VOLUME 5, ISSUE 1, 2020

TABLE OF CONTENTS

VOLUME 5, ISSUE 1, 2020

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Research Manuscript www.sciplatform.com

Effect of different priming treatments on seed germination of sago palm (*Cycas revoluta* L.)

Zia Ullah *, Imran Hassan, Ishfaq Ahmed Hafiz, Nadeem Akhtar Abbasi
Department of Horticulture PMAS, University of Arid Agriculture Rawalpindi, Pakistan

Authors' Contribution	**Z. Ullah**, conducted the survey; **I. Hassan** helped in green house experiment; **I.A. Hafiz** and **N.A. Abbasi** performed the statistical analysis and paper writing
Article History	**Corresponding email address**: dr.hamid@bzu.edu.pk **Received: 03 September 2019, Revised: 17 October 2019, Accepted: 06 December 2019, Published Online: 09 December 2019**

ABSTRACT

King sago palm or sago cycas are the other name of Kangi palm (*Cycas revoluta*) sago palm has been used as an indoor and outdoor landscape plant for centuries. The present study was conducted to estimate the effect of different priming treatments on seed germination of sago palm (*Cycas revoluta* L.) in the research area of Department of Horticulture PMAS, University of Arid Agriculture Rawalpindi, Pakistan. The Experiment consisted of ten treatments; the seeds without pulp were soaked in solution of 500, 750 and 1000 ppm GA_3 and 2%, 3% and 4% solution of KNO_3 for 24 hr at room temperature. In case of hot water treatment, seeds were primed at 80°C, 90°C and 100°C for 30, 20 and 10 minutes respectively. The effect of different concentrations of gibberellic acid (GA_3), potassium nitrate (KNO_3) and hot water on various parameters like germination rate, germination percentage, germination value, decayed seed percentage, time of germination, number of leaves and seedling height were studied. Significant results of germination rate (55.56 days), germination value (192.19) were achieved from 500 ppm GA_3. Maximum germination percentage (73.33%) and number of leaves (2) were observed in KNO_3 at 2% followed by 500 ppm GA_3. Similarly lowest decayed seed percentage (26.66%) and time of germination (59.41 days) were noted in 2% KNO_3. The seedling height was optimum (19.33 cm) in 3% KNO_3 followed by 2% KNO_3. Best germination results were obtained due to permeability of hard seed coat made by low concentrations of priming treatments (KNO_3 @ 3%).

Key word: *Cycas revoluta*, gibberellic acid, potassium nitrate, germination parameters.

INTRODUCTION: The sago palm *(Cycas revoluta* L.*)* is one of the important cycad commonly known as Kanghi palm or Japanese sago or simply sago palm. The cold hardy sago palm has been used as an indoor and outdoor landscape plant for centuries. It is used as a significant or focal point in any landscape design. Despite its importance in ornamental industry, it is facing certain problems regarding its germination due to its hard seed coat. It has been estimated that over 25% of all palm species require over 100 days for germination and they have less than 20% total germination (Tomlinson, 1990). So, there is a serious need of consideration to sort out this major issue. The reasons for this remain obscure, as little research work has been accomplished on seed dormancy in palms. Certain mechanical and chemical scarification, pretreatments were proved to be effective in germination of the hard-seeded species of *Cycas* and some other species (Frett, 1987; Chauhan *et al.*, 2009; Rouhi *et al.*, 2010). Cycad seeds respond to various pretreatment, including scarification, depulping and exposure to some chemical materials like gibberellic acid (GA_3), potassium nitrate (KNO_3) and soaking in hot water for specific period of time.

The overall development of plant is regulated by the growth hormones, nutrient and environmental factors. They also vary in their germination requirement (Chauhan *et al.*, 2009). KNO_3 is most widely used chemical for promoting germination. Solutions of 0.1 to 0.2% KNO_3 are common in routine germination testing and are recommended by the Association of Official Seed Analysts and the International Seed Testing Association for germination tests of many species (Copeland and McDonald, 1995).

OBJECTIVES: The objectives of the present research was to minimize the time period of seed germination and to enhance percentage of germination by breaking the external dormancy through different levels of chemicals including GA_3, KNO_3 and hot water.

MATERIALS AND METHODS: This study was conducted in the research field of Department of Horticulture PMAS, Arid Agriculture University Rawalpindi. An experiment was conducted by using Completely Randomized Design (CRD).The seeds of sago palm were collected from 10-15 years old female stocks growing at a commercial garden located in suburb of Islamabad city. Uniform, equal and the same weight and healthy seeds were selected. The seeds had diameter 2.54 to 5.08 cm. Seeds were soaked in fresh water for two weeks to remove pulp from the upper surface of hard seed coat. Seeds without pulp were soaked in solution of 500, 750 and 1000 ppm GA_3 and 2%, 3% and 4% solution of KNO_3 for 24 hr at room temperature. In case of hot water treatment, seeds were primed at 80°C, 90°C and 100°C for 30, 20 and 10 minutes respectively (Table 1). Then seeds were washed with few drops of tween twenty in order to remove surface tension. Seeds were dried at 24°C room temperature.

After sterilization, 10 seeds of sago palm were planted in each pot of 14 inch diameter containing sterilized soil media (Sand, soil, FYM 1:1:1) at 4-8 cm depth and incubated in a greenhouse at daytime temperature of 25±2°C and relative humidity of 60-80% and watered weekly depending on weather conditions. Germination was evaluated at the end of 10 months. Seed emergence was recorded as germination index. The data for germination rate (days), germination percentage (%), germination value, seed decayed percentage (%) and time of germination (days) were recorded during the course of study. After seed germination, observations were recorded for number of leaves an d seedling height. The data collected was compiled and analyzed statistically by using computer software germination; observations were recorded for number of leaves

(Steel and Torrie, 1980).

RESULTS AND DISCUSSION: Analysis of data showed that parameters related to germination significantly affected by

Table 1: Different priming treatments to enhance seed germination of Sago palm.

Treatment	Chemical	Concentration	Time
T_o	(Control/ untreated)	Tab water	24 hrs
T_1	GA_3 (Gibberellic acid)	500 ppm	24 hrs
T_2	GA_3 (Gibberellic acid)	750 ppm	24 hrs
T_3	GA_3 (Gibberellic acid)	1000 ppm	24 hrs
T_4	KNO_3(Potassium nitrate)	2 %	24 hrs
T_5	KNO_3 (Potassium nitrate)	3 %	24 hrs
T_6	KNO_3 (Potassium nitrate)	4 %	24 hrs
T_7	Hot water	80^0C	30 min
T_8	Hot water	90^0C	20 min
T_9	Hot water	100^0C	10 min

hr= hours and min= minutes.

hydro and chemical priming treatments (Table 2). Seeds treated with 500 ppm GA_3 showed maximum germination rate (55.56 days) which was statistically significant with control. Hot water treatments observed average germination rate. Minimum germination rate (159.88 days) was recorded in unprimed seeds. Gibberellin encourage germination by inducing hydrolytic enzymes that weaken the hurdle tissues such as the endosperm or seed coat, inducing mobilization of food reserves in seed and stimulating expansion of the embryo (Bewley and Black, 1994; Dhoran and Gudadhe, 2012).

Table 2: Effect of different priming treatments on germination parameters of sago palm.

Treatments	Germination rate (days)	Germination percentage (%)	Germination Value	Decayed seed percentage (%)	Time of germination (days)	No. of leaves	Seedling height (cm)
Control	159.8^{cd}	33.33^{e}	74.43^{h}	66.66^{a}	204.58^{a}	1.33^{ab}	12.33^{cd}
GA_3 @ 500 ppm	55.5^{abc}	70^{ab}	192.19^{a}	30.0^{de}	102.92^{f}	1.66^{ab}	16.33^{abc}
GA_3 @ 750 ppm	62.5^{ab}	63.33^{abc}	184.12^{b}	36.66^{cde}	114.81^{e}	1.66^{ab}	16.66^{ab}
GA_3 @ 1000 ppm	67.95^{ab}	56.66^{bcd}	166.57^{c}	43.33^{bcd}	123.62^{d}	1.33^{ab}	17.0^{ab}
KNO_3 @ 2%	62.06^{a}	73.33^{a}	186.42^{ab}	26.66^{e}	59.41^{h}	2.0^{a}	18.33^{a}
KNO_3 @ 3%	67.23^{a}	63.33^{abc}	166.92^{c}	36.66^{cde}	63.81^{h}	1.66^{ab}	19.33^{a}
KNO_3 @ 4%	75.38^{ab}	53.33^{cd}	152.04^{d}	46.66^{bc}	72.16^{g}	1.33^{ab}	16.66^{ab}
Hot water @ 80^o C	99.3^{abcd}	46.66^{de}	119.1^{g}	53.33^{ab}	161.1^{c}	1.0^{b}	15.3^{abcd}
Hot water 90^o C	106.38^{d}	46.66^{de}	127.72^{f}	53.33^{ab}	180.47^{b}	1.0^{b}	11.33^{d}
Hot water 100^o C	111.77^{bcd}	43.33^{de}	136.49^{e}	56.66^{ab}	185.2^{b}	1.0^{b}	13.33^{bcd}

Germination rate (days) and germination percentage (%): The data regarding germination percentage indicated that difference between primed and non-primed seeds was statistically significant. Lower concentrations of potassium nitrate (KNO_3) @ 2% and gibberellic acid (GA_3) @ 500 ppm treatments significantly affected the germination percentage 73.33% and 70% respectively as compared to control (33.33%). Significant improvement in seed germination might be due to enhanced breakdown of reserve metabolites present in seed. The lower concentration of KNO_3 has promoting effect on seed germination as compared to its higher concentration. This leads to supposition that higher concentrations exercise decreasing effects on seed germination by causing death of cells and ultimately result in loss of seed viability (Nascimento, 2003; Ramzan *et al.*, 2010).

Germination value: Analysis of variance revealed that germination value was affected by various priming treatments (Table 2). Result regarding germination value (192.19) was highest in T_1 (500 ppm GA_3) followed by 186.42 in T_4 (3% KNO_3) and 184.12 in T_2 (750 ppm GA_3). Minimum germination (74.43) was noted in control. The gibberellic acid has positive effect on germination value due to its hormonal regulation capability and retarding effect against abscisic acid present in dormant seeds (Var *et al.*, 2010; Zarchini *et al.*, 2013; Pipinis *et al.*, 2015).

Decayed seed percentage (%): Data regarding decayed seed percentage have displayed in Table 2. The difference between primed and non-primed seed was significant and primed seed have minimum decayed seed percentage as compared to non-primed seeds. Lowest decayed seed percentage (26.66%) was recorded when 2% KNO_3 was applied followed by 30% when 500 ppm GA_3) was applied. Whereas maximum decayed (66.66%) of seeds was occurred in untreated seeds. It is reported that scarified treatments have improved germination as compared to non-scarified seeds. Decayed seed percentage might be highest in control due to impermeability of hard seed coat (Fallahabadi *et al.*, 2012).

Time of germination (days): Potassium nitrate showed a statistically significant effect on reducing the germination time (Table 2). Minimum time of germination (59.41 days) was recorded in seeds treated with 2% KNO_3 followed by 3% and 4% KNO_3 levels which took 63.81 days and 72.15 days respectively while maximum time duration was taken by control (204.58 days). Reduction in seed germination time was occurred when seeds of *Descurainia sophia* and *Plantago ovate* were primed with 0.3% KNO_3 (Ali *et al.*, 2010; Gashi *et al.*, 2012). Stimulating effect of nitrate for seed germination might be due to dormancy breakage (Hilhorst, 1990). It stimulates oxygen uptake (Hilton and Thomas, 1986) and KNO_3 act as co-factor for phytochrome (Mavi *et al.*, 2006).

Number of leaves: Analysis of data showed that number of leaves influenced by different treatments. Hormonal priming

with 2% KNO_3 gave maximum number of leaves per seedling followed by priming with 3% KNO_3, 4% KNO_3 and in 500 ppm GA_3, 750 ppm GA_3 and 1000 ppm GA_3 in improving number of leaves per seedling as compared to other physical priming treatments, while results of minimum number of leaves were achieved in non-primed seeds. It was suggested that potassium is an important macronutrient that plays a key role in carbohydrate metabolism and photosynthesis (Marschner, 2011; Kazemi, 2013).

Seedling height (cm): Analysis of variance exposed that there was a significant difference between primed and non-primed seed for seedling height (Table. 2). It was found that maximum seedling height was 19.33 cm influenced by 3% KNO_3 while minimum 11.33 cm observed in 90°C hot water. It is reported that foliar application of K, improved the chlorophyll and fruits-NK content (Sarrwy *et al.*, 2010; Marschner, 2011; Kazemi, 2013).

CONCLUSION: The present study was undertaken to assess the effect of different priming treatments on seed germination of *Cycas revoluta* L. The results of the study clearly indicated that germination rate and germination value were maximum at lower concentration of gibberellic acid (500 ppm GA_3). While, germination percentage, maximum number of leaves, maximum seedling height , decayed seed percentage and time required for seed germination were observed minimum at lower concentration of potassium nitrate (2% and 3% KNO_3). Hot water treatments had least effect on seed germination.

REFERENCES

Ali, T., P. Hossein, F. Asghar, Z. Salman and Z. C. M. Ali, 2010. The effect of different treatments on improving seed germination characteristics in medicinal species of *Descurainia sophia* and *Plantago ovata*. African Journal of Biotechnology, 9(39): 6588-6593.

Bewley, J. and M. Black, 1994. Seeds: Physiology of development and germination plenum. Plenum Press New York, USA.

Chauhan, J., Y. Tomar, N. I. Singh and S. Ali, 2009. Effect of growth hormones on seed germination and seedling growth of black gram and horse gram. Journal of American Science, 5(5): 79-84.

Copeland, L. and M. McDonald, 1995. Seed science and technology (3rd eds.). Chapman and Hall.

Dhoran, V. and S. Gudadhe, 2012. Effect of plant growth regulators on seed germination and seedling vigour in Asparagus Sprengeri regelin. Research Journal of Biologicalical Sciences 1(7): 6-10.

Fallahabadi, P., D. Hashemabadi, R. Onsinejad, M. Zarchini and B. R. Kaviani, 2012. Improving germination rate of *Cycas revoluta* L. By using different cultivation media and scarification. Annals of Biological Research, 3(7): 3187-3191.

Frett, J. J., 1987. Seed germination of *Cyeas revoluta*. Journal of Environmental Horticulture, 5(3): 105-106.

Gashi, B., K. Abdullai, V. Mata and E. Kongjika, 2012. Effect of gibberellic acid and potassium nitrate on seed germination of the resurrection plants *Ramonda serbica* and *Ramonda nathaliae*. African Journal of Biotechnology, 11(20): 4537-4542.

Hilhorst, H. W., 1990. Dose-response analysis of factors involved in germination and secondary dormancy of seeds of *Sisymbrium officinale*: Ii. Nitrate. Plant Physiology, 94(3): 1096-1102.

Hilton, J. R. and J. A. Thomas, 1986. Regulation of pregerminative rates of respiration in seeds of various weed species by potassium nitrate. Journal of Experimental Botany, 37(10): 1516-1524.

Kazemi, M., 2013. Effect of foliar application of humic acid and potassium nitrate on cucumber growth bull. Bulletin of Environment, Pharmacology and Life Sciences, 2(11): 03-06.

Marschner, H., 2011. Marschner's mineral nutrition of higher plants. Academic Press.

Mavi, K., S. Ermis and I. Demir, 2006. The effect of priming on tomato rootstock seeds in relation to seedling growth. Asian Journal of Plant Sciences, 5(6): 940-947.

Nascimento, W. M., 2003. Muskmelon seed germination and seedling development in response to seed priming. Scientia Agricola, 60(1): 71-75.

Pipinis, E., E. Milios, M. Georgiou and P. J. F. I. Smiris, 2015. Effects of gibberellic acid and cold stratification on seed germination of two sorbus species. 21(1): 107-114.

Ramzan, A., I. Hafiz, T. Ahmad and N. Abbasi, 2010. Effect of priming with potassium nitrate and dehusking on seed germination of gladiolus (*Gladiolus alatus*). Pakistan Journal of Botany, 42(1): 247-258.

Rouhi, H., K. Shakarami and R. Afshari, 2010. Seed treatments to overcome dormancy of waterlily tulip (*Tulipa kaufmanniana* regel). Australian Journal of Crop Science, 4(9): 718.

Sarrwy, S., E. A. Mohamed and H. Hassan, 2010. Effect of foliar sprays with potassium nitrate and mono-potassium phosphate on leaf mineral contents, fruit set, yield and fruit quality of picual olive trees grown under sandy soil conditions. American-Eurasian Journal of Agricultural Environmental Science, 8(4): 420-430.

Steel, R. G. and J. H. Torrie, 1980. Principles and procedures of statistics, a biometrical approach. McGraw-Hill Kogakusha, Ltd.

Tomlinson, P. B., 1990. The structural biology of palms. Oxford University Press.

Var, M., B. Bekci and D. Dinçer, 2010. Effect of stratification treatments on germination of *Sorbus torminalis* L. Crantz (wild service tree) seeds with different origins. African Journal of Biotechnology, 9(34): 5535-5541.

Zarchini, M., D. Hashemabadi, N. Negahdar and S. Zarchini, 2013. Improvement seed germination of wild service tree (*Sorbus aucoparia* L.) by gibberellic acid. Annals of Biological Research, 4(1): 72-74.

© 2020 by the authors. Licensee Scientific Platform. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Research Manuscript www.sciplatform.com

Effect of potassium and sowing time on potato yield and quality

[a] Mujeeb Ur Rehman, [b] Muhammad Jamal Khan, [c] Mujib Ur Rehman, [d] Hanif Khan, [e] Ihsan Muhammad, Irfan Ullahe, [f] Hafiz Ullah, [d] Umar Zeb*
[a] Department of Soil and Environmental Sciences, the University of Agriculture, Peshawar Pakistan,
[b] Department of Soil and Environmental Sciences, the University of Agriculture, Peshawar Pakistan,
[c] College of Resource and Environment Science, Sichuan Agricultural University , Sichuan, China,
[d] Key Laboratory of Resource Biology and Biotechnology, College of Life Sciences, Northwest University, China,
[e] College of Urban and Environmental Sciences,Northwest University, Xi'an China,
[f] Department of Botany University of Chitral, Pakistan.

