FOLIAR NAPTHALENE ACETIC ACID MEDIATED BIOMASS, GROWTH AND YIELD REGULATIONS IN WHEAT (*Triticum aestivum* L.) UNDER CADMIUM STRESS

Running Title: Exogenously applied napthalene acetic acid under cadmium stress in wheat

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Abstract

Cadmium (Cd) is considered most toxic heavy metal because it is highly soluble in the water. Naphthalene acetic acid (NAA) is growth regulator from auxins family and having positive role under cadmium stress. A pot experiment was held to minimize the effect of heavy metal cadmium on wheat growth by using naphthalene acetic acid as foliar spray. The trial was conducted in the glass house, Faculty of Agriculture, University of Agriculture Faisalabad. The experimental design was completely randomized (CRD) and the replications were four. This experiment was consisted of two factors. Cadmium concentrations were 0, 50 and 75 ppm. Growth regulator naphthalene acetic acid was used at 0, 25, 50 and 75 ppm as foliar spray. Response of wheat to applied Cd and NAA was observed by studying various growth, biomass and yield parameters. Data was collected and analyzed using suitable statistical techniques. Growth, biomass and yield were positively affected by naphthalene acetic acid. Foliar applied naphthalene acetic acid in overcoming the negative effect of cadmium stress on growth, biomass and yield of wheat. The level of 50 ppm applied naphthalene acetic acid proved best in this regard.

Key words: Heavy metal, Stress, Growth regulators, Naphthalene acetic acid, Cadmium, Wheat

1. Introduction

Wheat is one of the world's largest growing grains. Wheat chips account for 21% of global eatables and are farmed on a 221 million-hectare global scale (Tao *et al*., 2015). Food security in Pakistan is linked to wheat production and consumption. Heavy metal toxicity in the rhizosphere is a potential health risk to the world's growing population and causes major concern among environmentalists worldwide (Sabir *et al*., 2020). Cadmium (Cd) is commonly emitted from businesses as a result of numerous anthropogenic activities, excessive fertilizer use (particularly phosphate) in agricultural soils, natural weathering of rocks and minerals (Xing *et al*., 2022). Wheat (*Triticum aestivum* L.) is known to absorb more Cd than other cereal crops (Zhou *et al*., 2021). In developing nations, more than 80% of wheat supply is used as a staple grain, while wealthy countries use less than half (Awika, 2011). Previous research discovered that Cd in wheat is absorbed from the roots and subsequently translocated to the aerial regions, interfering with main metabolic processes, preventing normal growth and reproduction activities (Abedi and Mojiri, 2020). Photosynthetic processes are the primary drivers of plant growth and development (Maishanu *et al*., 2017). An elevated level of Cd in the rhizosphere caused severe phytotoxic effects in plant tissues (Riaz *et al*., 2021b), which replicated themselves by interfering with plant morphological, physiological and biochemical processes (Wali *et al*., 2015), such as destroying the leaf chlorophyll structure to deteriorate photosynthetic and gas exchange relations (Malik *et al*., 2021). The photosynthetic system is extremely sensitive to environmental changes. Any environmental limits may directly harm the structural and functional capacities of the photosynthetic machinery, thereby disrupting plant growth and survival (Saleem *et al*., 2020). Under cadmium stress, growth regulators perform a variety of beneficial functions. Naphthalene acetic acid (NAA) is an auxin-based development controller. Naphthalene acetic acid has been commercialized in several countries to boost field crop productivity and improve the value of horticulture crops (Jahan and Fattah, 1991). The use of growth regulators is considered one method of improving yield. Naphthalene acetic acid, a synthetic growth regulator, has demonstrated that when used at the optimum concentration, it impacts the growth and yield of a variety of wheat plants. Water take-up is significant for cell prolongation, separation of vascular tissues, setting of foods grown from the ground (Alabadíet *et al*., 2009). Naphthalene acetic acid also has an important role in root acceptance and is used to propagate plants by leaf and stem cuttings (Dimitrios *et al*., 2008). The direct impact of naphthalene acetic acid on natural product formation has also been studied (Agusti *et al*., 1994). Naphthalene acetic acid works with other plant hormones, such as brassinosteriods, to reduce the pressure of heavy metals and other loads (Halliday, 2004). Exogenous usage of naturally occurring or created PGRs affects a plant's endogenous hormonal system by either increasing problematic levels or interfering with the combination, movement, or movement inactivation of previously existing hormone levels (Adam and Jahan, 2011). The combination of naphthalene acetic acid and brassinosteriods increased seedling hypocotyl length in *Arabidopsis thaliana* (Tanaka *et al*., 2003). Naphthalene acetic acid is used to promote growth and yield in a variety of grain crops. The objective of this study is to minimize the cadmium stress on wheat by foliar application of naphthalene acetic acid.

