *et al.*, 1991).

The protective effects of vitamin C on genotoxicity and cytotoxicity have also been confirmed in mice (Surjyo and Anisur, 2004). Another antioxidant property of the experiment is lutein, also can play an important role to minimizing meiotic abnormality, because, Alves-Rodrigues and Shao (2004) found that lutein can protects plants from photo-

induced free radical damage Lutein is an ocular antioxidant that can quench both singlet oxygen and lipid peroxy radicals (Bohm *et al.,* 2012). In other studies, it was shown that copper/ CuO-NPs increase CAT activity in tomato leaves (Mares-Perlman *et al.,* 2001). Furthermore, copper/ CuO-NPs increase the activity of antioxidant enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) (Beatty *et al.,* 2013) since these enzymes help to reduce oxidative stress (Bone *et al.,* 2003). Gaucin-Delgado *et al.* (2022) showed that CuO-NPs increased the amount of vitamin C in lettuce leaves which can lead to resist the oxidative stress.

5.1.2.5 Pollen sterility

Characteristics of both female and male reproductive organs and their interactions are closely related to setting of seeds. Therefore, ultrastructural characteristics change with pollen and anther development, and they are important in embryological development as well. Pollen sterility has been shown to be an important factor for reducing seed production because fertilization probability decreased considerably when sterile pollen grains were deposited on a stigma (Teng *et al.,* 2012; Wilcock and Neiland, 2002; Zhao *et al.,* 2004). Low pollen fertility was responsible for the poor seed yields in *Glycine max* (L.), *Nelumbo nucifera* and *Bambusa vulgaris* (Koshy and Jee, 2001; Teng *et al.,* 2012; Zhao *et al.,* 2004). In addition, pollen sterility has found to prove as a major factor leading to the endangered status of *Opisthopappus taihangensis* (Li *et al.,* 2009) and *Pancratium maritimum* L. (Konyar, 2016). Ripe yellow anthers of all the treatments along with control were collected to estimate the percentage of pollen sterility. In the present findings percentage of sterile pollen was high in control compared to pre and post treatments. It is due to the toxic effect of pesticide. In case of control pollen sterility increased with an increased of the pesticide concentration. Ignacimuthu and Babu (1989) found increased dose dependent increase in pollen sterility in wild and cultivated Urd and Mung beans using Ethylmethane sulphonate (EMS) and gamma rays. Mutagen induced pollen sterility has been reported by several workers (Kalloo, 1972; Kalloo and Das, 1973; Sinha and Godward, 1972; Nerkar, 1977).

Similar result was observed by Alam and Kabir (1983) due to the treatment with insecticide on barley. Pre-treatment showed low pollen sterility than post treatment and control. In case of pre-treatment low pollen sterility was observed both low and high concentration of pesticide.

5.1.2.6 Pollen abnormality

Pollen grain abnormality occurs both in angiosperm and gymnosperm plant. Plant response to either biotic or abiotic stresses cause abnormal pollen grain. The normal pollen grains in wheat are typically characterized by peg shaped bi-nucleate. The abnormal pollen grains were round shaped mono-nucleate, bi-nucleate, tri-nucleate and tetra-nucleate. However this study revealed that only pesticide treatment in control induced increased pollen grain abnormality with an increased concentration of pesticide on wheat seeds. Kabir (1981) found increased pollen grain abnormality using pesticide treatment (Carbicron-100 EC and Vapona-50) on barley (*Hordium vulgure* L.). Findings showed round shaped mono-nucleate, bi-nucleate and tri-nucleate types of abnormal pollen grains. Hamdi and Solaiman (1978) reported similar type of pollen grain abnormality in six cultivar of hexaploid wheat. Mean values of percentage of pollen abnormality of this study exhibiting that antioxidants treatment mitigated pollen abnormality in pre and post treatments. Anti-mutagenic effect of antioxidant was more appreciable in pre-treatment than that of post-treatment.

5.2 Study of M2 generation

Seed is a critically important basic input of agriculture, because sowing healthy seeds is essential for healthy production. But seed borne pathogens, soil borne pathogenic organisms and storage insects can reduce yield quantity and quality of the crops produced. That is why, seed treatment is an important measure to protect plant seedlings from pathogen and insects attacks at emergence and at the early growth stages, contributing to healthy crop plants and good yield. However, seed treatment refers to the application of fungicide, insecticide or a combination of both, to seeds disinfect from pathogens or insects. Literature

revealed that use of insecticides, pesticides or herbicides has many adverse effects to plant and environment. Thus, practice has been developed to use some beneficial chemicals/metal (for example-Ca, Se, Zn, etc.) combine with insecticides, pesticides or herbicides for seed treatments. In this study seeds of control treated with only pesticide, seeds of pre-treatment treated with antioxidants before pesticide, seeds of post-treatment treated seeds with antioxidant after pesticide in M1 generation and seeds from M1 were used in M2 generation without any further treatment. The study was aimed to investigate pesticide and antioxidant's combined effect in M1 generation and combined residual effect in M2 generation.