Authors' Contribution	**M. Rehman, M. J. Khan, M. Rehman** and **H. Khan** conducted the research; **I. Muhammad, I Ullahe, H. Ullah** and **U. Zeb** procced the data analysis and wrot the manuscript.
Article History	***Corresponding email address**: umar02mkd@gmail.com
	Received: 22 December 2019, Revised: 01 January 2020, Accepted: 05 January 2020, Published Online: 07 January 2020

ABSTRACT

The current study was conducted the effect of sowing time and different doses of a potassium supplement on yield attributes of potato at Gollen valley Chitral, the Northern Pakistan during summer 2018. Sowing Potato (cv. Roko) commenced from 5thMay and continued till 5thJune keeping an interval of 15 days among sowing times. Potassium (K) was applied at the rate of 100, 150, 200 and 250 kg ha^{-1} as potassium chloride. Basal doses of nitrogen (N) and phosphorus (P) were applied at the rate 120 and 100 kg ha^{-1} respectively, as urea and DAP. All yield attributes like number of leaves per plant, plant height, the number of tubers per plant, tuber volume and yield of potato were higher for May 5th (early sowing date) with potassium dose of 200 kg ha^{-1}. Interaction of sowing dates and Potassium (SD×K) for yield, tuber volume and soil potassium content was statistically significant (P≤0.05). The study showed that by delaying the sowing season, yield traits and yield of potato decreased significantly; hence early cultivation and K fertilization of 200 kg ha^{-1} resulted in maximum production of potato as well as improved soil properties under agroclimatic conditions of the region which is a dry temperate zone of Pakistan.

Key word: Potassium levels, sowing time, potato yield, Chitral, Pakistan.

INTRODUCTION: The appearance of the potato (*Solanum tuberosum* L) belongs to family *Solanaceae*, generally considered being an index of quality and often determines consumer choice. Great efforts have recently been focused in producing good appearance and quality of potato through the utilization of inexpensive and environ-mentally friendly resources. However, Pakistan is an agro-economy based country of Asia, depending on cash crops to meet the demands of the fast-growing population. Cultivating vegetables is not only a reliable source of essential nutrients but it also creates more livelihood opportunities than cereals (Ali and Tsou, 2000; AbdelGadir *et al.*, 2003). Among these vegetable crops potato occupies a significant position in revenue generation and food production. The yield of potat crop in Pakistan is very low due to several biotic and abiotic factors (Abbas *et al.*, 2012; Abbas *et al.*, 2014; Abbas and Madadi, 2016; Qamar *et al.*, 2016; Urooj *et al.*, 2016). Production of good quality fruits is controlled by the interaction of genetic diversity, environmental factors, including plant nutrients. Among significant plant nutrients, potassium (P) is the one that is absorbed by the potato plant in the major amounts and it is considered being the key to production of quality vegetables. Potato is one of the cash crops of Pakistan containing a large amount of starch and higher production per unit area. It occupies a prime position among all underground crops. This is the rich source of carbohydrates, vitamins B, C and minerals. Potato tubers contain 20.6% carbohydrates, 70-80% water, 2.1% proteins, 1.1% crude fibers, 0.3% fat and 0.9% ash (Banu *et al.*, 2007). Among the major crop's potato produces the highest biological yield and more edible protein per unit area, about 3-4 times more than cereals for the same productivity area. Pakistan has the low yield of potato about 10 tons ha^{-1} as compared to the other developing countries which needs improvement to meet the rapidly increasing demand (Singh *et al.*, 2019). Unavailability of quality seed, sowing time, application of fertilizers and climatic conditions are some major factors which determine yield per unit area. The NPK fertilizers govern its growth and development and required for the production of the higher yield of potato crops. The rates of NPK fertilizers are directly proportional to the yield of potato and quality of certain levels of application beyond which the yield decreases (Aggarwal *et al.*, 1976).

The twisted and extreme use of N and P fertilizer might heighten the situation in different cropping system because of insignificant K use in the country and continuous use of N and P would hasten drainage of soil natural K reserves (Akhtar and Khan, 2002; Khan *et al.*, 2014) It not only changes soil K level but also adversely affects crop yield. Planting dates influences leaf area index trend as well as an amount of absorbed radiation in crop population and is ultimately an important factor for determining potato yield. Delayed planting dates cause yield reduction (Chapagain *et al.*, 2003). Short-duration potatoes in temperate regions where the objective is to maximize sellable tuber yield, is significantly affected by frost action resulting in a reduction of the number of tubers per plant and decline of leaves (Krishnappa, 1993; Arab *et al.*, 2011). Among the fertilizer potassium fertilizer is considered most important for the growth and yield of potato crops. Increasing the doses of potassium also increase the tuber size and a number of tubers and ultimately the yield of potato (El-Gamal, 1985). The application of K either increases the tuber yield or increases the size of tubers per plant or both which ultimately increases the

production of potato crops (Humadi, 1986). Potassium is essential for photosynthesis, increasing enzymes activities, improving a synthesis of fats, carbohydrates and proteins and also translocation of photosynthesis which enable their ability to resist against pests and diseases (Jamro *et al.*, 2015). Potassium is sometime recorded as in indicator crop for K++ availability because of its high requirements (Humadi, 1986; Arab *et al.*, 2011).

Chitral is considered as a rich producing region of potato in Pakistan. It is the main cash crop of rural areas of Chitral like Garam Chashma and Gollen. More than 80% of the farmers grow potato as their main crop. Being off season, potatoes give much better financial returns in these areas. Moreover, the soil and climatic conditions are favorable for potato production and the average yield in these areas is greater than other parts of the country. These potato growing areas are single cropping regions and farmers can grow only one crop in a year. Due to climatic change in terms of global warming during recent years, the snowfall has considerably decreased. Low snowfall has encouraged an increase in temperature during spring season. According to meteorological department's forecast winter rains would further decrease in the next twenty years which could further increase the temperature in spring and summer seasons in these areas.

These single cropping zones could be converted into double cropping zones as summer will be prolonged with the increase in spring and autumn temperatures and could be a great opportunity for livelihood improvement of the poor people of these remote areas in terms of cultivation of a second crop in the same season. In this connection two experiments were laid out in Borbonu and Lohok villages of lower Goboor valley Chitral to determine the suitable sowing and harvesting dates for a potato crop which could provide sufficient space for a second crop without compromising on potato yield and income. The seed of Paramount and Roko cultivers stored by ARS Chitral was utilized in these experiments.

OBJECTIVES

The objectives of this study were as follows: (1) to study the effect of different sowing time on growth yield and quality of potato (2) to study the effect of potassium source and rate of application on potato yield. (3) To evaluate the combined effect of fertilizer application and harvest time on unit area production.

MATERIALS AND METHODS

Research description: Field experiment was conducted in village Ashgoor of Gollen valley, Chitral during summer 2018. The research experiment was planned in Randomized Complete Block design (RCB) in a split plot arrangement each in three replicas. The plot size was kept 5 m x 3 m (15m^2) and *S. tuberosum* was sown at three different dates with an interval of 15 days. Each plot was divided into six sub plots. Experiments were conducted in three different sowing times which were assigned to main plot while five potassium levels were applied to subplots. Potatoes were sown on ridges (30-40 cm high) and fertilizer was mixed before the formation of ridges. All P, K and half N of the recommended doses were applied at the time of sowing while the remaining half of N was applied 30 days after sowing. Regular irrigation was done according to the crop demand through spring water. Potassium was applied at the rate of 0, 100,150, 200 and 250 kg ha^{-1} as KCl whereas all other cultural practices, including weeding, insect control and irrigation were uniformly carried out for all plots.

Soil analysis of the experimental site: A composite soil samples comprising ten randomly collected soil cores (0–15 cm) each was collected at pre-sowing stage. The collected samples were then passed through a 2 mm sieve size to clean the soil from other trash, leaves and plant roots. After cleaning plant and soil samples were brought to Soil and Environmental Science laboratory, The University of Agriculture, Peshawar and were analyzed for various physcio-chemicals properties like soil texture, electrical conductivity (EC), pH, organic matter, lime content and AB-DTPA extractable P and K. Beside this soil and plant samples were also collected from the subplots at flowering and post-harvest stage for the P and K analysis.

Statistical analysis: In this experiment the data obtained from a randomized complete block design was analyzed using analysis of variance. The means were compared by using LSD test at 5% level of significance.

Physico-chemical properties of soil before sowing of potato crop: The physico-chemical properties of composite soil sample are given in the (Table 1).

Table 1: Physico-chemical properties of soil before sowing of potato crop

S. No	Soil Property	Units	Value
1	Soil pH	----	7.4
2	Electrical Conductivity	dSm^{-1}	0.9
3	Soil Texture	-----	Sandy loam
4	Organic Matter	%	2.5
5	Lime Content	%	10.5
6	Total Nitrogen	mg kg^{-1}	0.5
7	Potassium	mg kg^{-1}	74
8	Phosphorous	mg kg^{-1}	11.0

The soil of the experimental site was a sandy loam (Koehler *et al.*, 1984), with the slightly alkaline pH 7.4 (Page *et al.*, 1982), and sufficient organic content (Nelson and Sommers, 1982). The soil was non-saline and slightly calcareous. Both P and K are deficient.

RESULTS

Emergence m^{-2} and number of leaves plant^{-1}: The experimental data (table 1 and 2) showed that emergence m^{-2}and number of leaves per plant^{-1}of potato crop were significantly (P≤0.05) affected through showing dates while levels of potassium and their interaction with climate was found significant in case of a number of leaves per plant^{-1}. The mean values of the data reveal that maximum emergence 96.1% were recorded when the crop was sown on 5th May followed by sowing date 20thMay. Minimum emergence (69.7%) and leaves per plant^{-1} were recorded when the crop was sown in early June (5th June). In case of potassium level maximum emergence 77.2% and leaves per plant^{-1}101.1% were noted from the application of 250 kg K ha^{-1} whereas low emergence of 75.8% was seen in the control plots, but they are statistically non-significant (NS) while found significant in case of leaves per plant^{-1} (71.3%).

Plant height (cm) and number of tubers plant^{-1}: Analysis of the data regarding plant height, number of tuber plant^{-1} showed that significant result for sowing dates and potassium rates presented in table 4. Interactions of sowing dates and potassium levels remained non-significant in plant height, while significant in case of number of tuber plant^{-1} (figure 1). Taller

plants were produced (25.1) cm after 60 days of emergence for May 5th sowing date, while dwarf plants were found (19.5 cm) after 60 days of emergence from 20th May.

Table 2: Effect of potassium levels and sowing dates on emergence (%) and number of leaves plant^{-1} of potato Emergence Percentage (%) of potato crop

Potassium levels (kg ha^{-1})	Sowing Dates			Means
	5th May	20th May	5th June	
0	84.3	75.3	67.7	75.8
100	83.3	71.3	69.3	74.6
150	86.3	68.3	69.7	74.8
200	86.7	72.7	71.0	76.8
250	87.7	73.0	71.0	77.2
Means	85.7	72.1	69.7	
Number of leaves plant^{-1}of potato crop				
0	74.0	70.0	70.0	71.3 c
100	79.3	94.7	72.8	82.3 bc
150	100.0	101.0	69.3	90.1 ab
200	118.0	100.3	85.0	101.1a
250	109.3	102.7	84.7	98.9 a
Means	96.1	93.7	76.4 b	
LSD for Emergence % (0.05)			LSD for Leaves per plant $^{-1}$ (0.05)	
Sowing dates (S)	3.71		Sowing dates (S)	14.03
Potassium (K)	NS		Potassium (K)	16.87
S x K	NS		S x K	NS

Mean with different letters in the similar group are significantly varied from each other at ($p \leq 0.05$) by checking with LSD test.

Table 3: Effect of potassium and sowing dates on plant height (cm) and number of tuber plant^{-1}after 60 days plant height as influenced by sowing dates and K levels

Potassium (kg ha^{-1})	Sowing dates			Means
	5th May	20th May	5th June	
0	18.0	14.0	13.0	15.0 d
100	22.0	17.0	19.4	19.5 c
150	25.0	19.3	21.0	21.8 b
200	29.7	23.0	22.7	25.1 a
250	30.7	24.0	24.1	26.2 a
Means	25.1 a	19.5 b	20.0 b	
Number of tuber plant^{-1} as affected by sowing dates and K levels				
Potassium (kg ha^{-1})	Sowing dates			Means
	5th May	20th May	5th June	
0	7.7	9.3	7.3	8.1 d
100	12.3	13.0	10.7	12.0 c
150	13.0	12.3	11.7	12.3 c
200	15.0	13.7	12.0	13.6 b
250	16.0	14.3	13.7	14.7 a
Means	12.8 a	12.5 a	11.1 b	
LSD for plant height (0.05)			LSD for umber of tuber plant^{-1} (0.05)	
Sowing dates (S)	1.496		Sowing dates (S)	1.125
Potassium (K)	2.1469		Potassium (K)	0.845
S x K	NS		S x K	1.463

Means in the above with different subscripts differ significantly ($p \leq 0.05$) using LSD test.

Similarly, a greater number of tuber plant^{-1}(12.8) was verified from sowing date 5th May; while a smaller number of tuber plant^{-1} (12.5 and 11.1) was noted in 20th May and June 5th respectively. For potassium levels highest plant height (26.2 cm) was recorded for K fertilizer (250 kg K ha-1) which was statistically compared to 200 kg K ha-1 with plant height of (25.1 cm). Subsequently, maximum number of tuber plant-1 (14.7) was seen in the treatment of 250 kg K ha-1 while minimum plant height (15 cm) and number of tuber plant-1 (8.1) was recorded in control plots.

Volume of tuber (cm^3) and potato yield (ton ha^{-1}): Data analysis for tuber volume and yield of potato crop indicated that its volume and yield were considerably affected with potassium levels and sowing dates while the interaction of potassium and sowing date remained non-significant in case of tuber volume, and found significant for yield of potato (table 3 and 4). Maximum volume (114.7 cm^3) and tuber yield (38.3-ton ha^{-1}) were recorded from sowing date of 5th May, while sowing date 20th May and 5th June ranked 2nd and 3rd with tuber volume (110 and 108 cm^3) and tuber yield (37 and 36.9-ton ha^{-1}) respectively which were statistically similar to each other. With the increase in potassium levels, it was noted that the volume of

tuber and tuber yield also increased significantly. Maximum volume of tuber (129 cm^3) was observed in the treatments of 250 kg K ha^{-1} which is statistically at level with 200 kg K ha^{-1} (127 cm^3), while in the case of potato yield, highest tuber weight (39.8 ton ha^{-1}) was noted in the plots receiving 200 kg K ha^{-1} which is statistically similar with 250 and 150 kg K ha^{-1} with tuber yield of 39.4 and 39.7 ton ha^{-1} respectively. The minimum tuber volume (83 cm^3) and tuber yield (32.7-ton ha^{-1}) were recorded from control plots.

Soil and leaf potassium after 60 days of emergence (mg kg^{-1}): The results of K content in the soil and leaf after 60 days of emergence as influenced by applied K level and sowing dates is shown in (table 5). The analyzed data revealed sowing dates and applied potassium levels were significant at ($p \leq 0.05$), while their interaction was found also significant (figure 2) in case of soil K, while non-significant in the case of leaf K after 60 days of emergence. More K concentration (112 mg kg^{-1}) was recorded from sowing date 20th May, while lower K content (109 mg kg^{-1}) was noted when crop was planted on early May and June. Maximum leaf K concentration (1%) was given by the sowing date 5th May, while leaf K concentration (0.8% and 0.8%) was recorded for May 20th and June 5th sowing dates respectively. In case of different levels of K fertilizer significantly higher soil and leaf K content (149 mg kg^{-1} and 1%) were recorded when 250 kg K ha^{-1} was delivered to the potato crop. While lower soil and leaf K was observed in control plots after 60 days of emergence of the potato crop (table 6).

Potassium concentration in post-harvest soil: Effect of potassium and sowing dates on the potassium content after harvest is shown in Table 9. The data showed statistically that potassium content after harvest was significantly ($p \leq 0.05$) effected by applied potassium level along with sowing dates and their interaction (figure 4). Maximum potassium content in soil post-harvest was observed in plots receiving 250 kg K ha^{-1} followed by the treatments 200 and 150 kg K ha^{-1} with post-harvest potassium level of 127 and 108 mg kg^{-1} respectively. Minimum post-harvest potassium level (57.4mg kg^{-1}) was observed in the control plot.

DISCUSSION: The highest yield was noted in 5th May sowing date, because the climate of Chitral offers favorable growth conditions for potato vegetable is the month of May. Early sowing showed maximum emergence of seedlings, maximum number of leaves and tubers per plant. Similar fallouts were also stated by Jamro *et al.* (2015), who found that early sowing gives maximum return in comparison with late sowing. The results were also in judgment with Jamro *et al.* (2015) who recorded that highest emergence occurs in timely sowing as compared to late sowing.

The early sowing of the potato crop was found more efficient as compared to late sowing of potato crop due to the environmental condition, particularly temperature which has paramount effect on number of tubers per plant, volume of tuber and hence the yield (Khan *et al.*, 2010; Yenagi *et al.*, 2010). According to the judgments of, who reported maximum number of tubers when temperature is favorable, because during this month temperature of the area is low as temperature goes up growth of potato is also affected thus a decrease in volume of tubers results. Increase in temperature above optimum reduces growth of tubers and ultimately results in the minimum yield. Delayed sowing time from May to June decreased the plant height and other yield and growth parameter of the crop.

Our study findings are also in association with the results of (Putra *et al.*, 2019), who reported that, the application of the highest level of K i.e 300 kg K_2O ha^{-1}improved the numbers of tubers per plant. The increasing levels of K did not affect the emergence of potato because K is mainly involved in activation of enzyme and regulation of plant water (El-Latif *et al.*, 2011; Manolov *et al.*, 2015). Potassium plays a vital role in the photosynthetic action and regulation of the process due to which gives maximum energy which facilitated more number of leaves also regulating the conductance in leaves, water absorption and translocation assimilate in plants (Grzebisz *et al.*, 2013). Furthermore, K involved in various functions of plants including health of plants, resistant of plant against diseases and promoting the number of leaves (Chapagain and Wiesman, 2004; Misskire *et al.*, 2019).

Similarly, increasing levels of K also increased the plant height of the potato crop. Potassium maintains the nutrient balance in plants which in turn promote vegetative growth of the plants. Our results are comparable with (Pregno and Armour, 1992), who reported that application of K increase the efficiency of N which ultimately increase the plant growth of the crop because of more uptake of nitrogen. Similar results were also reported by Al-Moshileh and Errebi (2004) that increasing K levels increased the plant height of potato crop, chlorophyll content in plants, leaf area, K concentration and carbohydrate (Khan *et al.*, 2010).