2. Materials and Methods

The experiment was conducted to study the role of naphthalene acetic acid on wheat under cadmium stress conditions.

2.1. Plant material:

Seed of variety Anaj-2017 was collected from Wheat Research Institute, AARI, Faisalabad for this study.

2.2. Experimental site:

The experiment was conducted at glass house, Faculty of Agriculture, University of Agriculture Faisalabad.

2.3. Physiochemical traits of experimental site:

Soil samples were randomly collected before sowing and after harvesting from the collected soil. Analyzed the samples to quantify different physiochemical attributes (ICARDA, 2013). Soil analysis was carried out in Environmental Sciences lab, Institute of soil and environmental science, UAF (Table 1).

2.4. Weather elements:

Weather data were collected from metrological observatory cell, Department of Crop Physiology, University of Agriculture; Faisalabad situated at latitude 31° north, longitude 73° east and at altitude of 184.4 meter are presented graphically (Table 2). The experimental site was semiarid with annual mean rain fall of 375 mm.

2.5. Treatments:

The experiment was comprised of two variables a) soil applied cadmium (control, 50, 75 ppm) and b) four levels of naphthalene acetic acid (control, 25, 50, 75 ppm).

2.6. Experimental design:

The experiment was laid out in completely randomized design (CRD) with factorial arrangement having four replications.

2.7. Imposition of treatments:

Cadmium chloride was mix with soil during the time of pot filling with soil and naphthalene acetic acid was foliar spray at wheat crop.

2.8. Statistical analysis:

The recorded data was analyzed by Fisher's analysis of variance (ANOVA) technique. All treatments mean was compared by honestly significant difference (HSD) test at 1% level of probability.

2.9. Agronomic practices:

The experiment was conducted in pots. The size of pot was 30 cm length and 20 cm height. Crop was sown with hands at recommended rate was 12 seeds per pot. Nitrogen and phosphorus were applied at rate of 150-100 kg ha^{-1} to all treatments. Whole of phosphorus and half of nitrogen were applied at sowing while remaining nitrogen was applied at first irrigation. $CdCl_2$ was mixed in the soil (as per treatment) before filling the pot. Crop was irrigated per requirement of the crop. All other agronomic practices were kept normal and uniform for all the treatments.

2.10. Observations recorded:

Shoot length of primary tillers, selected randomly from each pot was measured from base to head, root length from each pot was measured from soil surface to the final growing point and spike length were measured randomly with the help of a meter rod and then average were calculated. Number of spikes per plant, number of spikelet per spike, grains per spike, number of productive tillers per plant and 100-grain weight were counted by randomly selected three plants from each pot and means were calculated. Plant height of selected plants were recorded with the help of meter rod. Plants of each pots were harvested, thrashed manually and sundry for grain yield per plant.

Characteristics	**Unit**	**Value**	
Time of sampling		Before sowing	After harvesting
Texture	-	Loam	
pH	-	8.00	8.00
Sand	%	33.78	33.78
Silt	%	34.12	34.12
Clay	%	32.23	32.23
EC	dSm^{-1}	1.05	0.96
Organic matter	%	0.35	0.77
Nitrogen (N)	%	0.058	0.053
Phosphorus (P)	ppm	3.2	2.9
Potassium (K)	ppm	180	180

Table. 1: Chemical analysis of soil before sowing of crop

Weather elements	November	December	January	February	March	April	May
Average temperature (°C)	18.9	12.2	11.7	16.5	19.1	27.0	31.8
Relative humidity (%)	61.7	75.0	75.3	66.0	64.0	43.9	27.5
Rainfall (mm)	10.0	0.0	12.2	20.5	67.9	32.8	17.0
Pan evaporation (mm)	1.8	1.5	1.0	2.1	13.0	5.3	7.6
Sunshine duration (hours)	7.6	4.7	5.0	5.6	4.9	9.1	10.4
Evapotranspiration (mm)	1.5	1.3	0.7	1.8	2.8	3.7	5.3
Wind speed (km h^{-1})	3.1	2.0	3.6	5.3	5.6	6.2	5.7