5.2.1 Morphological features

To see the residual effect of antioxidant and pesticide wheat seeds were allowed to germinate both in laboratory and in pot. Then morphological and cytological data were collected on same parameters following the previous methods as it was for M1 generation.

The morphological study of M2 generation indicates that all the morphological character like germination percentage, plant height at different DAG (in pot and in lab), root length at different DAG (in lab), coleoptile length, flag leaf length , number of tiller/plant, number of spikelet/spike, number of seeds/spike, length of inflorescence (with awn), length of inflorescence (without awn), 100 seed weight, total seed weight showed lowest value in control with exception of flag leaf length of post treatment than that of pre and post treatment.

Almost every morphological character revealed maximum value in M1 generation than that of M2. But plant height at 7 and 14 DAG in laboratory only post treatment of M2 generation showed higher value than that of M1. Similarly germination percentage in pot, post-treatment of M2 generation showed higher value than that of M1 and pre-treatment of M2 generation showed similar value with M1.

5.2.2 Cytological features

Cytological studies were conducted to observe the residual effect of antioxidant and pesticide. Result revealed that control showed minimum value for mitotic index whereas pre- and post-treatment showed maximum value. For mitotic index in M1 generation showed higher value than that of M2. The results for ICV revealed highest value at control in both cases of M1 and M2 generation but pre and post treatment showed lower ICV value in M1 than that of M2.

Various type of mitotic and meiotic abnormality was observed in M2 generation namely fragments, lagging chromosome, bridges, ring chromosome. Results for mitotic and meiotic abnormality in M1 and M2 generation indicate that control showed highest value than that of pre and post treatment. M1 generation showed lower abnormalities compared to M2. In case of pollen abnormality control showed maximum value than that of pre and post treatment for both of M1 and M2 generation. But pre-treatment of M2 showed maximum value than that of M1 and post-treatment of M1 showed maximum value than that of M2. So it is clear that pre and post-treatments of M1 and M2 generation did not show any linear relation with each other.

Maximum pollen sterility was observed at control than that of pre and post treatments for both M1 and M2 generation. M1generation showed higher value for pollen sterility than that of M2.

From the above mentioned characteristics it is clear that control showed minimum value for all the morphological and cytological parameters except ICV, mitotic, meiotic and pollen abnormality and pollen sterility. It is because control was treated only with pesticide. Thus ICV, mitotic and meiotic abnormality were increased in M1 and M2 generation compared to antioxidant treatment because of pesticide toxicity. Different pesticides have harmful effect on different plants (Rank *et al.,* 2002; Ruiz *et al.,* 2003). Due to the antioxidant, pre and post

treatment showed different result than that of control for both M1 and M2 generation. Between M1and M2 generations, M1 showed expected result of antioxidant than that of M2. Because antioxidant played a pivotal role in M1 to mitigate the pesticide toxicity. Supplementation with exogenous antioxidants or boosting of endogenous antioxidant defenses of the body has been found to be a promising method of countering the undesirable effects of oxidative stress (Kasote *et al.,* 2013). In absence of antioxidant and pesticide, result of M2 generation varied slightly from M1. The frequencies of chromosomal abnormalities were substantially reduced in M2 generation as compared to M1 at all dose treatments for all the three species of Bean and the frequencies of micronuclei and laggards were not markedly different between M1 and M2 generations (Ignacimuthu and Babu, 1989). The percentage of meiotic cells with chromosome irregularities observed for most treatments in the Cl generation was higher than that found in the C2 generation and there was little correlation between the percentage of cells found with chromosome abnormalities for treatments in the C1 generation and with those found in the C2 generation (Wuu, and Grant, 1967).

Suggesting that, pesticide has some sort of residual effect in M2 generation. Kabir and Alam, (1986) found that chromosomal abnormalities induced in M1 was carried over in M2 generation. It is now evident that the use of these insecticides has many secondary consequences (Wuu and Grant 1966, Amer 1965, Amer and Ali 1968, 1969). The variation in chromosomal aberrations was found from generation to generation, from treatment to treatment, from plant to plant, and from floret to floret. Unrau (1953) noticed variation within spike and between plants after spraying barley plants with 2, 4-D. Kabir and Alam (1986) also reported that radiomimetic effect of insecticides observed in M1 and M2 barley plants and it appears that their effect may continue up to some generations. This research reveals that M2 generation showed more morphological and cytological abnormalities than that of M1 generation but not more than control. Since these values are minimum than that

of control indicating antioxidants also have residual effect in M2 generation. According to the literature due to the pesticide residual effect and absent of antioxidant supplementation, M2 could have showed similar abnormalities as control. Present investigation showed that M2 generation produced less abnormalities compared to control, suggesting that antioxidants also have some sort of residual effect in M2 generation.