Likewise, K significantly increased the volume of tuber as K play an important role in size, yield and tuber weight of potato. The results were also in the agreement of Hailu *et al.* (2017) who reported that by increasing K levels improved the total yield, the highest being 200 kg K_2O ha^{-1} ultimately increased the size of large and medium sized tubers and was significantly higher as compared to lower levels of K_2O. These results were also supported by (Adhikari and Karki, 2006) who reported that increase in tuber size requires maximum K as function of K is to transform sugars from leaves to tubers resulting in an increase in the volume of tuber.

These results showed similarity with Winsor and (Nilson and Abrahamson, 2018), who reported that plant reared sufficient K may produce much more yield even in stress conditions. The findings were in the line with the results which suggested that at the maximum K application rates, the successive crop might be benefited from a positive residual K, instead of the more effectiveness of potato production at that particular combination. It is notable; however, that potassium convenience in the soil was limited to a depth of 60 cm only (Grzebisz *et al.*, 2013).

Conclusions

In this study, we concluded that emergence m^{-2}, leaves $plant^{-1}$, plant height, volume of tuber, tuber yield and number of tubes per plant are significantly influenced by early sowing date i.e 5th May. The number of leaves $plant^{-1}$, plant height, and volume of tuber, tuber yield and number of tubes per plant significantly increased with the application of 250 and 200 kg K ha^{-1}. Maximum soil and plant potassium at post-harvest and sixty days after emergence was increased by application of 250 and 200 kg K ha^{-1} and similarly leaf P and K concentration was also found more at the same levels of K while sowing dates

Table: 4 Effects of potassium rates and sowing dates on volume of tubers (cm^3) and yield of potato (ton ha^{-1}) Volume of tubers (cm^3) as affected by potassium rates and sowing dates

Potassium (kg ha^{-1})	Sowing dates			Means
	5th May	20th May	5th June	
0	85.7	82.7	80.7	83.0 d
100	103.0	98.0	100.7	100.6 c
150	118.3	117.7	108.3	114.8 b
200	130.7	125.7	126.7	127.7 a
250	136.0	126.7	125.3	129.3 a
Means	114.7 a	110.1 b	108.3 b	
Potato Yield (ton ha^{-1}) as influenced by K levels and Sowing Dates				
0	34.7	31.8	31.8	32.7 c
100	36.8	34.8	34.4	35.3 b
150	40.0	39.2	39.7	39.7 a
200	40.3	39.8	39.2	39.8 a
250	39.5	39.3	39.3	39.4 a
Means	38.3 a	37.0 b	36.9 b	
LSD for volume of tuber (0.05)			LSD for yield of potato (0.05)	
Sowing dates (S)	2.027		Sowing dates (S)	0.480
Potassium (K)	6.815		Potassium (K)	0.984
S x K	NS		S x K	1.704

Means in the above with different subscripts are significantly different ($p \leq 0.05$) using LSD test.

Table: 5 Effect of potassium and sowing date on soil potassium after 60 days of emergence Soil K after 60 days of after emergence as affected by K levels and sowing dates

Potassium (kg ha^{-1})	Sowing dates			Means
	5th May	20th May	5th June	
0	82	77	79	80 e
100	90	89	91	90 d
150	102	102	99	101 c
200	128	138	125	130 b
250	141	155	150	149 a
Means	109 b	112 a	109 b	
Leaf potassium after 60 days of emergence of potato plants				
0	0.9	0.7	0.6	0.7 d
100	0.9	0.7	0.7	0.8 c
150	1.1	0.8	0.8	0.9 b
200	1.1	0.8	0.8	0.9 b
250	1.2	0.9	0.9	1.0 a
Means	1.0 a	0.8 b	0.8 b	
LSD for soil K(0.05)			LSD for leaf K (0.05)	
Sowing dates (S)	1.926		Sowing dates (S)	0.206
Potassium (K)	2.81		Potassium (K)	0.071
S x K	4.88		S x K	NS

Means in the above with different subscripts significantly different ($p \leq 0.05$) using LSD test.

Table 6: Effect of potassium and sowing date on potassium concentration in soil after harvest of potato plants

Potassium (kg ha^{-1})	Sowing dates			Means
	5th May	20th May	5th June	
0	59.1	54.7	58.4	57.4 e
100	68.2	66.6	67.8	67.5 d
150	83.5	82.3	76.3	80.7 c
200	106.3	114.7	103.0	108.0 b
250	117.5	136.5	128.6	127.5 a
Means	86.9	90.9	86.8	
LSD (0.05)				
Sowing dates (S)	3.03			
Potassium (K)	4.50			
S x K	7.79			

Means of the matching type followed by different letters are significantly different from each other at $p \leq 0.05$ using LSD test.

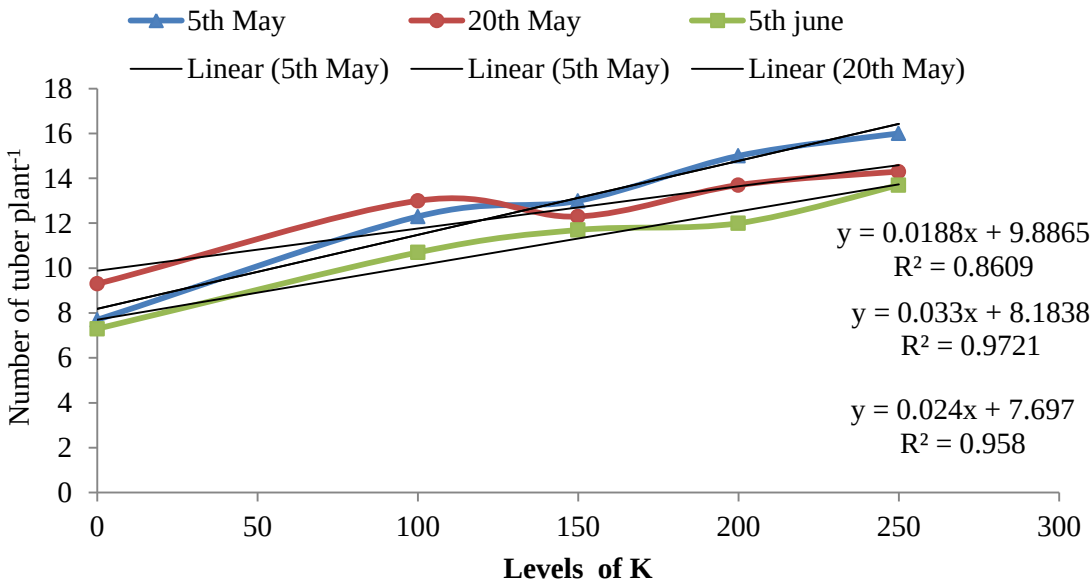

Figure 1: Effect of potassium and sowing date on number of tuber plant^{-1}

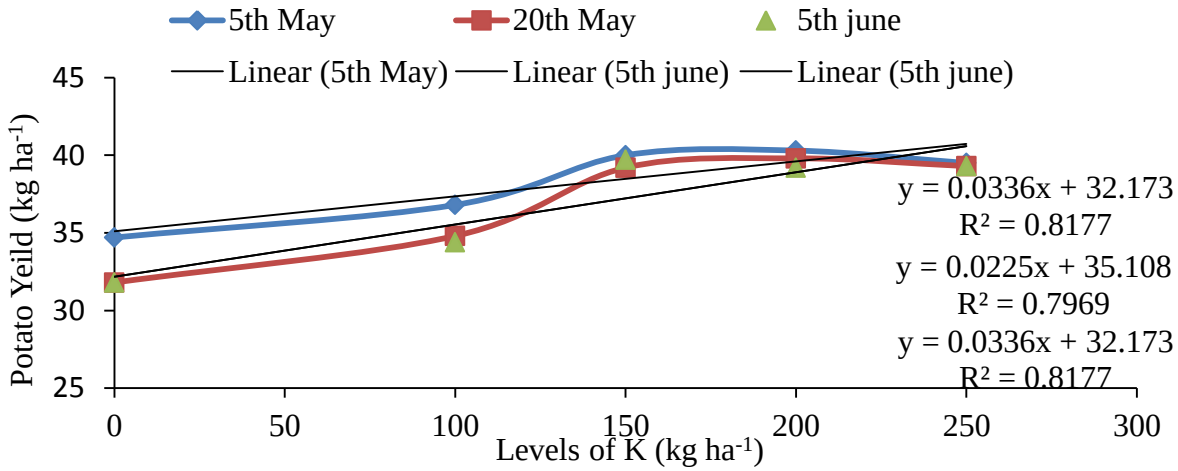

Figure 2: Effect of potassium and sowing date on crop yield (ton ha^{-1}) of potato.

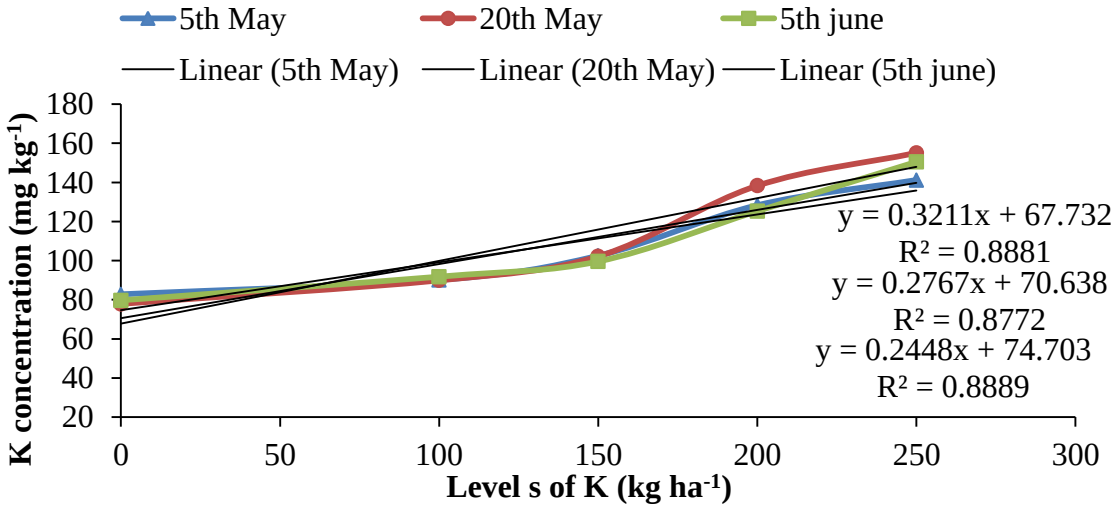

Fig. 3: Effect of potassium and sowing date on soil potassium after 60 days of emergence

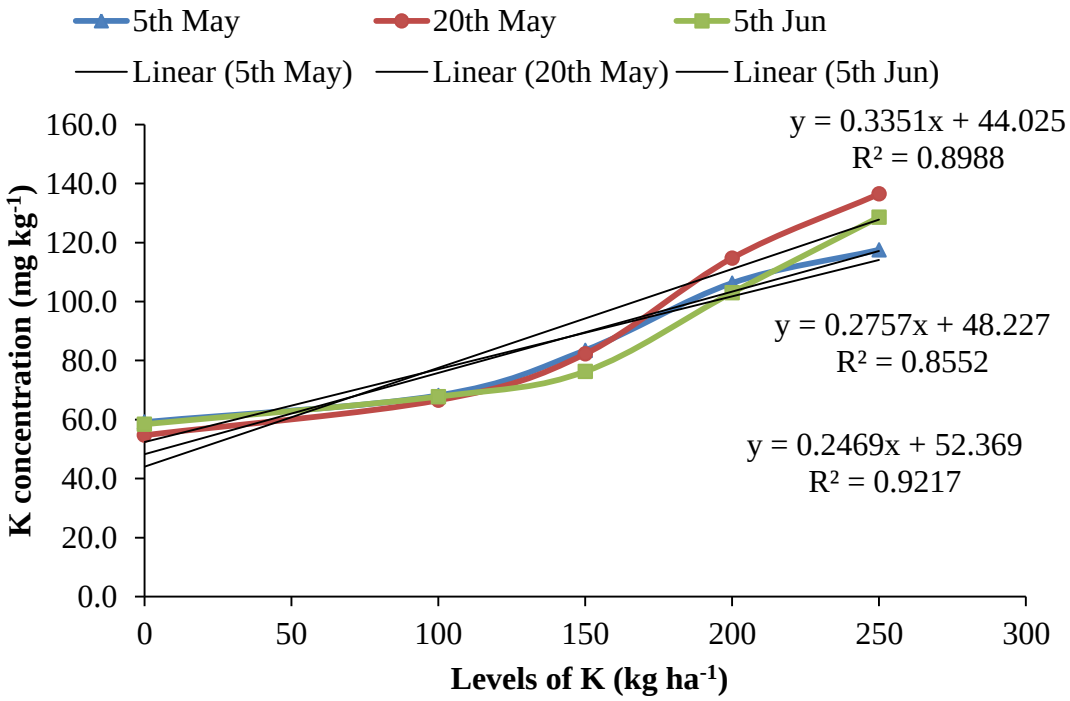

Figure 4: Effect of potassium and sowing date on potassium concentration in soil after harvest

produced non-significant result on soil and leaf P and K concentration. Among sowing dates, the early sowing of potato crop was found more efficient as compared to late sowing so early sowing i.e 5th May is recommended for obtaining more yield. Similarly, the application of K at the rate 150 kg ha^{-1} is strongly recommended for the good yield of potato crop in the agro-climatic condition of Chitral. Further research work should be conducted to identify more sowing times as changing the climatic condition of the selected area.

REFERENCES

Abbas, M. F., S. Hameed, A. Rauf, Q. Nosheen, A. Ghani, A. Qadir and S. Zakia, 2012. Incidence of six viruses in potato growing areas of Pakistan. Pakistan journal of phytopath, 24(1): 44-47.

Abbas, M. F., S. Hameed and C. A. Rauf, 2014. Presence of newstrain of potato virus Y in Pakistan. International journal of agriculture and biology, 16(5): 941-945.

Abbas, A. and M. Madadi, 2016. A review paper on potato mop-top virus (PMTV): Occurrence, properties and management. World journal of biology and biotechnology, 1(3): 129-134.

AbdelGadir, A., M. Errebhi, H. Al-Sarhan and M. Ibrahim, 2003. The effect of different levels of additional potassium on yield and industrial qualities of potato (*Solanum tuberosum* L.) in an irrigated arid region. American Journal of Potato Research, 80(3): 219-222.

Adhikari, B. and K. B. Karki, 2006. Effect of potassium on potato tuber production in acid soils of Malepatan, Pokhara. Nepal Agriculture Research Journal, 7: 42-48.

Aggarwal, R., J. Gupta, S. Saxena and K. Muthana, 1976. Studies on soil physico-chemical and ecological changes under twelve years old five desert tree species of Western Rajasthan. Indian Forester, 102(12): 863-872.

Akhtar, M. and M. Khan, 2002. Nutrient mining-a cause of soil degradation. Farming Outlook, 2: 22-24.

Al-Moshileh, A. and M. Errebi, 2004. Effect of various potassium sulfate rates on growth, yield and quality of potato grown under sandy soil and arid conditions. In: International Potash Institute. Regional workshop on potassium and fertigation development in West Asia and North Africa, Rabat, Morocco. pp: 24-28.

Ali, M. and S. Tsou, 2000. The integrated research approach of the asian vegetable research and development center (avrdc) to enhance micronutrient availability. Food Nutrition Bulletin, 21(4): 472-481.

Arab, H. R., H. Afshari, D. M. Sam, G. Laei and S. R. Toudar, 2011. The effect of planting date, depth and density on yield and yield components of potato in Shahrood (Iran). Journal of Research in Agricultural Science 7(2): 141-149.

Banu, S. S., T. M. Thiyagarajan and P. Malarvizhi, 2007. Effect of graded levels of fertilizers on quality aspects of potato. Potato Journal, 34(3-4).

Chapagain, B., Z. Wiesman, M. Zaccai, P. Imas and H. Magen, 2003. Potassium chloride enhances fruit appearance and improves quality of fertigated greenhouse tomato as compared to potassium nitrate. Journal of Plant Nutrition, 26(3): 643-658.

Chapagain, B. P. and Z. Wiesman, 2004. Effect of potassium magnesium chloride in the fertigation solution as partial source of potassium on growth, yield and quality of greenhouse tomato. Scientia Horticulturae, 99(3-4): 279-288.

El-Gamal, A., 1985. Effect of potassium level on potato yield and quality. Journal of Agriculture Sciences Mansoura University, 10(4): 1443-1476.