Table 2: Weather conditions during cotton growing period

3. Results:

3.1. Biomass accumulating traits:

Shoot length is important morphological trait of combined effects of genetic makeup of plant, environmental conditions and nutrient status of soil in which plant is grown. Root length is the part of a plant that connects it to the ground or support, usually underground, transporting water and nutrients to the rest of the plant. Data regarding shoot length, root length, plant height of wheat as affected by cadmium stress and foliar application of naphthalene acetic acid (Figure 1). It showed that different naphthalene acetic acid levels and cadmium stress levels had significant effects on shoot, root length and plant height of wheat. As regards cadmium stress maximum shoot (6.614 cm), root (8.739 cm) length and plant height (106.05 cm) were observed with no cadmium stress (control). While minimum shoot, root length and plant height were observed in cadmium stress with (75 ppm). Among different levels of naphthalene acetic acid maximum shoot length (6.449 cm), root length (8.449 cm) and plant height (101.94 cm) were observed where naphthalene acetic acid was applied at rate of (50 ppm) followed by application of (75 ppm) naphthalene acetic acid. While minimum shoot, root length and plant height were observed in control. Interaction of naphthalene acetic acid with cadmium stress did not significantly affected the shoot, root length and plant height of wheat plant (Figure 1).

3.2. Yield components and grain yield:

A tiller is a stem made by grass plants and refers to the shoots which develop from a seed after the initial shoot. Tillers are segmented and have their own two-part leaf in each segment. They lead to the plant distribution and also to the production of seeds. Interaction of naphthalene acetic acid with cadmium stress was non-significant for number of productive tillers, grains per spike, 100-grain weight and grain yield per plant in wheat. Number of productive tillers, grains per spike, 100-grain weight and grain yield per plant were significantly affected by cadmium stress and napthalene acetic acid improves these traits. The maximum number of productive tillers (4.24), grains per spike (42.08), 100-grain weight (4.48 g) and grain yield per plant (3.78 g) with control (no cadmium stress) were observed with respect to cadmium stress levels. While minimum number of productive tillers, grains per spike, 100-seed weight and grain yield per plant were recorded with (75 ppm) of cadmium stress. Maximum number of productive tillers (3.85), grains per spike (38.11), 100-grain weight (4.23 g) and grain yield per plant were observed (3.40 g) where naphthalene acetic acid was applied at a rate of (50 ppm) followed by the application of (75 ppm) naphthalene acetic acid (Figure 2, 3).

3.3. Growth of spike

An ear is the grain-bearing tip component of a cereal plant's stem, for either wheat or maize. It might also refer to "a prominent lobe in some leaves." The ear is a spike, consisting of a central stem on which flowers develop in tightly packed rows. Number of spikes per plant and spikelet per spike of wheat as affected by cadmium stress and foliar application of naphthalene acetic acid. It showed that different levels of naphthalene acetic acid and cadmium stress had major effects on no of spikes per plant and spikelet per spike. The maximum number of spikes per plant (3.2) and spikelet per spike (13.85) with no cadmium stress (control) were counted with respect to cadmium stress. While minimum number spikes per plant and spikelet per spike were counted with (75 ppm) of cadmium stress. Maximum numbers of spikes per plant was observed among different rates of naphthalene acetic acid (3.1) and spikelet per spike (13.43) where naphthalene acetic acid was applied at a rate of (50 ppm) closely followed by the application of (75 ppm) naphthalene acetic acid (Figure 3, 4). While minimal number of spikes per plant and spikelet per spike were observed in pots where no spray of naphthalene acetic acid was done. Interaction of naphthalene acetic acid with cadmium stress did not significantly affected both spike growth traits.