CHAPTER-VI

SUMMARY

6. SUMMARY

The objective of this investigation was to see the effects of antioxidants on pesticide induced cytomorphological consequences in wheat (L). This study was comprised of M1and M2 generation following two different treatments pre- and post-treatment .Three antioxidants and one pesticide was used in this study following three different concentrations and duration of time.

Morphological characters such as germination percentage, coleoptile length, root length at different DAG (in lab), plant height at different DAG (in pot and in lab), flag leaf length, number of tiller/plant, number of spikelet/spike, number of seeds/spike, length of inflorescence (with awn), length of inflorescence (without awn), 100 seed weight and total seed weight were considered for this investigation. Most of the morphological characters significantly showed decreased value in control both M1 and M2 generation. Among all the treatments both in case of M1 and M2 generation pre-treatment showed increased morphological value compared to control and post treatments. Between M1and M2, M1 showed increased morphological value.

The mitotic index (MI) is a cytogenetic test which is a reliable predictor of cell proliferation in the tissue that is used to characterize proliferating cells and to identify the compounds that inhibit mitotic progression resulting in a decrease in the MI of that population. In the present study, cytological observation revealed that the pesticides reduced mitotic index in root tip cells of wheat. MI decreased with an increased pesticide concentration. Pre-treatment showed high value for MI compared to post-treatment and control in case of both M1 and M2 generation. MI showed higher M1 than that of M2 generation.

Control showed high value for ICV in case of both pre- and post- treatment of M1 and M2. This increase of ICV, in general, might be due to alteration of cell membrane configurations, modification of chromosomal protein and changes in sensitivity of chemicals. Antioxidant may be play a role to decreased ICV value of pre- and post-treatment in case of both M1 and M2 generation. ICV of M1 was higher than M2 in case of both pre- and post-treatment.

Various types of mitotic abnormality were produced by pesticide. High concentration of pesticide creates high value for mitotic abnormality in control for both M1and M2 generations. Mitotic abnormality was decreased in both pre- and post-treatment due to the pivotal role of antioxidants. Between M1 and M2, M2 showed increased mitotic abnormality in absence of antioxidant.

Meiotic abnormality also produced the same result like mitotic abnormality. Pre- and post-treatment showed decreased meiotic abnormality than control in both M1 and M2.

Pollen sterility and pollen abnormality decreased in pre- and post-treatment compared to control in both M1 and M2 generation it may be antioxidants mitigate the toxic effect of pesticide. Pre-treatment showed maximum pollen sterility and pollen abnormality.

From the above it is evident that antioxidant can reduce the mutagenic efficiency of pesticide significantly. As literature revealed that certain chemicals properties possess that directly or indirectly or immediately reduce or eliminate the mutagenic activity of other chemicals.

CHAPTER-VII

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7. REFERENCES

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APPENDIX

PLANT ENVIRONMENT DEVELOPMENT
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ACCEPTANCE LETTER

Dear **Golam Kabir and co-authors**

I am pleased to inform you that your paper entitled, "**Effect of antioxidant on pesticide induced morphological consequences in hexaploid wheat (*Triticum aestivum* L.)**" by Afroja Akter, Abu Bakkar Shiddiq Shah, Md. Mamunur Rashid Sarkar, M. Saifur Rahman, Mosleh Uddin, Rezaul Karim and Golam Kabir* has been accepted as an article for publication in the journal of Plant Environment Development.

Your article will be published in Volume 9, Issue 2 (December 2023).

Sincerely yours

Prof. Dr. Mohammad Shahidul Alam
Editor-in-Chief

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Author(s) Name: Afroja Akter, Abu Bakkar Shiddiq Shah, Md. Mamunur Rashid Sarkar, Md. Saifur Rahman, Mosleh Uddin, Rezaul Karim and Golam Kabir*

Prof. Sultanul Alam Cytogenetics Laboratory, Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh.

Paper Title: **Effect of antioxidant on pesticide induced cytological consequences in hexaploid wheat (*Triticum aestivum* L.)**

Dear Prof. Dr. G. Kabir,

We are pleased to inform you that your paper entitled "**Effect of antioxidant on pesticide induced cytological consequences in hexaploid wheat (*Triticum aestivum* L.)**" has been accepted for publication in the forthcoming issue (Volume 18, 2023) of the Journal of Life & Earth Science.

Thank you very much indeed for your contribution to the Journal.

17. 01. 24

(Professor Dr. M. Golam Mortuza)
Chief Editor

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