El-Latif, K. A., E. Osman, R. Abdullah and N. A. El Kader, 2011. Response of potato plants to potassium fertilizer rates and soil moisture deficit. Advances Applied Sciences Research,

2(2): 388-397.
Grzebisz, W., A. Gransee, W. Szczepaniak and J. Diatta, 2013. The effects of potassium fertilization on water-use efficiency in crop plants. Journal of Plant Nutrition Soil Science, 176(3): 355-374.
Hailu, G., D. Nigussie, M. Ali and B. Derbew, 2017. Nitrogen and phosphorus use efficiency in improved potato (Solanum tuberosum L.) cultivars in southern ethiopia. American Journal of Potato Research, 94(6): 617-631.
Humadi, F. J. Z., 1986. Influence of potassium rates on growth and yield of potato (*Solanum tuberosum*)(in Iraq).
Jamro, M. R., S. Tunio, U. Buriro and Q. Chachar, 2015. Effect of planting dates on growth and yield of true potato seed (tps) in nursery raising approach. ournal of Basic Applied Sciences, 11: 318-322.
Khan, I., M. Zaman, M. Khan, M. Iqbal, M. J. J. o. s. s. Babar and p. nutrition, 2014. How to improve yield and quality of potatoes: Effects of two rates of urea n, urease inhibitor and cytozyme nutritional program. 14(2): 268-276.
Khan, M. Z., M. E. Akhtar, M. N. Safdar, M. M. Mahmood, S. Ahmad and N. Ahmed, 2010. Effect of source and level of potash on yield and quality of potato tubers. Pakistan Journal of Botany, 42(5): 3137-3145.
Koehler, F., C. Moodie and B. McNeal, 1984. Laboratory manual for soil fertility, washington state uni. Pullman Washington.
Krishnappa, K., 1993. Effect of planting time on the yield and yield attributes of potato in eastern karnataka. Field Crop Absttract, 46(11).
Manolov, I., N. Neshev, V. Chalova and N. Yordanova, 2015. Influence of potassium fertilizer source on potato yield and quality. In: Proceedings. 50th Croatian and 10th International Symposium on Agriculture. Opatija. Croatia. pp: 363-367.
Misskire, Y., T. Mamo, A. M. Taddesse and U. Yermiyahu, 2019. The effect of potassium on yield, nutrient uptake and efficiency of teff (*Eragrostis tef zucc*. Trotter) on vertisols of North Western Ethiopian Highlands. Journal of Plant Nutrition, 42(4): 307-322.
Nelson, D. and L. E. Sommers, 1982. Total carbon, organic carbon, and organic matter 1. Methods of soil analysis. Part 2. Chemical Microbiological Properties(methodsofsoilan2): 539-579.
Nilson, M. A. and K. Abrahamson, 2018. Potato seed planting apparatus and method of planting potato seed using the apparatus. Google Patents.
Page, A., R. Miller and D. Keeney, 1982. Methods of soil analysis, part 2. Chemical Microbiological Properties, 2.
Pregno, L. and J. Armour, 1992. Boron deficiency and toxicity in potato cv. Sebago on an oxisol of the atherton tablelands, north queensland. Australian Journal of Experimental Agriculture, 32(2): 251-253.
Putra, F. P., S. Saparso, S. Rohadi and R. Ismoyojati, 2019. The response of potato plants (*Solanum tuberosum* L.) to various thicknesses of cocopeat media and sundstrom nutrition time. Jurnal Ilmiah Pertanian, 15(2): 57-66.
Qamar, M. I., S. Batool, W. Aurangzeb, R. Zainab and S. Menghwar, 2016. Different techniques for diagnostic of potato viruses: A brief review. World journal of biology and biotechnology, 1(3): 123-128.
Singh, V., B. Dwivedi, R. Mishra, A. Shukla, J. Timsina, P. Upadhyay, K. Shekhawat, K. Majumdar and A. Panwar, 2019. Yields, soil health and farm profits under a rice-wheat system: Long-term effect of fertilizers and organic manures applied alone and in combination. Agronomy, 9(1): 1-6.
Urooj, M., U. Arif and A. Intikhab, 2016. A brief review for identification and detection of potato viruses. World journal of biology and biotechnology, 1(1): 33-37.
Yenagi, B., S. Meli and S. Angadi, 2010. Effect of row spacing, planting date and nitrogen level on tuber grade, yield and economics of potato. Karnataka Journal of Agricultural Sciences, 17(2).

© 2020 by the authors. Licensee Scientific Platform. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Review Manuscript www.sciplatform.com

Advanced applications of nanotechnology in agriculture: an overview

Saira Younas, Hunza Asghar, Iqra Shaukat, Sheeba Riaz, Maham Zaib, Maryam Ishaq, Amna Jamshaid, Zoqia Tariq, Saqib Hussain Hadri*
Department of Biochemistry and Biotechnology, University of Gujrat, Punjab, Pakistan

Authors' Contribution: **S. Younas**, **H. Asghar** and **I. Shaukat** wrote the introduction, nanotechnology and agriculture, nano-encapsuled fertilizers; **S. Riaz** and **M. Zaib** wrote liquid nano-fertilizers, engineered nanoparticles, gold nanoparticles, nano-encapsulate pesticides; **M. Ishaq, A. Jamshaid, Z. Tariq** and **S. H. Hadri** worked on the development of nano-encapsulated pesticides with different materials and biosensors

***Corresponding email address**: saqib.hussain@uog.edu.pk

Article History: **Received: 22 December 2019, Revised: 13 January 2020, Accepted: 19 January 2020, Published Online: 31 January 2020**

ABSTRACT

As population of world is increasing at an enormous rate, so this increase in population exerts pressure on farmers to increase food production from available area of agriculture. Conventional methods used to increase food production like formation of genetically modified plants as well as use of fertilizers such as urea fertilizer have large number of disadvantages. In order to increase food quantity and quality, nanotechnology is being preferred now a days. Nanotechnology involves Nano encapsulation of agro chemicals such as fertilizers and pesticides so that their accurate and desired amount is released in plants when needed. This nano encapsulation prevents toxicity which is caused by excessive use of these chemicals when these are directly applied to plants. Nano sensor is another application of nanotechnology, which is used to detect pathogen attack on plants. Diagnosis and treatment of the disease at Nano scale is acquiring a lot of attention these days. Inspite of all that nanoparticles can cause chemical as well as physical poisonousness and phytotoxicity to plants affecting their development. Even then, Scientists are expecting that in future, use of nanotechnology in agriculture will be helpful to solve all the problems that are related to food shortage.

Key word: Urbanization, nano-encapsulated fertilizers, nano-encapsulated pesticides, nano-microcapsules, biostimulator.

INTRODUCTION: The process of Agriculture which is also called as farming is basically a science in which food is produced by using the ability of plants to convert inorganic energy to organic compounds. In the agriculture certain other food compounds are also produce by raising the livestock. Agriculture is the basic corner stone of food production in most of the developing countries. Agriculture food production is basic driver of economy of a country (Sekhon, 2014). In 1950, global population was about 2.5 billion in number which has grown up to 7.2 billion in the year 2014. It is estimated that world's population will increase up to 9.6 billion by 2025 (Grillo *et al.*, 2016). Food and Agriculture Organization (FAO) has forecasted that annually 200 million tons of meat will be required by 2050 in order to fulfill the food requirements of growing population. This rapid growth in population intensifies the food demand produced through agriculture means. This increase in food demand exerts pressure on farmers to increase the growth of crops which are used for animal feed (Sekhon, 2014). In the developing countries there is high rate of urbanization which has also reduced the area for agriculture. Satellite images of earth have also shown that Earth is running out of fertile land and in future food production will be inefficient to satisfy needs of growing world's population (Naderi and Danesh-Shahraki, 2013). About 13.5% of the world's population in 2012-2014 is suffering from undernourishment. Mortality caused by food shortage is estimated to be greater than due to different diseases like tuberculosis, AIDS and malaria in total (Grillo *et al.*, 2016). In order to fulfill the food requirements of large population with limited agriculture resources it is necessary to increase the yield of food production from same area of agricultural land (Khati *et al.*, 2018).

Conventional techniques used to increase food yield: There is urgent need of developing effective strategies that could help in increasing agricultural productivity (Grillo *et al.*, 2016). There are many methods which can be used to increase the yield and quality of agricultural food. Genetically modified plants (GMPs) are one of alternative method use to increase yield of food in the land available for cultivation. GMPs are not accepted worldwide because they have altered genetic makeup and because of human health and environmental concerns (Arora *et al.*, 2012). In order to increase food yield large number of fertilizers such as urea fertilizers, herbicides and pesticides are being used. Phosphorus and nitrogen are the main constituents of the fertilizers. Efficiency of nitrogen fertilizers is 20-50% and that for phosphorus fertilizers is 10-25% so there is a need to make food production more efficient (Naderi and Danesh-Shahraki, 2013). There are certain disadvantages associated with use of chemicals such as pesticides, herbicides and fungicides. This results in bad effects on human health, on domestic animals and also causes soil and water pollution (Mousavi and Rezaei, 2011). When nitrogen phosphorus fertilizers are applied in excessive amount then these fertilizers can affect the growth of plant, decreases organic matter present in soil and also they can move to ground water where excessive nutrient causes eutrophication, water contamination and also affect aquatic life (Naderi and Danesh-Shahraki, 2013).

Nanotechnology and agriculture: Nanotechnology can be used to solve all the problems related with agricultural food quality and quantity. Nanotechnology being an emerging field, opens up wide range of opportunities in different fields like electronics, safety and security, energy conservation, medicine, pharmaceutical industry and also plays a significant role in agriculture area (Ragaei and Sabry, 2014). Agro chemicals can be prepared in Nano-formulation for supplying accurate and

desired amount of fertilizers and pesticides to plants which result in crop improvement. Application of biotechnology in agriculture is important (Khan *et al.*, 2017) Nano sensor is another application of Nano biotechnology which is used to detect any disease produced in plant so that it can be identified and cured. For example silver nanoparticles are used for detection and diagnosis of pathogens in plants. Several nano-devices have also made which are being used for genetic manipulation of plants. With the help of these devices foreign DNA can be injected inside of plant cells to make plants insect resistant or herbicide resistant (Sekhon, 2014). Gold nanoparticles are specifically use for the bombardment of foreign DNA into plant cells because they easily take up DNA and are not toxic to plants and they do not harm them (Arora *et al.*, 2012). Nano-technology is also used for processing of food, storage of food and increasing shelf life of processed food. Nano biotechnology is also used to improve animal health, poultry production and animal breeding (Sekhon, 2014).

Nano technology particularly focuses on the use of nanoencapsulated pesticides that aims at increasing solubility of active ingredients and then their release in targeted manner preventing their premature degradation (Ragaei and Sabry, 2014). Another important application of Nano biotechnology is nano-sensor. Network of wireless Nano sensors help in real time monitoring of crop growth and field conditions like moisture content of soil, pests, viruses, temperature, crop nutrient status, weeds and soil fertility etc (Ditta, 2012). Nowadays, Nanotechnology is used for the disease management in plant. Engineered Nanoparticles (e.g. metalloids, nanomaterial and metallic oxide nanoparticles) and lipid derived nano-sensors are used to eliminate plant diseases and observe nutrient deficiency (Karny *et al.*, 2018).

Nano-encapsulated fertilizers Fertilizers have a crucial role in the betterment of crop yields, yet connate inabilities of conventional fertilizer treatments may result in drastic economic and ecological consequences. Almost half of the nitrogen supplied to the plants in the form of the fertilizer is lost to air, water, and other channels, resulting in negative environmental effects such as nitrates leaching into aquatic ecosystem and discharge of nitrogen oxides into the atmosphere. Usage of phosphorus fertilizers is evenly discouraging, a huge concern taking into account the fact that its runoff exasperates eutrophication in marine ecosystems (Mastronardi *et al.*, 2015).

Regarding sustainable agriculture, the application of productive nanotechnology in agriculture is considered as one of the propitious approaches for fundamentally increasing crop yield and to feed the world's fast-growing population. Despite of the interest taken by the agricultural experts towards advancement and use of nanomaterial-related fertilizers, there is a lack of directly-related research in this field (Liu and Lal, 2015). Nanomaterial or nanoparticles can procure time-controlled, estimated, target-specific, automated, and multifunctional abilities to fertilizers (Wang *et al.*, 2016)

Liquid nano-fertilizers: Ekinci, et al. in 2014 carried out experiments on cucumber plant (*Cucumis sativus* A-21F_1) in two years. Ferbanat and Nanonat are liquid nanofertilizers. Ferbanat is composed of amino acids, natural biological substances, vitamins, micro hamates and micro floras. These components are in appropriate agricultural values to improve the existence of the crops produced. Moreover, Nano Nat is a source of mineral and vitamins for the crops which is used to bring about chemical dressing. For this purpose, 30-50% nanonat, with elements in it, is used with phosphorus, calcium, potassium, magnesium and biological nitrogen. Ferbanat is used as a biostimulator against soil microorganisms and stress, for the new generation plants. These liquid Nano fertilizers significantly affected the fruit weight, total yield, fruit length, yield per plant, and dry matter statistically. In both the years, the control gave the lowest value of yield (Ekinci *et al.*, 2014).

Engineered nanoparticles: Engineered nanoparticles (ENPs), such as mesoporous silica nanoparticles (MSNs) can be used to deliver proteins or enzymes that may be helpful for biochemical analysis and genome modifications. Other types of ENPs, such as quantum dots (QDs) or gold nanoparticles (Au NPs) can be used to deliver plasmid having a GFP gene and efficient recombinase enzyme into plant tissues, resulting in effective genome editing. Compared to the conventional delivery techniques, this NP-facilitated methodology can be carried out easily and is very much sensitive (Wang *et al.*, 2016).

Gold nanoparticles: In an experiment to study the Gold-nanoparticles (AuNPs), five experimental groups of the plant *Brassica juncea* were sprayed with different concentrations of Gold-nanoparticles. With increasing concentration of Gold-nanoparticles that were applied, the bio-accumulation of AuNPs was recorded to be increased when calculated by atomic absorption spectroscopy. In the seeds sprayed with 25 ppm of AuNPs, percent germination was recorded to be maximum i.e. 97%. While it was only 80% in the control seeds. This may be because seed capsule now has enhanced permeability. Gold-nanoparticles have an antagonistic effect on the ethylene, causing an increased number of *Brassica* leaves. The AuNPs-treated seedlings also exhibited greater height and stem diameter, as compared to the control. Treatment with Gold-nanoparticles also affected the biochemical markers such as increased chlorophyll, more CO_2 fixation, and higher level of hydrogen peroxide (H_2O_2) (Arora *et al.*, 2012).

Nano encapsulated pesticides: In agriculture, pesticides are used for controlling insects and to minimize the loss caused by the damage to the crops by the insects (Ramadass and Thiagarajan, 2017)**.** Studies have revealed that there are 20-40% losses in global agricultural production due to damage by pests (Grillo *et al.*, 2016). The use of traditional strategies in agriculture like integrated pest management are insufficient. Furthermore, the use of conventional chemical pesticides causes adverse effects on human beings and animals (Ragaei and Sabry, 2014). Pesticide poisoning can lead to abnormalities in fetus, cancer, female infertility, and many problems in immune, nervous and endocrine systems (Grillo *et al.*, 2016)**.** Excessive pesticide usage in agriculture leads to incorporation of toxic materials in ground and surface water. So, ultimately water supplies get contaminated (Chaturvedi, 2014). Therefore, there is need of pesticides that can be employed efficiently and more safely (Grillo *et al.*, 2016).

Nanoencapsuled pesticides are the novel products targeting pests more effectively in smaller concentrations. Active ingredients are encapsulated in polymer material allowing them to be enclosed in protective matrix that protects sensitive core material from physical factors like light or air (Chaturvedi, 2014). Nanopesticides are more advantageous than

conventional pesticides because efficiency of active ingredients is increased, also reduces the hazard to the human health and environment safety profiles are enhanced (Mohd Firdaus *et al.*, 2018).

Development of new pesticides: Nano-encapsulation is the process in which substances are coated by various materials in nano range. Substances being coated or encapsulated material are commonly known as core material, the fill or filler, or internal phase, for example pesticides. Materials used for encapsulation are referred to as coating, external phase, membrane or shell, for instance Nano capsules (Auffan *et al.*, 2009).

To encapsulate, different nanomaterial that have been used already includes inorganic porous nanomaterial, polymer based nanomaterial, layered double hydroxides, solid lipid nanoparticles, Nano clays. These nanomaterial that can either be used as carrier material or pesticides exhibiting excellent properties like thermal stability, permeability, stiffness, biodegradability. Active ingredients encapsulated within Nano carrier material and uniformly spreads over the surface of soil or on leaves, are readily ingested by chewing insects. Insects also absorbs it by physiosorption process through cuticular lipid layer as a result of which water protection barrier of insects breaks causing death of insect from desiccation. Nanopesticides larger surface area results in greater affinity to susceptible species thus helps in pest control. Active materials are being protected from premature degradation and also get released in controlled manner by using these nano-carrier materials (Nuruzzaman *et al.*, 2016).

Huang suggested that different polymer materials that are humidity sensitive, light sensitive, enzyme sensitive, pH-sensitive, and thermo sensitive can be used to prepare Nano-microcapsules for pesticide delivery. Nanopesticides formulated using these materials can detect environmental responses and then causes pesticide release in targeted manner. Because of their ability of responding to external stimuli, these intelligent microcapsules have gained considerable attention (Huang *et al.*, 2018)**.**

Encapsulation with polymer based materials: Polymer based materials can be used to encapsulate active ingredients. For example, Polymer Nano composites in which Nano fillers or nanoparticles being dispersed in matrix form polymer. Nano capsules that are used for encapsulating active ingredients generally have core-shell arrangement in which shell is made of polymer material while liquid core contains dissolved active substances. Pesticide formulations are loaded on inner core which may have polymeric matrix absorbing active ingredients. Nano spheres are also being used as Nano carrier system. Active compounds here are distributed uniformly and embedded in polymer material (Ezhilarasi *et al.*, 2013). Nano gels are composed of hydrogel particles dispersed in aqueous solution through chemical and physical cross linking polymers can also be used (Kabanov and Vinogradov, 2009).

Encapsulation with lipid-based nanomaterial: Lipid-based nanomaterial are being used for encapsulation of active ingredients that are hydrophobic, lipophilic and hydrophilic in nature. Hydrophobic active ingredients dispersion in aqueous solutions and bioactive compounds absorption through insect's cuticle is facilitated by them. Example includes Nano liposomes and solid lipid nanoparticles. Nano liposomes are actually vesicles composed of lipid bilayer having watery interior. Phospholipids present in aqueous solution form basis of colloidal structure (Mozafari, 2010). Solid Lipid Nanoparticles are composed of constituents like Lipids (solid at physiological temperatures), emulsifiers, surfactants and water. These nanostructure substances are either crystalline or semi-crystalline. Lipids being used here can be fatty acids, triglycerides, waxes, steroids stabilized by emulsifiers (Potta *et al.*, 2011).

Encapsulation with other materials: Porous inorganic nanomaterial can be used for encapsulation of bioactive compounds. For example, mesoporous silica nanoparticles in which deposition of pesticides is done by suspending them in solution of pesticides followed by evaporation of solvent (Popat *et al.*, 2012) Layered Double Hydroxides (LDHs) are synthetic and natural material composed of metal hydroxide layers that are positively charged can be used for encapsulation (Bi *et al.*, 2014). Clay-Based Nanomaterial's, for example Nano clays are being used for development of Nano carrier materials as well. Nano clays refers to material that is finely grained and belong to class of minerals occurring naturally as hydrous silicate or aluminium silicates (Majeed *et al.*, 2013). Many other Materials can be used for Nano encapsulation such as noisome, nano-zeolite, carbon nanotubes, polymersomes, dendrimers etc (Nuruzzaman *et al.*, 2016)**.**

Formulation of Nano-encapsulated Pesticide: Formulation of pesticide include combination of different materials like active ingredients, stabilizers, solvents and surface active ingredients. Different nanoformulations are available include micro emulsions, Nano emulsions and Nano suspensions (Nuruzzaman *et al.*, 2016).