4. Discussion:

Minimizing cadmium (Cd) uptake in wheat plants is critical for reducing Cd toxicity and, ultimately, ensuring food safety (Wu *et al*., 2020). Its growth qualities address crop health and vigour. The negative effects of several heavy metals, including Cd, may be related to the modification of the plant's morpho-physiological and biochemical pathways, which indirectly influence plant growth (Turan, 2020). It is widely recognized that heavy metal contamination can reduce plant development and physiological properties (Kamran *et al*., 2019a). Previous research has shown that cadmium stress reduces wheat development and shoot length, resulting in lower grain yield (Hussain *et al*., 2019). Current findings demonstrate that naphthalene acetic acid at 50 ppm improves crop development and yield under heavy metal stress conditions (Godha *et al*., 2020). Cadmium stress reduces wheat development and root length, ultimately lowering yield (He *et al*., 2019). The current study's findings are congruent with those of Alam *et al*. (2020), who discovered that naphthalene acetic acid had favorable effects on root growth and yield under heavy metal stress conditions in some crop species. Cadmium accumulation in shoot and root tissues increased with increasing Cd levels, as previously observed (Bashir *et al*., 2020). However, Cd buildup was higher in roots than in shoots. Similar results were obtained in our prior tests (Fozia *et al*., 2021). Plant height was defined as the vertical distance between the base of the root and the plant's tallest point. Cadmium stress reduced plant height, however applying naphthalene acetic acid (NAA) increased chlorophyll content, plant height and flag leaf area. Cadmium stress reduces wheat growth and the number of productive tillers per plant, resulting in a lower yield (Shafi *et al*., 2009). The plant growth regulator NAA considerably increases the number of productive wheat tillers (Lue *et al*., 2011). Cadmium, a heavy metal, affects wheat development and the number of grains per spike, as well as the 1000 grain weight, reducing grain production. Under heavy metal stress circumstances, naphthalene acetic acid treatment has the ability to boost wheat output and yield-contributing characteristics (Rizvi *et al*., 2011). According to Saleh *et al*. (2020), cadmium stress affects wheat development, the number of spikes per plant, and the number of spikelets per spike, all of which reduce output. Our findings are consistent with those of Yue *et al*. (2017), who found that naphthalene acetic acid can increase yield in specific crop species under heavy metal stress circumstances. Antioxidant enzymes and other self-defense systems play a significant role in protecting crop plants from oxidative damage produced by abiotic stressors (Kamran *et al*., 2021). It has been proposed that increased Cd contamination can cause increased levels of oxidative damage (H_2O_2), ionic leakage and lipid peroxidation, resulting in an increase in the activities of non-enzymatic antioxidants(Alharby *et al*., 2021). The use of NAA, which is physiologically active and low molecular weight osmolytes serve as defensive chemicals to prevent protein decay and cell structure breaking while not interfering with the plant's normal metabolic processes (Dumanovic *et al*., 2021).

5. Conclusion

In this study Cd caused a significant reduction in biomass accumulating, yield related traits and growth of spike. Based on results acquired during this study, it tends to be presumed that naphthalene acetic acid reacted well under cadmium stress conditions. Doses differ in their belongings however over all application of naphthalene acetic acid at 50 ppm showed most extreme enhancements in all development and yield parameters of wheat.