Micro emulsions are pesticides translucent and transparent dispersions in oil or water and to solubilize them, certain additives like surfactants are added (Langevin, 1991). Nano emulsions also called as ultrafine emulsions or mini-emulsions consists of two immiscible liquid phases. One liquid phase exists as droplets of smaller spherical size that are dispersed in second liquid phase. Less Surfactant is used here as compared to micro emulsions (Kah and Hofmann, 2014). Nano suspensions refers to solid nanoparticles dispersion in liquid solution. It is colloidal dispersion in sub-micrometer range in which particles of drug get stabilized by polymer or surfactant or sometimes by both (Chingunpituk, 2011). This technology increases pesticides bioavailability by facilitating the dispersion of poorly soluble pesticides (Nuruzzaman *et al.*, 2016).

Nano biosensors: Nano biosensors are the sensors consisting of immobilized bio receptor probes for the detection of target analyte molecules. Nano biosensors not only enhances the research opportunities but also act as a potential instrument in the monitoring of biological and physiochemical changes in soil and gives an electrical signal which can be easily determined by humans (Singh and Prasad, 2017). These are the devices designed for the detection of biological or chemical component with Nano scale sensitivity by giving a digital electronic signal. Nano biosensors are reported as mini-laboratories with high potencies for monitoring seasonal and temporal changes in precision agriculture (Subramanian and Tarafdar, 2011). These detect a range of analytes including pathogens, pesticides, environmental pollutants, glucose, urea, metabolites etc.

Nano biosensors usually have three components including a

probe, a transducer and a detector. Probes could be any biologically sensitive component such as enzymes, lectins, cells, tissues, receptors, amino acids, molecular imprints etc. which are either directly obtained from biological components or are agonist molecules. These detect the biological change and transmit signal to next component named as a transducer. The transducer then measures the physical change that occurs at probe-analyte interaction site and convert this change into an electrical signal. The detector then perceives the signal and transmit it to microprocessor for amplification, after this data is analyzed and displayed in the form of output (Kaushal and Wani, 2017).

A research reported the first nanowire field biosensor based on transistor which was able to detect DNA methylation in the form of electronic signal, thus avoiding PCR amplification or bisulphite treatments which were used previously for methylated DNA detection. In the same way, Nano biosensors having protein molecules as a probe can sense the presence of special proteins. The ultra-sense detection of protein and DNA molecules play a vital role in the detection of biomarkers, plant microbes, mineral deficiencies in plants and for to differentiate one kind of plant species from the other one (Maki *et al.*, 2008).

Microorganisms produce a number of beneficial and devastating compounds for human beings such as alcohol produced by anaerobic respiration of bacteria. These pathogens cause the rotting of food and results in the production of foul odor which sometimes cannot be detected by humans and thus enhances the food poisoning. So, for these reasons rapid detecting bio elements are required which for to detect microbes in water supplies, food goods and raw food contents. Rapid detecting bio elements also saved time and cost of laborious immunoassays and microbial testing (Ditta, 2012). Latest, Nano biosensors are introduced which can rapidly detect metabolites and IgG (Dasgupta *et al.*, 2017).

By the quantitative measurement of differential oxygen consumption of bad and good microbes in the soil, Nano sensors help a lot in diagnosing the soil diseases caused by fungus, viruses, bacteria and other microorganisms. This measurement involves two sensors_impregnated with bad and good microbes respectively and then these are dipped in a soil solution and differential oxygen consumption of these two sensors are detected. By comparing the oxygen consumption data, the suitable organism for the soil can be determined. Forzani manufactured a device known as noncontact sensor to sense heavy metals in water. In this device a silicon chip is fabricated with an array of electrode rods separated from each other by a few nanometers distance. When Nano sensors are placed in a soil solution then the heavy metal ions in the solution deposit on silicon chip between electrode pairs. This deposition of ions forms a bridge between electrode rods enabling quantum jump conductance in them and thus detection of metal ions.

Nano sensors linked with zeolites also enhance the status of sustainable agriculture. As zeolites enable efficient plant growth by improving the value and efficiency of fertilizer and also by improving the infiltration and retention of water, so by linking Nano sensors with zeolites the release of nutrients and water in plants and soil can be monitored (Singh and Prasad, 2017).

Nano-sensors for disease management: The effective diagnosis and management of plant diseases has fundamental importance for food production, many plant-derived products, and for the sustainability of natural environment. Financial cost on conventional plant pathogen management is very high. The emergence of wide range pathogens and cultural context is critical. To make the successful disease-control strategies, policy makers and farmers tend to communicate and engage (Almeida, 2018).

Nanotechnology now a days is being used for the (Agrawal and Rathore, 2014) disease management in plants. Diagnosis and treatment of the disease at nano scale is acquiring attention these days. Engineered Nanoparticles (NPs) like metalloids, nanomaterial and metallic oxides have activity in elimination of plant diseases. For example, Ag, Zn and Cu NPs are toxic to microorganisms directly (Elmer *et al.*, 2018). Lipid derived Nanosensors are used to observe malnutrition level in diseased plants. These nanoparticles are lipid soluble. So, these Nano-particles can easily penetrate from membranes (Karny *et al.*, 2018). Wade H. Elmer described a new way to manage the plant diseases. According to them, engineered nanoparticles can be used for this purpose. The most used nano-particles are of Ag, Cu and Zn. These nanoparticles act as nano-sensors and also have fungicidal and bactericidal properties to enhance plant health. Other nanoparticles of B, Mn, Cu and silicon have appeared to function in host defense system as fertilizers. These NPs are more competent against pathogens than commonly used fertilizers.

Problems associated with the use of nano-biotechnology in agriculture: Most recently and commonly used engineered Nano particles are classified into five subsequent classes: zerovalent, metal oxides, quantum dots, nano-polymers and carbonaceous nanoparticles. These engineered nanoparticles can stick to roots of plants and they can cause chemical as well as physical poisonousness to plants. Nanoparticles injected to plant cells can also leach to soil and they can cause agricultural land contamination which can also contaminate and spoil food chain (Handy and Shaw, 2007).

In a study, phytotoxicity caused by five different varieties of nanoparticles (MWCNTs, zinc, zinc oxide, alumina and aluminum) on germination of seeds and roots progression of six higher plants (rye-grass, rape, radish, corn, lettuce and cucumber) was examined. Germination of seeds were not exaggerated except for reticence of Nano zinc on ryegrass and Nano zinc oxide on corn plant at a concentration of 2000mg/L. Inhibition of the growth of roots varies among different plants and nanoparticles. Nano zinc and Nano zinc oxide suspensions at concentration of 2000mg/L fired the elongation of roots of different tested plants. Low level of Nano cerium oxide reduced the soybean pods size and growth of plant. Nano-cerium oxide enter into roots of plants and are important in a process of nitrogen fixation performed by soybean crops. When amount of nano-cerium oxide is higher in soil then soybean crops are incapable to perform nitrogen fixation.

It has been also observed that very high meditation of Nano silica silver can produce chemical injuries to the plants such as cucumber leaves. Some plants which are grown hydroponically are also affected by myriad synthetic Nano materials, increasing disquiets concerning the long term effects of these synthetic materials on food supply (Lin and Xing, 2007).

CONCLUSION: Nanotechnology will suitably enhance agricultural inputs and will ensure the safety of human

beings and environment by decreasing byproducts that will ultimately improves the agricultural productivity. Much anticipated applications of nanotechnology include detections of pathogens using Nano sensors and monitoring of the plant health. Furthermore, most of the exertions are oriented for the reduction of negative influence of different agrochemical products on human health. Besides limited implementations, nanotechnology will revolutionize the future precision agricultural methodology. Nanotechnology integrated with different emerging technologies like chemical biology would confront the biological problems that cause hindrances in further development.

CONFLICT OF INTEREST

The Author has no conflict of Interest

ACKNOWLEDGEMENTS

We are highly obliged and thankful to the faculty of department of Biochemistry and Biotechnology, University of Gujrat for their encouragement and support in compiling of this article.

REFERENCES

Agrawal, S. and P. Rathore, 2014. Nanotechnology pros and cons to agriculture: A review. International Journal of Current Microbiology and Applied Sciences, 3(3): 43-55.

Almeida, R. P., 2018. Emerging plant disease epidemics: Biological research is key but not enough. PLoS biology, 16(8): e2007020.

Arora, S., P. Sharma, S. Kumar, R. Nayan, P. Khanna and M. Zaidi, 2012. Gold-nanoparticle induced enhancement in growth and seed yield of *Brassica juncea*. Plant growth regulation, 66(3): 303-310.

Auffan, M., J. Rose, J.-Y. Bottero, G. V. Lowry, J.-P. Jolivet and M. R. Wiesner, 2009. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nature Nanotechnology, 4(10): 634.

Bi, X., H. Zhang and L. Dou, 2014. Layered double hydroxide-based nanocarriers for drug delivery. Pharmaceutics, 6(2): 298-332.

Chaturvedi, D. A., 2014. Study of nano-particle based pesticides as an alternative to conventional pesticides. International Joural of Multidisciplinary Approach and Studies, 1(1): 71-75.

Chingunpituk, J., 2011. Nanosuspension technology for drug delivery. Walailak Journal of Science and Technology (WJST), 4(2): 139-153.

Dasgupta, N., S. Ranjan and C. Ramalingam, 2017. Applications of nanotechnology in agriculture and water quality management. Environmental Chemistry Letters, 15(4): 591-605.

Ditta, A., 2012. How helpful is nanotechnology in agriculture? Advances in Natural Sciences: Nanoscience and Nanotechnology (ANSN), 3(3): 033002.

Ekinci, M., A. Dursun, E. Yildirim and F. Parlakova, 2014. Effects of nanotechnology liquid fertilizers on the plant growth and yield of cucumber (*Cucumis sativus* L.). Acta Science Pol Horticulture, 13(3): 135-141.

Elmer, W. H., C. Ma and J. C. White, 2018. Nanoparticles for plant disease management. Current Opinion in Environmental Science & Health. 6 (2018): 66-70

Ezhilarasi, P., P. Karthik, N. Chhanwal and C. Anandharamakrishnan, 2013. Nanoencapsulation techniques for food bioactive components: A review. Food and Bioprocess Technology, 6(3): 628-647.

Grillo, R., P. C. Abhilash and L. F. Fraceto, 2016. Nanotechnology applied to bio-encapsulation of pesticides. Journal of Nanoscience and Nanotechnology, 16(1): 1231-1234.

Handy, R. D. and B. J. Shaw, 2007. Toxic effects of nanoparticles and nanomaterials: Implications for public health, risk assessment and the public perception of nanotechnology. Health, Risk & Society, 9(2): 125-144.

Huang, B., F. Chen, Y. Shen, K. Qian, Y. Wang, C. Sun, X. Zhao, B. Cui, F. Gao and Z. Zeng, 2018. Advances in targeted pesticides with environmentally responsive controlled release by nanotechnology. Nanomaterials, 8(2): 102.

Kabanov, A. V. and S. V. Vinogradov, 2009. Nanogels as pharmaceutical carriers: Finite networks of infinite capabilities. Angewandte Chemie International Edition, 48(30): 5418-5429.

Kah, M. and T. Hofmann, 2014. Nanopesticide research: Current trends and future priorities. Environment international, 63: 224-235.

Karny, A., A. Zinger, A. Kajal, J. Shainsky-Roitman and A. Schroeder, 2018. Therapeutic nanoparticles penetrate leaves and deliver nutrients to agricultural crops. Scientific Reports, 8(1): 7589.

Kaushal, M. and S. P. Wani, 2017. Nanosensors: Frontiers in precision agriculture. In: Nanotechnology. Springer: pp: 279-291.

Khan, F. F., K. Ahmad, A. Ahmed and S. Haider. 2017. Applications of biotechnology in agriculture-review article World Journal of Biology and Biotechnology, 2(1): 139-142.

Khati, P., S. Gangola, P. Bhatt, R. Kumar and A. Sharma, 2018. Application of nanocompounds for sustainable agriculture system. In: Microbial biotechnology in environmental monitoring and cleanup. IGI Global: pp: 194-211.

Langevin, D., 1991. Microemulsions-interfacial aspects. Journal of Colloid and Interface Science, 34: 583-595.

Lin, D. and B. Xing, 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environmental Pollution, 150(2): 243-250.

Liu, R. and R. Lal, 2015. Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. Science of the Total Environment, 514: 131-139.

Majeed, K., M. Jawaid, A. Hassan, A. A. Bakar, H. A. Khalil, A. Salema and I. Inuwa, 2013. Potential materials for food packaging from nanoclay/natural fibres filled hybrid composites. Material Design, 46: 391-410.

Maki, W. C., N. N. Mishra, E. G. Cameron, B. Filanoski, S. K. Rastogi and G. K. Maki, 2008. Nanowire-transistor based ultra-sensitive DNA methylation detection. Biosensors and Bioelectronics, 23(6): 780-787.

Mastronardi, E., P. Tsae, X. Zhang, C. Monreal and M. C. DeRosa, 2015. Strategic role of nanotechnology in fertilizers: Potential and limitations. In: Nanotechnology in food and agriculture. Springer: pp: 25-67.

Mohd Firdaus, M. A., A. Agatz, M. E. Hodson, O. S. Al-Khazrajy and A. B. Boxall, 2018. Fate, uptake, and distribution of nanoencapsulated pesticides in soil–earthworm systems and implications for environmental risk assessment. Environmental Toxicology and Chemistry, 37(5): 1420-1429.

Mousavi, S. R. and M. Rezaei, 2011. Nanotechnology in agriculture and food production. Journal of Applied

Environmental Biologyical Sciences, 1(10): 414-419.
Mozafari, M., 2010. Nanoliposomes: Preparation and analysis. In: Liposomes. Springer: pp: 29-50.
Naderi, M. and A. Danesh-Shahraki, 2013. Nanofertilizers and their roles in sustainable agriculture. International journal of Agriculture and Crop sciences, 5(19): 2229.
Nuruzzaman, M., M. M. Rahman, Y. Liu and R. Naidu, 2016. Nanoencapsulation, nano-guard for pesticides: A new window for safe application. Journal of Agricultural and Food Chemistry 64(7): 1447-1483.
Popat, A., J. Liu, Q. Hu, M. Kennedy, B. Peters, G. Q. M. Lu and S. Z. Qiao, 2012. Adsorption and release of biocides with mesoporous silica nanoparticles. Nanoscale, 4(3): 970-975.
Potta, S. G., S. Minemi, R. K. Nukala, C. Peinado, D. A. Lamprou, A. Urquhart and D. Douroumis, 2011. Preparation and characterization of ibuprofen solid lipid nanoparticles with enhanced solubility. Journal of Microencapsulation, 28(1): 74-81.
Ragaei, M. and A.-k. H. Sabry, 2014. Nanotechnology for insect pest control. International Journal of Science and Environmental Technology, 3(2): 528-545.
Ramadass, M. and P. Thiagarajan, 2017. Effective pesticide nano formulations and their bacterial degradation. In: IOP Conference Series: Materials Science and Engineering. IOP Publishing: pp: 022050.
Sekhon, B. S., 2014. Nanotechnology in agri-food production: An overview. Nanotechnology, Science and Applications, 7: 31.
Singh, A. and S. Prasad, 2017. Nanotechnology and its role in agro-ecosystem: A strategic perspective. International Journal of Environmental Science and Technology, 14(10): 2277-2300.
Subramanian, K. and J. Tarafdar, 2011. Prospects of nanotechnology in indian farming. Indian Journal of Agricultural Sciences, 81(10): 887-893.
Wang, P., E. Lombi, F.-J. Zhao and P. M. Kopittke, 2016. Nanotechnology: A new opportunity in plant sciences. Trends in Plant Science, 21(8): 699-712.

© 2020 by the authors. Licensee Scientific Platform. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Research Manuscript www.sciplatform.com

Effect of silicon and gibberellic acid on growth and flowering of gladiolus

[a] Muhammad Umer Farooq *, [a] Khalid Masood Ahmad, [a] Muhammad Adnan Sadique, [b] Farhan Shabbir, [c] Mirza Muhammad Waseem Khalid, [d] Muhammad Shahzad

[a] Department of Horticulture, Bahauddin Zakariya University Multan, Pakistan,
[b] Department of Horticulture, University of Agriculture Faisalabad, Sub-campus Burewala, Pakistan,
[c] Department of Agronomy, University of Agriculture Faisalabad, Sub-campus Burewala, Pakistan,
[d] Department of Agronomy, University of Agriculture, Faisalabad, Pakistan

Authors' Contribution	**M. U. Farooq, K. M. Ahmad and M. A. Adnan;** developed the concepts, designed the experiments and wrote the manuscript, **F. Shabbir, M. M. W. Khalid and M. Shahzad** helped in data collection and in statistical analysis
Article History	*Corresponding email address: mr.umrfarooq@gmail.com **Received: 24 December 2019, Revised: 13 February 2020, Accepted: 29 February 2020, Published Online: 04 March 2020**

ABSTRACT

Gladiolus grandiflorus is known and grown for its high profit and excellent cut flower. To compete with other growers and to meet the consumer demand the grower should adopt new techniques and apply effective chemicals to the plant precisely which in result gives good quality flowers. For this an experiment was carried out on *Gladiolus grandiflorus* cv. Rose supreme in experimental area of department of horticulture, Bahauddin Zakariya University, Multan. There were 6 treatments and 3 replications and the corms were planted in pots. Each treatment in replications is replicated four times to get best results. The sowing was done on 3rd December 2018 and the first application of chemicals was applied on 15/02/2019. The total number of applications was 6 and each application was applied by foliar application after one week interval. The chemicals were silicon and gibberellic acid. Silicon is applied as T0 (0g), T1 (1g), T2 (2g), T3 (3g), T4 (4g) and T5 (5g) while gibberellic acid has a fixed dose of 200ppm in each treatment. The best results related to vegetative and floral parameters were observed in T4 treatment plants which showed best result and an increase in stalk length, spike length, diameter of floret, diameter of spike, number of leaves per plant, vase life, number of florets per spike, fresh weight of complete flower stalk and plant height.

Key word: GA3, silicon, rose supreme

INTRODUCTION: Gladiolus is commonly known and grown for its high aesthetic and economical value, especially in Pakistan economy. It is placed second important cut flower in Pakistan while fourth most important cut flower in the world. The cultivated area of gladiolus is only 970 acre and is too small as compared to rose which is 9200 acre and tuberose which is 2787 acre (Khan, 2005). Gladiolus belongs to *Iridaceae* family; holds about 260 species but many of them are wild. Native to Africa but some species are also from Mediterranean, South Africa and from Europe (Dole and Wilkins, 1999). Progressive farmers in Pakistan are now converting to floriculture industry instead of growing traditional crops, for this rose, gladiolus, tuberose and carnation are the best flowers that give maximum profit in low time period. The total cultivated area of gladiolus in Punjab is more than 450 acres. Plant growth regulators are responsible especially for the physical attributes of a plant in an effective way. Treating plants with plant growth regulators is very mandatory to enhance the growth and yield of plants (Nuvale *et al.*, 2010). Different doses of GA3 can affect significantly on the vegetative as well as reproductive growth of gladiolus (Umrao *et al.*, 2007). GA3 can increase the height, number of florets and can initiate early sprouting of flowers (Taiz and Zeiger, 2002). Silicon is the 2nd most available element on Earth's crust; about 32 percent silicon is present in soil by weight. 1% to 10% silicon is present in plant dry matter. The available form of silicon that plant can easily uptake is called as Mono salicylic acid $Si(OH)_4$. Silicon is mostly required during vegetative as well as reproductive growth of the plant to attain healthy and maximum yield from plant (Savant *et al.*, 1997). Farmers now a days do not have proper knowledge of cultivating flowers that is the reason they apply extra chemicals to get maximum yield but cannot achieve it because the amount and type of chemical they are applying are used for traditional crops, ornamentals and flowers have their own need of different chemicals for this, the research is done to describe the role of chemicals on gladiolus to attain maximum yield with high quality flowers.

MATERIALS AND METHODS: The research was carried out at experimental area of Department of Horticulture, Bahauddin Zakariya University Multan, Pakistan. The research was done to get and to elaborate the outcome of foliar use of GA_3 and Silicon on growth, yield and flowering of *Gladiolus grandiflorus* cv. Rose supreme in pots. Soil samples were taken from various pots and then collected to check the soil properties i.e. its acid: base ratio, Electrical conductivity, form and the amount the nutrients present in the soil. The combination of soil media used in the research was 1:1 (Silt: Leaf manure) and the pots were placed according to the statistical design which was Randomized Complete Block Design (RCBD).

The corms of *Gladiolus grandiflorus* cv. Rose supreme was imported from Netherlands. The treatments were applied as 200 ppm of Gibberellic acid (GA_3) and 1, 2, 3, 4, 5 g/L of silicon as sodium meta-silicate. The treatments were applied by foliar application with different combination in a Randomized complete block design (RCBD) which is as follows in table 1.

On 3rd December, corm sowing was done. One corm was in each pot. There were total 6 treatments one was control which has only 200 ppm GA_3 while others have different silicon doses as well as has fixed dose of GA_3. Each treatment was divided into 4 pots thus total No. of pots were (6 × 3 × 4) = 72 having three replications. GA_3 and silicon was applied by foliar application with one week interval. The first foliar application was done

before stick formation and the date was 15/02/2019, while the last application was done on 22/03/2019. The total number of applications was six and was applied through foliar implementation of chemicals. The cultural practices, integrated pest management and fertilizer application were done thoroughly on each replication with equal amount of dose.

Data collection: Following were the some parameters in table 2 taken to elaborate the outcome of foliar use of GA_3 and silicon on the growth, flowering and yield of *Gladiolus grandiflorus* cv. Rose supreme.

RESULT AND DISCUSSION: Stalk length (cm): Table 3 showed the best effect of silicon and Gibberellic acid as T4 which showed maximum stalk length T4 (80.543) after that T1 (78.833), T0 (76.667), T3 (73.417) and T5 (73.167) while T2 (72.833) showed minimum stalk length. The stalk length was taken in cm. The length of stalk was approximately similar to each other by the application of silicon and Gibberellic acid. On the other hand table 4 showed the ANOVA for stalk length of gladiolus. Maximum stalk length will give maximum profit to flower growers that's why it is important to choose efficient chemicals that enhance the flower growth as well as the accurate dose of chemical is also important to get maximum results. Increase in stalk length was also reported in anthurium through foliar allocation of GA_3 (Dhaduk *et al.*, 2007).

Spike length (cm): The best results were shown in different concentrations among those T4 concentration showed best result and then T1, T3, T0, T2 and T5 respectively. The results of the chemicals on spike length are shown in table 5. Different concentrations imparts favorable impact on spike length and increase their size as T4 (33.350), T1 (31.750), T3 (29.707), T0 (28.833), T2 (28.267) and T5 (27.303). More spike length increased the profit ratio of flower grower and meets the consumer demand more precisely. Good spike length is an important constituent to increase the quality of flower. The statistical analysis i.e. ANOVA for spike length of gladiolus is shown in table 6. To increase the quality of flower it is mandatory to choose best and most effective chemical and applied with the recommended dose which in result gives maximum quality flower. It was reported that spike length and stalk length can be increased via foliar allocation of GA_3 on anthurium (Dhaduk *et al.*, 2007).

Diameter of spike (cm): The best results were shown in different concentrations among those T4 concentration showed best result and then T1, T3, T2, T0 and T5 respectively. Different concentrations imparts favorable impact on spike length and increase their size as T4 (0.5990), T1 (0.5990), T3 (0.5887), T2 (0.5867), T0 (0.5700) and T5 (0.5443) as shown in table 7. In table 8 ANOVA for diameter of spike of gladiolus showed significant results. More diameter of spike increased the profit ratio of flower grower and meets the consumer demand more precisely. Good diameter of spike is an important constituent to increase the quality of flower. To increase the quality of flower it is mandatory to choose best and most effective chemical and applied with the recommended dose which in result gives maximum quality flower.

Diameter of floret (cm): The best results were shown in different concentrations among those T4 concentration showed best result and then T1, T2, T3, T5 and T0 respectively. Different concentrations imparts favorable impact on diameter of floret and increase their size as T4 (0.6890), T1 (0.6817), T2 (0.6700), T3 (0.6600), T5 (0.6300) and T0 (0.6100) as shown in table 9. Significant results were seen in ANOVA table 10.

Quality of flower i.e. its size and color is very important to get maximum profit and to sustain in a competitive market. To achieve best flower size different chemicals and plant growth regulators are applied which have positive effects on the growth and nourishment of flower. Silicon and Gibberellic acid showed their best result at the concentrations as 200ppm Gibberellic acid and 4g of silicon.

Number of leaves per plant: Table 11 showed the best results in different concentrations among those T4 concentrations showed best result and then T1, T2, T3, T0 and T5 respectively. Different concentrations imparts favorable impact on number of leaves per plant and increase their size as T4 (8.9167), T1 (8.8333), T2 (8.6667), T3 (8.6333), T0 (8.4667) and T5 (7.5100). Number of leaves in each treatment from T0-T4 was approximately same but in T5 the number of leaves decreased. Number of leaves in any plant was most important because they are responsible for the photosynthesis which in result provides energy to the plant body to grow well. Table 12 showed statistical approach of number of leaves per plant. More number of plants will cause more photosynthesis and in result the plant grow well with good quality flowers for the consumer thus gives maximum profit to the flower grower. It is reported in different experiments that Gibberellic acid is responsible to increase the number of leaves in chrysanthemum and other cut flowers (Naira *et al.*, 2003).

Vase life (days): The best results were shown in different concentrations among those T4 concentration showed best result and then T5, T2, T0, T1 and T3 respectively. Different concentrations imparts favorable impact on vase life and increase as T4 (10.580), T5 (8.777), T2 (8.763), T0 (8.750), T1 (8.583) and T3 (8.5800). Table 13 and table 14 showed the significant results.

Concentration showed best result and then T1, T2, T3, T0 and T5 respectively. Different concentrations imparts favorable impact on days to spike emergence and the results are as T5 (122.40), T0 (115.83), T3 (114.92), T2 (114.58), T1 (113.83) and T4 (112.33). By the application of silicon and Gibberellic acid the days to spike emergence decrease significantly in each treatment while the best and early results were shown in T4 and the dose was 200ppm Gibberellic acid along with 4g of silicon. Table 15 showed different treatments and their result while table 16 showed significant results of days to spike emergence. The flower grower can get maximum profit by introducing its flowers earlier than other growers in the market, thus less competition will give more profit. It was reported that Gibberellic acid is responsible to maximum spike length and it is observed that minimum number of days required for spike emergence when Gibberellic acid is sprayed on plants (Devadanam *et al.*, 2007).

Number of florets per spike: The best results were shown in different concentrations among those T4 concentration showed best result and then T1, T3, T2, T0 and T5 respectively. The results were significantly described in table 17 and table 18. Different concentrations imparts favorable impact on number of florets per spike and the results are as T4 (10.583), T1 (9.660), T3 (9.333), T2 (9.250), T0 (8.550) and T5 (8.167). More number of florets on a single flower stalk will give more profit because it met the demand of consumer. Consumer will

pay more to get more flowers on a single flower stalk. A significant increase in number of florets per spike was noted. An increase in number of flowers was reported on some flowering plants by the foliar application of gibberellic acid (Kumar *et al.*, 2003).

Fresh weight of complete flower stalk (g): The best results were shown in different concentrations among those T4 concentration showed best result and then T1, T3, T2, T0 and T5 respectively. The results were significantly described in table 19 and table 20. Different concentrations imparts favorable impact on weight of newly harvested whole inflorescence stalk and results are as T4 (39.000), T1 (34.750), T3 (31.040), T2 (30.833), T0 (29.200) and T5 (25.320). More fresh weight of flower stalk is considered to be a good indicator for good quality flower which in result give consumer mental satisfaction as well as more profit to flower grower. Fresh weight of anthurium flower increase by the application of gibberellic acid as well as the increase the flower yield to some extent (Kumar *et al.*, 2003). It was reported in chrysanthemum that an increase in fresh weight, dry weight and size of flower was observed significantly.

Dry weight of complete flower stalk (g): Table 21 showed the best results in different concentrations among those T5 concentration showed best result and then T4, T0, T1, T2 and T3 respectively, while table 22 showed ANOVA for dry weight of complete flower stalk. Different concentrations imparts favorable impact on dry weighing of whole inflorescence stalk and the results are as T5 (13.423), T4 (13.363), T0 (13.313), T1 (13.280), T2 (13.160) and T3 (12.420). Dry weight of complete flower stalk of all treatments was approximately same. There is a very minute difference among them. It was reported in chrysanthemum that an increase in fresh weight, dry weight and size of flower was observed significantly (Nagarjuna *et al.*, 1983).

	R1	R2	R3
T0	200+0	200+1	200+3
T1	200+2	200+3	200+5
T2	200+5	200+2	200+1
T3	200+4	200+5	200+2
T4	200+1	200+4	200+0
T5	200+3	200+0	200+4

Table 1: Treatments and replications design layout of research plan.

Stalk length (cm)	Spike length (cm)	Diameter of floret (cm)
Diameter of spike (cm)	Number of leaves per plant	Vase life (days)
Days to spike emergence	Number of florets per spike	Plant height (cm)
Fresh weight of complete flower stalk (g)	Dry weight of complete flower stalk (g)	

Table 2: Parameters which are taken to evaluate the research.

Treatments	Stalk length
T0 (control)	76.667 AB
T1 (200ppm GA_3+1g silicon)	78.833 A
T2 (200ppm GA_3+2g silicon)	72.833 B
T3 (200ppm GA_3+3g silicon)	73.417 B
T4 (200ppm GA_3+4g silicon)	80.543 A
T5 (200ppm GA_3+5g silicon)	73.167 B

Table 3: Effect of silicon and gibberellic acid on stalk length of *Gladiolus grandiflorus* L. CV. rose supreme.

Source	Df	SS	MS	F	P
Blocks	2	0.709	0.3545		
Treatments	5	161.384	32.2768	5.31	0.0122
Error	10	60.780	6.0780		
Total	17	222.873			

Table 4: Analysis of variance for stalk length of *Gladiolus grandiflorus* L. CV. rose supreme as affected by silicon and gibberellic acid.

Treatments	Spike length (cm)
T0 (control)	28.833 BC
T1 (200ppm GA_3+1g silicon)	31.750 A
T2 (200ppm GA_3+2g silicon)	28.267 BC
T3 (200ppm GA_3+3g silicon)	29.707 B
T4 (200ppm GA_3+4g silicon)	33.350 A
T5 (200ppm GA_3+5g silicon)	27.303 C

Table 5: Effect of silicon and gibberellic acid on spike length of *Gladiolus grandiflorus* L. CV. rose supreme.

Source	Df	SS	MS	F	P
Blocks	2	2.8272	1.4136		
Treatments	5	77.7138	15.5428	17.84	0.0001
Error	10	8.7135	0.8713		
Total	17	89.2545			

Table 6: Analysis of variance for spike length of *Gladiolus grandiflorus* L. CV. rose supreme as effected by silicon and gibberellic acid.

Treatments	Diameter of spike (cm)
T0 (control)	0.5700 AB
T1 (200ppm GA_3+1g silicon)	0.5990 A
T2 (200ppm GA_3+2g silicon)	0.5867 A
T3 (200ppm GA_3+3g silicon)	0.5887 A
T4 (200ppm GA_3+4g silicon)	0.5990 A
T5 (200ppm GA_3+5g silicon)	0.5443 B

Table 7: Effect of silicon and gibberellic acid on diameter of spike on *Gladiolus grandiflorus* L. CV. rose supreme.

Source	Df	SS	MS	F	P
Blocks	2	0.00024	0.00012		
Treatments	5	0.00586	0.00117	2.68	0.0870
Error	10	0.00438	0.00044		
Total	17	0.01047			

Table 8: Analysis of variance for diameter of spike of *Gladiolus grandiflorus* L. CV. rose supreme as affected by silicon and gibberellic acid.

Treatments	Diameter of floret
T0 (control)	0.6100 C
T1 (200ppm GA_3+1g silicon)	0.6817 A
T2 (200ppm GA_3+2g silicon)	0.6700 A
T3 (200ppm GA_3+3g silicon)	0.6600 AB
T4 (200ppm GA_3+4g silicon)	0.6890 A
T5 (200ppm GA_3+5g silicon)	0.6300 BC

Table 9: Effect of silicon and gibberellic acid on floret diameter of *Gladiolus grandiflorus* L. CV. rose supreme.

Source	Df	SS	MS	F	P
Blocks	2	0.00101	0.00051		
Treatments	5	0.01424	0.00285	8.31	0.0025
Error	10	0.00343	0.00034		
Total	17	0.01869			

Table 10: Analysis of variance for diameter of floret of *Gladiolus grandiflorus* L. CV. rose supreme as affected by silicon and gibberellic acid.

Treatments	Number of leaves per plant
T0 (control)	8.4667 A
T1 (200ppm GA_3+1g silicon)	8.8333 A
T2 (200ppm GA_3+2g silicon)	8.6667 A
T3 (200ppm GA_3+3g silicon)	8.6333 A
T4 (200ppm GA_3+4g silicon)	8.9167 A
T5 (200ppm GA_3+5g silicon)	7.5100 B

Table 11: Effect of silicon and gibberellic acid on number of leaves per plant of *gladiolus grandiflorus* L. CV. rose supreme,

Source	Df	SS	MS	F	P
Blocks	2	1.44521	0.72261		
Treatments	5	3.93411	0.78628	3.65	0.0386
Error	10	2.15372	0.21537		
Total	17	7.53304			

Table 12: Analysis of variance for number of leave per plant of *Gladiolus grandiflorus* L. CV. rose supreme as effected by silicon and gibberellic acid.

Treatments	Vase life
T0 (control)	8.750 B
T1 (200ppm GA_3+1g silicon)	8.583 B
T2 (200ppm GA_3+2g silicon)	8.763 B
T3 (200ppm GA_3+3g silicon)	8.500 B
T4 (200ppm GA_3+4g silicon)	10.580 A
T5 (200ppm GA_3+5g silicon)	8.777 B

Table 13: Effect of silicon and gibberellic acid on vase life of *Gladiolus grandiflorus* L. CV. rose supreme.

Source	Df	SS	MS	F	P
Blocks	2	0.7283	0.36416		
Treatments	5	9.2641	1.85282	3.59	0.0405
Error	10	5.1587	0.51587		
Total	17	15.1511			

Table 14: Analysis of variance for vase life per spike of *Gladiolus grandiflorus* L. CV. rose supreme as affected by silicon and gibberellic acid.

Treatments	Days to spike emergence
T0 (control)	115.83 B
T1 (200ppm GA_3+1g silicon)	113.83 B
T2 (200ppm GA_3+2g silicon)	114.58 B
T3 (200ppm GA_3+3g silicon)	114.92 B
T4 (200ppm GA_3+4g silicon)	112.33 B
T5 (200ppm GA_3+5g silicon)	122.40 A

Table 15: Effect of silicon and gibberellic acid on days to spike emergence on *Gladiolus grandiflorus* L. CV. rose supreme.

Source	Df	SS	MS	F	P
Blocks	2	16.043	8.0217		
Treatments	5	184.717	36.9433	0.69	0.0014
Error	10	38.125	3.8125		
Total	17	238.885			

Table 16: Analysis of variance for days to spike emergence of *Gladiolus grandiflorus* L. CV. Rose supreme as effected by silicon and gibberellic acid.

Treatments	Number of florets per spike
T0 (control)	8.550 BC
T1 (200ppm GA_3+1g silicon)	9.660 AB
T2 (200ppm GA_3+2g silicon)	9.250 BC
T3 (200ppm GA_3+3g silicon)	9.333 B
T4 (200ppm GA_3+4g silicon)	10.583 A
T5 (200ppm GA_3+5g silicon)	8.167 C

Table 17: Effect of silicon and gibberellic acid on number of floret per spike on *Gladiolus grandiflorus* L. CV. Rose supreme.

Source	Df	SS	MS	F	P
Blocks	2	0.0305	0.01527		
Treatments	5	10.8484	2.16967	5.63	0.0100
Error	10	3.8505	0.38505		
Total	17	14.7294			

Table 18: Analysis of variance for number of florets per spike of *Gladiolus grandiflorus* L. CV. rose supreme as effected by silicon and gibberellic acid.

Treatments	Fresh weight of complete flower stalk
T0 (control)	29.200 C
T1 (200ppm GA_3+1g silicon)	34.750 B
T2 (200ppm GA_3+2g silicon)	30.833 C
T3 (200ppm GA_3+3g silicon)	31.040 C
T4 (200ppm GA_3+4g silicon)	39.000 A
T5 (200ppm GA_3+5g silicon)	25.320 D

Table 19: Effect of silicon and gibberellic acid on fresh weight of complete flower stalk of Gladiolus grandiflorus L. CV. Rose supreme.

Source	Df	SS	MS	F	P
Blocks	2	3.924	1.9620		
Treatments	5	332.199	66.4398	48.23	0.0000
Error	10	13.775	1.3775		
Total	17	349.898			

Table 20: Analysis of variance for fresh weight of complete flower stalk of *Gladiolus grandiflorus* L. CV. rose supreme as affected by silicon and gibberellic acid.