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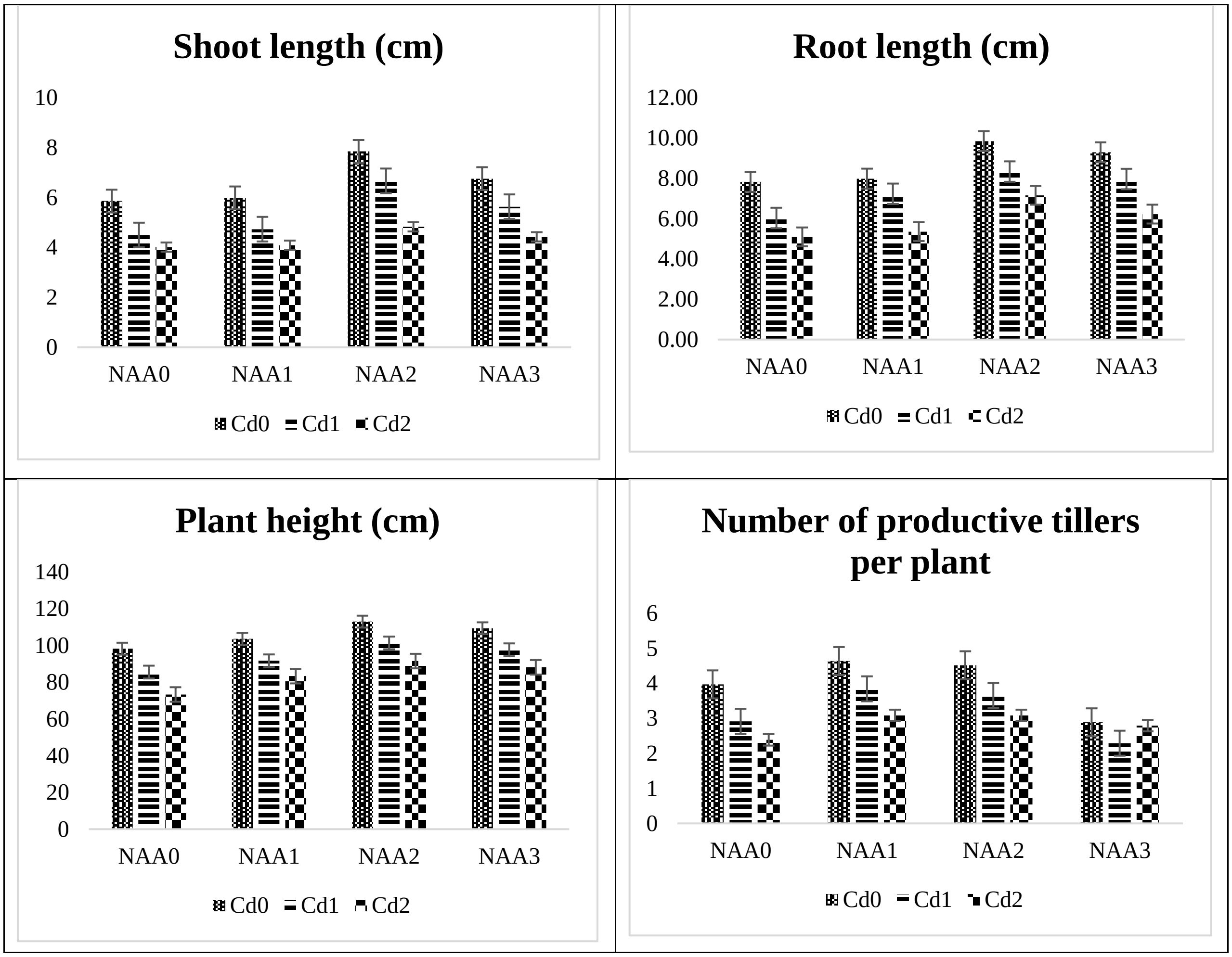

Figure 1: Role of naphthalene acetic acid on biomass accumulation and number of productive tillers of wheat under cadmium stress conditions

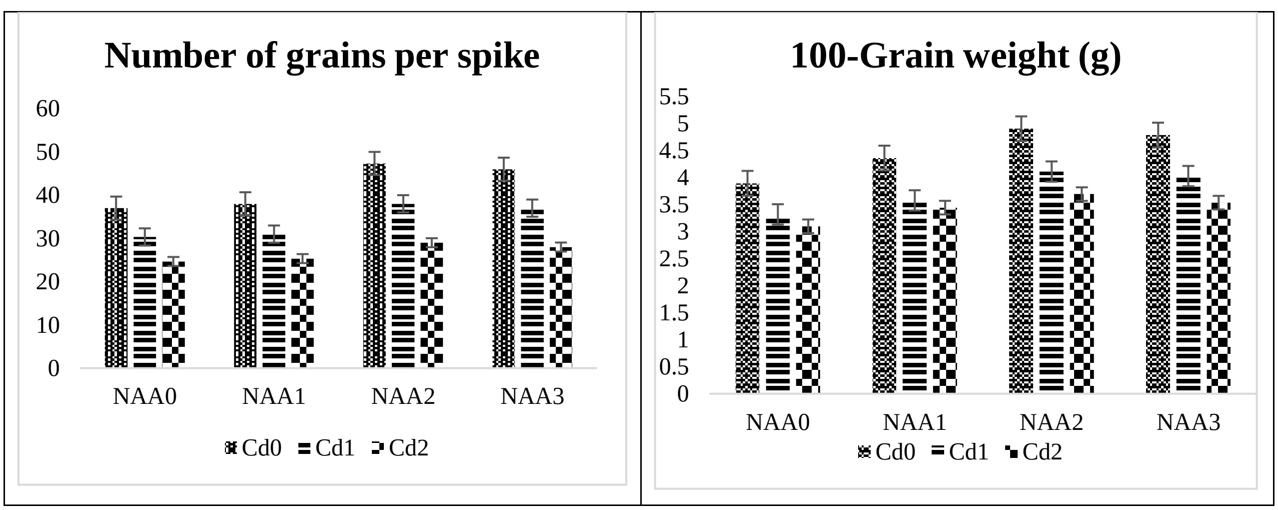

Figure 2: Role of naphthalene acetic acid on number of grains per spike and 100-grain weight of wheat under cadmium stress conditions