Treatments	Dry weight of complete flower stalk
T0 (control)	13.313 A
T1 (200ppm GA_3+1g silicon)	13.280 A
T2 (200ppm GA_3+2g silicon)	13.160 A
T3 (200ppm GA_3+3g silicon)	12.420 A
T4 (200ppm GA_3+4g silicon)	13.363 A
T5 (200ppm GA_3+5g silicon)	13.423 A

Table 21: Effect of silicon and gibberellic acid on dry weight of complete flower stalk of *Gladiolus grandiflorus* L. CV. rose supreme.

Source	Df	SS	MS	F	P
Blocks	2	1.4700	0.73500		
Treatments	5	2.0886	0.41772	0.41	0.8295
Error	10	10.1152	1.01152		
Total	17	13.6738			

Table 22: Analysis of variance for dry weight of complete flower stalk of *Gladiolus grandiflorus* L. CV. Rose supreme as effected by Silicon and Gibberellic acid.

Plant height (cm): The best results were shown in different concentrations among those T4 concentration showed best result and then T1, T0, T3, T2 and T5 respectively. The results were significantly described in table 23 and table 24. Different concentrations imparts favorable impact on plant height and the results are as T4 (65.350), T1 (64.070), T0 (61.340), T3 (58.383), T2 (57.633) and T5 (57.513). Plant height is one of the most important parts of any plant. Consumers like the flowers which have more height because more flower height will increase the number of floret per spike. 4g silicon along with 200ppm of Gibberellic acid is recommended to increase the plant height effectively. An increase in plant height, number of leaves and branches was reported by the foliar application on Gibberellic acid on chrysanthemum and on other cut flowers (Kumar *et al.*, 2003; Naira *et al.*, 2003).

Treatments	Plant height
T0 (control)	61.340 B
T1 (200ppm GA_3+1g silicon)	64.070 A
T2 (200ppm GA_3+2g silicon)	57.633 C
T3 (200ppm GA_3+3g silicon)	58.383 C
T4 (200ppm GA_3+4g silicon)	65.350 A
T5 (200ppm GA_3+5g silicon)	57.513 C

Table 23: Effect of silicon and gibberellic acid on plants height of *Gladiolus grandiflorus* L. CV. rose supreme.

Source	Df	SS	MS	F	P
Blocks	2	2.545	1.2727		
Treatments	5	174.942	34.9883	34.15	0.0000
Error	10	10.247	1.0247		
Total	17	187.734			

Table 24: Analysis of variance for plant height of *Gladiolus grandiflorus* L. CV. Rose supreme as effected by silicon and gibberellic acid.

Conclusion

The research was done in research area of Horticulture department, Bahauddin Zakariya University Multan. Randomized complete block design (RCBD) was the model on which the experiment was laid out. Total number of treatments were 6 which are as T0 (200ppm GA3), T1 (200ppm GA3+ 1g Silicon), T2 (200ppm GA3+ 2g Silicon), T3 (200ppm GA3+ 3g Silicon), T4 (200ppm GA3+ 4g Silicon) and T5 (200ppm GA3+ 5g Silicon). The best results were observed in T4 plants which has maximum effect of silicon as well as gibberellic acid. In most parameters T5 showed repellent effects due to high amount of silicon dose.

CONFLICT OF INTEREST

The Author has no conflict of Interest

REFERENCES: Devadanam, A., B. Shinde, P. Sable and S. Vedpathak, 2007. Effect of foliar spray of plant growth regulators on flowering and vase life of tuberose (*Polianthes tuberosa* L.). Journal of Soils Crops, 17(1): 86-88.

Dhaduk, B., S. Kumari, A. Singh and J. Desai, 2007. Response of gibberellic acid on growth and flowering attributes in anthurium (*Anthurium andreanum* Lind.). Journal of Ornamental Horticulture, 10(3): 187-189.

Dole, J. M. and H. F. Wilkins, 1999. Floriculture: principles and species. Prentice Hall, Upper Saddle River, U.S.A. pp. 578-581.

Khan, M., 2005. Development of commercial floriculture in Asia and the pacific-issues, challenges and opportunities. In: The national seminar on streamlining: Production and export of cut-flowers and house plants, march 2nd to 4th, 2005, Islamabad, Pakistan. HFP.

Kumar, P., S. Raghava and R. Misra, 2003. Effect of biofertilizers on growth and yield of *China aster*. Journal of Ornamental Horticulture, 6(2): 85-88.

Nagarjuna, B., V. Reddy, M. Rao and E. Reddy, 1983. Effect of growth regulators and potassium nitrate on growth flowering and yield of chrysamthemum. South Indian Horticulture, 36: 136-140.

Naira, Sujatha A and K. Shiva, 2003. Performance of selected gladiolus (*Gladiolus floribundus*) varieties under bay island conditions. Indian Journal of Agricultural Science, 73(7): 397-398.

Nuvale, M., S. Aklade, J. Desai and P. Nannavare, 2010. Influence of pgr's on growth, flowering and yield of chrysanthemum (*Dendranthem grandiflor*a Tzvelev) cv.'Iihr-6'. International Journal Pharmancy Bioscience, 1(2): 1-4.

Savant, N. K., L. E. Datnoff and G. H. Snyder, 1997. Depletion of plant-available silicon in soils: A possible cause of declining rice yields. Communications in Soil Science Plant Analysis, 28(13-14): 1245-1252.

Taiz, L. and E. Zeiger, 2002. Plant physiology (sunderland: Sinauer). Sunderland, Sinauer Associates: 559-590.

Umrao, V. K., R. Singh and A. Singh, 2007. Effect of gibberellic acid and growing media on vegetative and floral attributes of gladiolus. Indian Journal of Horticulture, 64(1): 73-76.

© 2020 by the authors. Licensee Scientific Platform. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Research Manuscript www.sciplatform.com

Prediction of live body weights in dromedary camels (*Camelus dromedarius*) from morphometric body measurements

[a b] **Abdelaziz Ahmed Fadlelmoula***, [a] **Mohammed Saeed Mudarris and** [a] **Mohammed Salih Hariri**
[a] Department of Biology, College of Sciences and Arts at Al-Kamel, University of Jeddah, Saudi Arabia,
[b] Department of Genetics and Animal Breeding, Faculty of Animal Production, Shambat-Sudan.

Authors' Contribution	**Saleem, A. A. F.** conducted the experiment **and M. S. Mudarris** and **M.S. Hariri** helped in data analysis and wrote the manuscript
Article History	*__Corresponding email address__: aasalem2@uj.edu.sa **Received: 13 January 2020, Revised: 22 January 2020, Accepted: 25 January 2020, Published Online: 27 January 2020**

ABSTRACT

To predict the live body weight (LBW) of a dromedary camel from morphometric body measurements (MBM) a regression equation was developed. A total of 223 camels (54 males and 169 females) from the Makkah region of Saudi Arabia were classified into three age groups; first (<5 years old), second (5-8 years old) and third (>8 years old); two groups according to sex (male, female). LBW and 10 MBM were obtained. Data were subjected to statistical analysis. Results showed a significant ($p<0.05$) high mean LBW and MBM in males compared to females. A significant ($p<0.05$) positive correlations were encountered between LBW and HRG in all age groups, BG in third age group and males in the second age group, HH and WH in the first age group. The best fir regression equations were found to include HRG, BG and HG in the first and third age groups with R^2 account for 90.59% and 93.82% respectively. Whereas in the second age group as well as pooled data the equation included HRG, BG, HH, HG and WH with R^2 at a level of 99.69% and 99.48% respectively. Multi-collinearity problem of MBM was not encountered as determined by VIF which was found to be less than 10. These formulas could be used for predicting LBW where weighing scales are not available.

Key word: Live body weight, morphometric body measurement; regression equation; variance inflation factor; correlation coefficient

INTRODUCTION: Traditionally and historically camel is considered as the major livestock in the Kingdom of Saudi Arabia, where the arid environment favors the breeding and growth of the animal. Camel is also a major source of meat and milk in the kingdom where in recent years the production of both meat and milk has increased by 5.4% and 6.4%, respectively (Abdallah and Faye, 2012). Most of the farmers here use traditional techniques for production and the traders rely on visual inspection for evaluation of body weight and pricing. However, a number of studies have shown that live body weight played an important role in many livestock production systems. Assan (2013) reported a direct relationship between LBW and production and profitability. Pesmen and Yardimci (2008) determined several important economic characteristics of farm animals from LBW. LBW can be used for selecting animals for meat (Van *et al.*, 2000; Mendeş *et al.*, 2005; Abbasi and Ghafouri-Kesbi, 2011), as an index for health and production, as management tool to assess growth rate and feeding systems, prediction of carcass characteristics and body conformation (Abdallah and Faye, 2012) and a criteria for description of phenotypic characteristics. The use of MBM in estimating the LBW was reported to be more practical in areas where accurate weighing scales and animals restraining facilities are not available to livestock farmers and breeders. Several studies explained the use of linear body measurements as a tool for estimating and predicting the LBW of livestock animals (Tadesse and Gebremariam, 2010; Ishag *et al.*, 2011; Oke and Ogbonnaya, 2011). Live body weight was found to be closely correlated with body measurements (Singh and Mishra, 2004; Hamayun *et al.*, 2006; Mungai *et al.*, 2010). Multiple regression models were developed for predicting live body weight in various livestock using MBM, with ultimate interpretation of the relationship between LBW and MBM (Ozkaya and Bozkurt, 2009; Tadesse and Gebremariam, 2010; Yakubu *et al.*, 2012).

OBJECTIVES

The current study was conducted to develop predicting regression equations for the LBW from some MBM of Saudi camels and to explore the relationship between body weight and linear body measurements.

MATERIALS AND METHODS: Study site and data collection: The study was carried out in the Makkah region of Saudi Arabia and it covers an area extending between latitude 22^0 N to 23^0 N. Residents of the area, their activities, the methods of body measurements and definitions were described previously by Fadlelmoula *et al.* (2015). A total number of 223 Saudi camels (54 male and 169 females) aged between 4-13 years were investigated. Data were classified into three groups according to age (First=> 5 years, second=5-8 years, and third=< 8 years), two groups according to sex (males and females). Body traits measured were; live body weight (LBW), neck length (NL), heart girth (HRG), barrel girth (BG), hip girth (HG), body length (BL), leg length (LL), hip height (HH), wither height (WH), body height (BH) and arm length (AL). Morphometric body measurements (MBM) were determined using a measuring metric tape, while the age estimation was based on dentition, owner and livestock attendant's experience.

Data analysis: SAS-Package was used to perform the following statistical analyses: Descriptive statistics of LBW and MBM in males, females and combined male and female of the three age groups (first, second and third). Correlation between body weight and MBM to determine the traits showing strong correlation with body weight to be included in the regression model. Pearson's correlation coefficients were attained for each age group, sex and for the pooled data.

Best predictive regression equations of LBW as dependent variable and other MBM as independent variables for each age group and the pooled data irrespective of age and sex were

obtained according to the following regression model:

$Y_{ij}=b_0+bx_i+e_i$ Where,

Y_{ij}= the LBW of the j^{th} animal, b_0= the intercept;

b= the regression coefficient of live body weight (Y) on MBM (x),

x_i= the MBM (HRG, BG, HG, HH, WH, BL),

e_{ij}= the residual error.

Variance inflation factors (VIF) as multicollinearity diagnostic tool of the independents variables (MBM) incorporated in the multiple regression models.

Linear, quadratic and cubic effects of HRG and BG (independent variables) on live body weight (dependent variable) which were included in the following best fitted regression model:

$Y_{ij}=b_0+b_1x_i+b_2x_i^2+b_3x_i^3+e_{ij}$

Where,

Y_{ij}= the LBW of the j^{th} animal,

b_0= the intercept;

b_1,b_2 and b_3= the corresponding linear, quadratic and cubic regression coefficients,

x_i= the MBM (HRG, BG, HG, HH, WH, BL),

e_{ij}= the residual error.

RESULTS: table 1 showed the descriptive statistics of LBW and MBM in dromedary camels. It was observed that the traits studied were significantly higher in males than in females ($p<0.05$) and shows an increasing trend in males of all age groups compared to females. Age was found to exert a significant effect ($p<0.05$) on LBW and MBM measured except for LL and AL. The correlation coefficients of LBW and MBM are presented in table 2. Output results indicated that HRG, BG, HG and WH had moderate to strong positive correlations with LBW in all age groups. However, HRG had stronger significance ($p<0.05$) and a positive correlation with LBW in females and combined males and females in all age groups compared to males. The predictive regression equations and coefficient of determination of variation (R^2) expressed as percentage of variation for LBW using HRG, BG, HG, HH, WH and BL in the three age groups were shown in table 3. The model excluded the negatives as well as weakly correlated variables. Results indicated that using a combination of HRG, BG and HG account for 93.82% of the variation in LBW in camels aged more than 8 years old and those with less than 5 years old. The VIF ranges for both the age groups were 1.00 -1.49 and 1.16 – 1.50, respectively, indicating lack of multi-collinearity problem among the independent variables (Table 4). Whereas, using HRG, HH, WH, BG explained 99.69% of the total variation in LBW in individuals aged 5-8 years old, and the addition of BL seems not to affect the model in this group as the R^2 was not changed (99.69%). The VIF calculated for this age group was in the range 1.14 – 3.30 (Table 4).

Still no multi-collinearity problems among the independent variables were detected. With ignorance of sex and age; the best fit regression model was found to include HRG, BG, HG, HH and WH with R^2 account for 99.48% of the variation in LBW and VIF ranged 1.19–3.04, revealing the inexistence of multi-collinearity problem. It has been observed that R^2 increased as more independent variables added to the model; therefore R^2 alone could not be used to judge the accuracy and precision of the model. Hence; variance inflation factor (VIF) was used to detect the problem of multicollinearity and in this study only independent variables with VIF less than 10 and positively correlated with LBW were included in the model. Linear, quadratic and cubic coefficients of HRG and BG was found to be higher as determined by R^2 for camels with 5-8 years old followed by the camels of age more than 8 years old and then camels less than 5 years old (Table 5).

Discussion: Inclusion of MBM in linear regression models has recently indicated as a useful tool for estimation and prediction of LBW in livestock animals (Keith *et al.*, 2009; Mungai *et al.*, 2010). In this study, the mean LBW and MBM were found to be significantly affected by sex and age of the animal; males were heavier and scored high measures than females and the same trend was shown by older animals compared to younger and middle age individuals, this variation could be attributed to the fact that at this age animals attained their mature body size and measures, the variations between sexes could be due to sexual dimorphism among camel types. Comparable results were reported by Yohannes and Gebru (2006), Ishag *et al.* (2011) and Yosef *et al.* (2014). From the correlation coefficients it was observed that HRG was the only MBM that shows significant positive correlation in males, females and their combination for all the three age groups. And the absolute high correlations between LBW, and HRG were recorded for females less than 5 years old, males, females and their combination with 5-8 years old and females more than 8 years old indicating that those individuals are likely to have high LBW, and also could be an explanation to the fact that at maturity HRG and LBW remain unchanged. LBW was also strongly positively correlated with BG, HG in individual camels more than 8 years old and males less than 5 years old, respectively, indicating that such body measurement could be good predictors of LBW. However, variable positive correlation coefficients ranged from medium to moderate were obtained for the rest of MBM.

This finding goes in line with Mungai *et al.* (2010) and Abdallah and Faye (2012) in dromedary camel. Similar observations in other livestock were demonstrated by Ozkaya and Bozkurt (2009) and Mahmud *et al.* (2014) in beef cattle, and in sheep by Boujenane and Halhaly (2015). Prediction of LBW from MBM was recognized using different multiple regression models for individual age group. It could be noticed that with the inclusion of HRG alone R^2 ranged between 70.00% - 76.20% in all the three age groups and increased up to 93.82% when BG and HG were included in the model in the first and third age groups. However, R^2 reached 99.69% of the total variation for the model, including HH, WH and BL in addition to HRG and BG in the second age group individuals.

This finding indicated that the model which includes HRG, BG and HG was the best fit for prediction of LBW in dromedary camels with less than fiver years old as well as individuals with more than eight years old; the result was also confirmed with VIF which was found to be less than 10. Comparable findings were reported by Kuria *et al.* (2007) and Mungai *et al.* (2010). For the pooled data (regardless of age and sex); a best fit regression model that include HRG, BG, HG, HH and WH was found to cause 99.48% of the total variation in LBW, which was in close resemblance to the best fit model in the second age group, indicating that in this study age played a little role in changing the structure of the model with inclusion of more MBM. The findings were like the one of Tadesse and Gebremariam (2010).