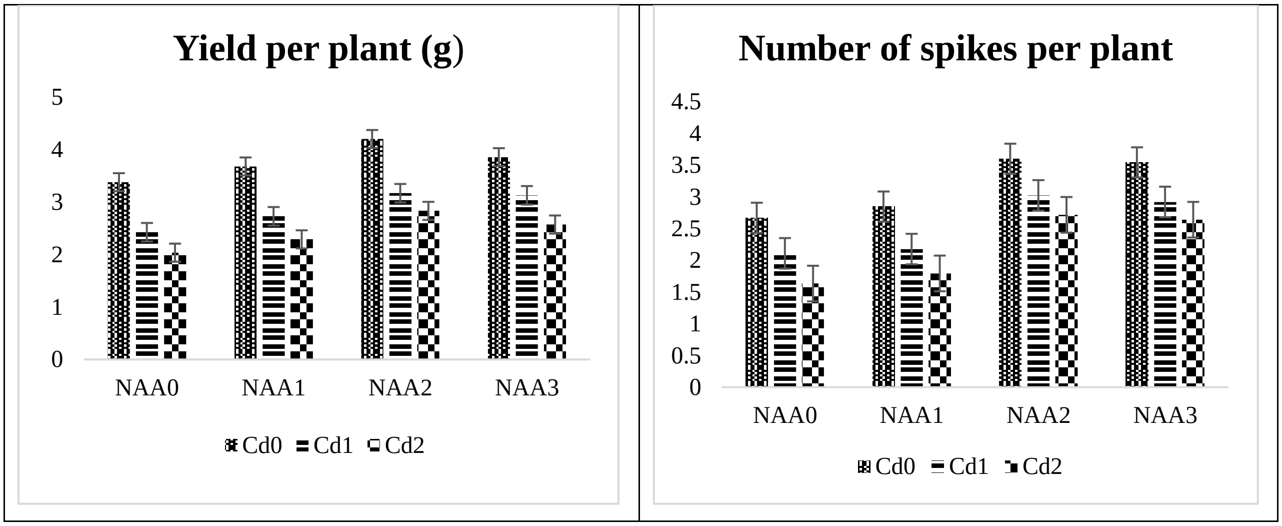

Figure 3: Role of naphthalene acetic acid on yield per plant and number of spikes per plant of wheat under cadmium stress conditions

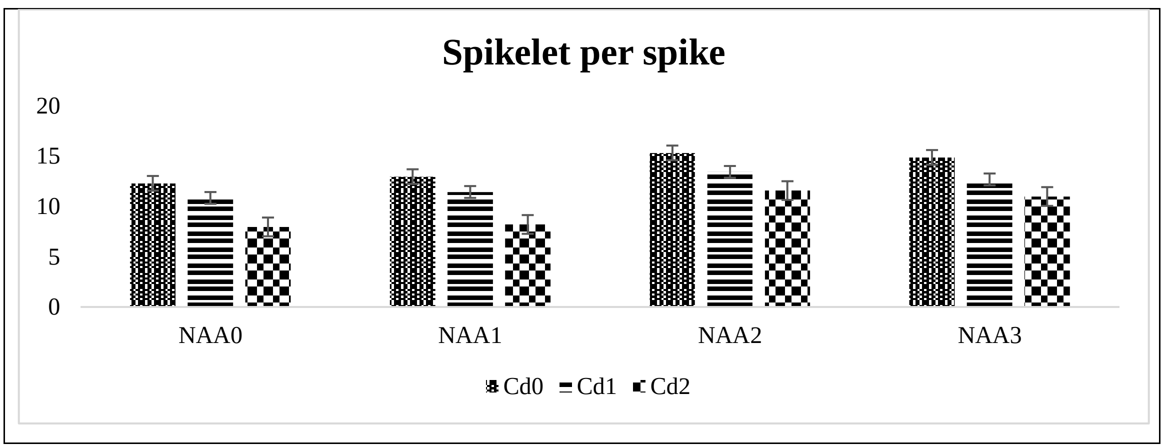

Figure 4: Role of naphthalene acetic acid on spikelet per spike of wheat under cadmium stress conditions