9	Sex	BWT (KG)	NL (M)	HRG (M)	BG (M)	HG (M)	BL (M)	LL (M)	HH (M)	WH (M)	BH (M)	AL (M)
< 5	Male	526.71±	1.35±	2.11±	2.53±	1.54±	1.61±	1.41±	1.81±	1.85±	2.22	1.31±
	(13)	108.25^{a}	0.81^{a}	0.32^{a}	0.14 ^{a}	0.20^{ab}	0.09^{a}	0.19^{NS}	0.15 ^{a}	0.15 ^{a}	±0.29	0.15 ^{NS}
	Female	465.69±	1.25±	2.02±	2.42±	1.52±	1.52±	1.40±	1.74±	1.78±	2.08±	1.29±
	(46)	86.38^{ab}	0.34^{ab}	0.27 ^{ab}	0.19^{ab}	0.12^{ac}	012^{ab}	0.18 ^{NS}	0.11 ^{ac}	0.10 ^{ac}	0.34 ^{ac}	0.18 ^{NS}
	Male+Female	479.13±	1.31±	2.04±	2.45±	1.57±	1.55±	1.47±	1.77±	1.80±	2.12	1.30±
	(59)	94.15^{ac}	0.58^{ac}	0.28 ^{ac}	0.18^{ac}	0.15^{a}	0.12^{ac}	0.19 ^{NS}	0.13^{ab}	0.11^{ab}	±0.34 ^{ac}	0.18 ^{NS}
	Male	603.00±	1.41±	2.30±	2.62±	1.64±	1.67±	1.51±	1.86±	1.89±	2.24±	1.39±
5-8	(24)	101.19^{a}	0.76^{a}	0.26 ^{a}	0.16 ^{a}	0.11^{a}	0.16 ^{a}	0.12 ^{NS}	0.11 ^{a}	0.11 ^{a}	0.29^{a}	0.14 ^{NS}
	Female	509.67±	1.27±	2.09±	2.51±	1.63±	1.60±	1.49±	1.80±	1.82±	2.18±	1.39±
	(83)	115.64^{ab}	0.49^{ab}	0.35 ^{ab}	0.21 ^{ab}	0.18 ^{ab}	0.16 ^{ab}	0.20 ^{NS}	0.10 ^{ab}	0.10 ^{ac}	0.25 ^{ac}	0.19 ^{NS}
	Male+Female	530.75±	1.34±	2.14±	2.53±	1.63±	1.61±	1.49±	1.81±	1.84±	2.19±	1.39±
	(107)	118.96^{ac}	0.59^{ac}	0.34 ^{ac}	0.20 ^{ac}	0.17 ^{ab}	0.16 ^{ab}	0.19 ^{NS}	0.11 ^{ab}	0.10 ^{ab}	0.26 ^{ac}	0.18 ^{NS}
	Male	636.19±	1.39±	2.35±	2.66±	1.74±	1.96±	1.54±	1.89±	1.90±	2.52±	1.48±
> 8	(17)	118.51^{a}	0.48^{a}	0.17 ^{a}	0.22 ^{a}	0.12^{a}	0.33 ^{a}	0.18 ^{NS}	0.14 ^{a}	0.14 ^{a}	0.36^{a}	0.19 ^{NS}
	Female	559.17±	1.29±	2.18±	2.62±	1.64±	1.67±	1.45±	1.81±	1.84±	2.28±	1.37±
	(40)	116.25^{ab}	0.64^{ab}	0.37 ^{ab}	0.16 ^{ac}	0.11 ^{ac}	0.15 ^{ac}	0.11 ^{NS}	0.13 ^{ac}	0.10 ^{ab}	0.37 ^{ac}	0.10 ^{NS}
	Male+Female	582.15±	1.34±	2.23±	2.64±	1.67±	1.76±	1.48±	1.84±	1.86±	2.35±	1.40±
	(57)	121.19^{ac}	0.62^{ac}	0.34 ^{ac}	0.18 ^{ac}	0.12 ^{ab}	0.20 ^{ab}	0.14 ^{NS}	0.12 ^{ab}	0.12 ^{ab}	0.38 ^{ab}	0.14 ^{NS}

Table 1: Means and standard deviations (M±SD) of live body weight and MBM in dromedary camels at various age groups.
a, ab, ac Means bearing different superscript letters are significantly different (p<0.05), NS= Not significant, values between brackets represent number of records.

Age (years)	Sex	NL (M)	HRG (M)	BG (M)	HG (M)	BL (M)	LL (M)	HH (M)	WH (M)	BH (M)	AL (M)
	Male (13)	**0.58****	**0.64*****	**0.84*****	**0.82*****	-0.02 ^{NS}	**0.75*****	**0.74*****	**0.82*****	0.49*	**0.68****
< 5	Female (46)	0.38*	**0.91*****	0.28^{NS}	0.19^{NS}	0.24 ^{NS}	0.40*	**0.50****	**0.51****	-0.15 ^{NS}	0.36*
	Male+Female (59)	0.47*	**0.84*****	0.48*	0.45*	0.24 ^{NS}	**0.57****	**0.63****	**0.65****	0.12 ^{NS}	0.54**
	Male (24)	0.24^{NS}	**0.90*****	**0.64****	0.40*	0.30 ^{NS}	0.27 ^{NS}	0.31*	**0.55****	0.25 ^{NS}	0.44*
5-8	Female (83)	0.40*	**0.92*****	0.49*	0.39*	**0.56****	0.41*	**0.52****	**0.55****	0.14 ^{NS}	0.32*
	Male+Female (107)	0.42*	**0.92*****	**0.55****	0.38*	**0.54****	0.39*	**0.51****	**0.59****	0.19 ^{NS}	0.31*
	Male (17)	0.47*	**0.83*****	**0.51****	0.39*	0.38*	0.29 ^{NS}	0.48*	0.49*	-0.20 ^{NS}	**0.61****
> 8	Female (40)	0.19^{NS}	**0.90*****	**0.56****	**0.54****	0.41*	0.45*	0.34*	0.35*	0.04 ^{NS}	-0.18 ^{NS}
	Male+Female (57)	0.39*	**0.88*****	**0.57****	**0.54****	0.45*	0.42*	0.45*	0.44*	0.04 ^{NS}	-0.02 ^{NS}

Table 2: Correlation coefficients® of body weight and body morphometric measurements in dromedary camels at various age groups.
®Bold figures indicate moderate to strong positive correlation between the LBW and the corresponding MBM.
*=p<0.05, **=p<0.01, ***p=<0.001, ^{NS}= Not significant (p>0.05), values between brackets represent number of records.

Age (years)	Regression equations	R^2(%)
	Y= -111.61+289.17HRG*	76.20
<5 (59)	Y= -535.88+258.93HRG+198.47BG^{NS}	90.21
	Y= -553.35+252.41HRG*+180.78BG*+47.34HG^{NS}	90.59
	Y= -148.52+317.71HRG*	71.00
5-8 (107)	Y= -491.27+292.62HRG*+218.54HH*	87.27
	Y= -572.20+283.08HRG*-68.76HH^{NS}+203.16WH*	87.77
	Y=-1066.60+247.22HRG*-10.75HH^{NS}+307.23WH*+206.99BG*	99.69
	Y= -1064.20+247.17HRG*-12.22HH^{NS}+306.91WH*+206.34BG*+2.77BL^{NS}	99.69
	Y= -91.76+301.89HRG*	71.00
>8 (57)	Y= -871.41+297.03HRG*+299.59AG*	91.78
	Y= -1011.30+294.06HRG*+218.90BG*+215.16HG*	93.82
	Y= -148.77+317.74HRG*	78.86
	Y= -644.25+283.55HRG*+224.07BG*	92.72
All age groups (223)	Y= -687.12+278.17HRG*+201.30BG*+69.09HG*	93.34
	Y= -923.73+261.94HRG*+200.21BG*+02.52HG^{NS}+211.21HH*	96.53
	Y= -1083+252.15HRG*+210.41BG*-08.43HG^{NS}-12.30HH^{NS}+315.10WH*	99.48

Table 3: . Best prediction regression equations and coefficient of determination of variation (R^2) of live body weight in dromedary camels at different age groups
*= significant at p<0.05, ^{NS}= Not significant, values between brackets represent number of records.

Age (Years)	Independent variables (MBW)	Variance Inflation Factors (VIF)
	HRG*	1.00
<5 (59)	HRG*+BG*	1.06, 1.06
	HRG*+BG*+HG^{NS}	1.16, 1.36, 1.49
	HRG*	1.00
	HRG*+HH*	1.16, 1.16
5-8 (107)	HRG*+HH^{NS}+WH*	1.10, 2.67, 2.61
	HRG*+HH^{NS}+WH*+BG*	1.12, 2.89, 2.61, 1.24
	HRG*+HH^{NS}+WH*+BG*+BL^{NS}	1.14, 3.30, 2.64, 1.30, 1.27
>8 (57)	HRG*	1.00
	HRG*+BG*	1.00, 1.00
	HRG*+BG*+HG*	1.00, 1.50, 1.50
	HRG*	1.00
	+HRG*+BG*	1.07, 1.07
All age groups (223)	HRG*+BG*+HG*	1.10, 1.31, 1.33
	HRG*+BG*+HG^{NS}+HH*	1.17, 1.31, 1.57, 1.37
	RG*+BG*+HG^{NS}+HH^{NS}+WH*	1.19, 1.32, 1.58, 3.04, 2.87

Table 4: Variance inflation factors (VIF) of multiple regression models for MBM
*= significant at p<0.05, ^{NS}= Not significant, values between brackets represent number of records.

Age (years)	Body measurement (m)	Intercept (b_0)	Linear (x_1)	Quadratic (x_1^2)	Cubic (x_1^3)	R^2 (%)
< 5 (59)		-91.76	301.89*	-	-	70.00
5-8 (107)	HRG	-148.52	317.71*	-	-	84.02
> 8 (57)		-111.61	289.19*	-	-	76.61
< 5 (59)		214.38	-17.03^{NS}	79.66*	-	72.10
5-8 (107)	HRG	25.00	143.59^{NS}	42.40*	-	84.76
> 8 (57)		325.69	-204.18^{NS}	134.15*	-	81.32
< 5 (59)		1332.35	-1892.60^{NS}	1057.61^{NS}	-161.90^{NS}	72.94
5-8 (107)	HRG	1387.04	-2131.04*	1225.54*	-194.75*	87.66
> 8 (57)		2536.04	-4163.77*	2368.45*	-403.49*	85.32
< 5 (59)		-250.69	315.64*	-	-	23.00
5-8 (107)	BG	-277.46	319.16*	-	-	30.01
> 8 (57)		-248.41	297.08*	-	-	32.35
< 5 (59)		3028.45	-2191.93^{NS}	477.07^{NS}	-	24.68
5-8 (107)	BG	392.31	-271.52^{NS}	127.99^{NS}	-	31.78
> 8 (57)		1157.39	-847.36^{NS}	231.68^{NS}	-	33.36
< 5 (59)		-101615.00	116536.00*	-44276.00*	5608.76*	31.27
5-8 (107)	BG	646.77	-657.91^{NS}	313.14^{NS}	-28.49^{NS}	31.80
> 8 (57)		-11287.00	14106.00^{NS}	-5729.02^{NS}	788.21^{NS}	33.84

Table 5: Regressions of live body weight on linear, quadratic and cubic effects of HRG and BG at different age groups. *= significant at p<0.05, ^{NS}= not significant, values between brackets represent number of records.

CONCLUSION: The study concluded that LBW in the dromedary camel could be predicted using MBM. MBM with moderate to high positive correlation with LBW can be included in the best fit regression model; as such relationship could predict the LBW fairly accurately.

ACKNOWLEDGMENTS: This Project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University-Jeddah, under grant no. (42/856/1434). The authors, therefore, acknowledge with thanks DSR for technical and financial support.

REFERENCES: Abbasi, M.-A. and F. Ghafouri-Kesbi, 2011. Genetic (CO) variance components for body weight and body measurements in makooei sheep. Asian-Australasian journal of animal sciences, 24(6): 739-743.

Abdallah, H. R. and B. Faye, 2012. Phenotypic classification of saudi arabian camel (*Camelus dromedarius*) by their body measurements. Emirates journal of food agriculture, 24(3): 272-280.

Assan, N., 2013. Bioprediction of body weight and carcass parameters from morphometric measurements in livestock and poultry. Scientific journal of review, 2(6): 140-150.

Boujenane, I. and S. Halhaly, 2015. Estimation of body weight from heart girth in sardi and timahdite sheep using different models. Iranian journal of applied animal science, 5(3): 639-646.

Fadlelmoula, A. A., M. S. Mudarris and M. S. Hariri, 2015. Phenotypic classification based on body measurements and body features of some saudi camel types (*Camelus dromedarius*). Journal of camel practice research, 22(2): 265-270.

Hamayun, K. F. M., R. Ahmad, G. Nawaz and M. Zubair, 2006. Relationship of body weight with linear body measurements in goats. In: Agriculture and biological Sciences. Journal of Agricultre and Biology Science, 1(3), 51-

55.
Ishag, I., M. Eisa and M. Ahmed, 2011. Effect of breed, sex and age on body measurements of sudanese camels (*Camelus dromedarius*). Australian journal of basic applied sciences, 5(6): 311-315.
Keith, L., C. Okere, S. Solaiman and O. Tiller, 2009. Accuracy of predicting body weights from body conformation and testicular morphometry in pubertal boer goats. Research journal of animal sciences, 3(2): 26-31.
Kuria, S., R. Wahome, C. Gachuiri, M. Wanyoike and J. Mwangi, 2007. Use of linear body measurements in estimating live weight of camel (*Camelus dromedarius*), calves in kenya. Journal of camel practice research, 14(1): 21-25.
Mahmud, M. A., P. Shaba, W. Abdulsalam, H. Y. Yisa, J. Gana, S. Ndagi and R. Ndagimba, 2014. Live body weight estimation using cannon bone length and other body linear measurements in nigerian breeds of sheep. Journal of advanced veterinary animal research, 1(4): 169-176.
Mendeş, M., A. Karabayir and A. Pala, 2005. Path analysis of the relationships between various body measures and live weight of american bronze turkeys under three different lighting programs. Tarım bilimleri dergisi, 11(2): 184-188.
Mungai, I. P., W. R. Githaiga and M. Wanyoike, 2010. Correlation of actual live weight and estimates of live weights of camel calves (*Camelus dromedarius*) in samburu district of northern kenya. Journal of camelid science, 3: 26-32.
Oke, U. and E. Ogbonnaya, 2011. Application of physical body traits in the assessment of breed and performance of wad sheep in a humid tropical environment. Livestock research for rural development, 23(2): 15-25.
Ozkaya, S. and Y. Bozkurt, 2009. The accuracy of prediction of body weight from body measurements in beef cattle. Archiv tierzucht, 52(4): 371-377.
Pesmen, G. and M. Yardimci, 2008. Estimating the live weight using some body measurements in saanen goats. Archiva zootechnica, 11(4): 30-40.
Singh, P. and A. Mishra, 2004. Prediction of body weight using conformation traits in barbari goats. The Indian journal of small ruminants, 10(2): 173-173.
Tadesse, A. and T. Gebremariam, 2010. Application of linear body measurements for live body weight estimation of highland sheep in tigray region, North-Ethiopia. Journal of the dry lands, 3(2): 203-207.
Van, M.-K., E, B. E. Mostert and Van der Westhuizen, 2000. Body measurements as selection criteria for growth in south african hereford cattle. Archive furit tierzucht, 43(1): 5-16.
Yakubu, A., S. A. Okunsebor, A. A. Kigbu, A. O. Sotolu and T. D. Imgbian, 2012. Use of factor scores for predicting body weight from some morphometric measurements of two fish species in nigeria. Journal of agricultural science, 4(8): 60.
Yohannes, M. Z. M. and G. Gebru, 2006. Camel and camel product marketing in babilie and kebribeyah woredas of the jijiga zone, somali region, ethiopia. Livestock research for rural development, 19(4): 1-9.
Yosef, T., K. Kefelegn, Y. Mohammed, U. Mengistu, A. Solomon, D. Tadelle and J. Han, 2014. Morphological diversities and eco-geographical structuring of ethiopian camel (*Camelus dromedarius*) populations. Emirates journal of food agriculture, 26(4): 371-389.

© 2020 by the authors. Licensee Scientific Platform. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Short Communication www.sciplatform.com

Occurrence of Jellyfish *Crambionella orsini* (Vanhöffen, 1888) (Cnidaria: Scyphozoa) along the coast of Pakistan

Shahnawaz Gul
Jamia Millia Government Degree College, Malir, Karachi, Pakistan.

Authors' Contribution: "**S. Gul** identified the species and wrote the manuscript".

***Corresponding email address**: gulshahnawaz@yahoo.com

Article History
Received: 26 December 2019 Revised: 23 February 2020, Accepted: 23 February 2020 Published Online: 24 March 2020

ABSTRACT

This study reports jellyfish *Crambionella orsini* from the coast of Pakistan for the first time. The species is briefly described and its distribution is given. This is an edible jellyfish in Southeast Asia. Its abundance in the region may benefit fishermen in terms of fishery.

Key word: *Catostylidae, Crambionella*, Arabian Sea, Pakistan, first record, fishery

INTRODUCTION: *Crambionella orsini* (Vanhöffen, 1888) is a scyphozoan jellyfish originally described from Ethiopia, Red Sea and is known to have commercial value as a food delicacy in Southeast Asian countries (Omori and Nakano, 2001). In the Arabian Sea, the species was reported to form a widespread bloom in the Gulf of Oman and the Persian Gulf in 2002-2003, which markedly affected the fishing operations in the area (Daryanabard and Dawson, 2008). Pollution, eutrophication, overfishing, and climate changes are considered the main causes of such blooms (Purcell, 2005). Despite nuisance, such aggregations of large jellyfishes also draw fishermen's attention in favor of economic benefits. Currently, two species of scyphozoan jellyfish, *Catostylus perezi* Ranson, 1945, and *Rhopilema hispidum* (Vanhöffen, 1888) are being commercially exploited in Pakistan (Gul *et al.*, 2015).

OBJECTIVES: The aim of this study is to document first record of a jellyfish species identified as *Crambionella orsini* (Vanhöffen, 1888) from the coast of Pakistan.

MATERIALS AND METHODS: On 11th February, 2020, a local seafood company which deals with harvesting, processing and export of jellyfish provided eight specimens of a jellyfish to the author on voluntary basis as a contribution to the knowledge of jellyfish in Pakistan. The company collected some samples on the mentioned date for the first time for preliminary inspection regarding its fishery. The samples were collected from Char Dhoro along east of the Pakistani coast near to Indian coastline, where, according to fishermen this brown jellyfish were in abundance. Eight specimens were examined; morphological features were studied, measurements were noted and photographs were made before preservation in formaldehyde solution. One specimen was deposited in the Museum of Department of Zoology, Jamia Millia Government Degree College, Malir, Karachi.

RESULT AND DISCUSSION: Following Nishikawa *et al.* (2015), the jellyfish specimens were identified as *Crambionella orsini* (Vanhöffen, 1888). The genus *Crambionella* Stiasny, 1921, belongs to the family Catostylidae Stiasny, 1921, in the order Rhizostomeae Cuvier (1800). To date, four species: *Crambionella annandalei* Rao, 1931, *C. helmbiru* Nishikawa, Mulyadi & Ohtsuka, 2015, *C. orsini* (Vanhöffen, 1888) and *C. stuhlmanni* (Chun, 1896) are recognized in the genus based upon few characters which include coloration, presence of tubercles on marginal lappets, the presence of foliaceous appendages among mouth openings and size of terminal club in relation to the total length of oral arm. The given specimens had bell diameter ranging from 170 mm to 200 mm with uniform dark brown umbrella and whitish oral arms; umbrella smooth, shiny and without any tubercles on marginal area; number of velar lappets in the largest specimen 16 per octant; three winged orals arms bearing short terminal clubs not more than one-fourth of the length of oral arm; four crescent-shaped subgenital ostia, and intracircular network of canals connecting only to the ring canal (Figure 1). Portion of the oral arm dyed with red stain was examined under stereomicroscope but no foliaceous appendages were noticed. *C. orsini* was reported in different color varieties with or without a dark band on the umbrella margin. The species has a distribution range from Red Sea to western Indian Ocean: Arabian Sea to east of Indian coast and along east African coast to South Africa. In Pakistan, however, no information is available about the presence of this species in the scientific literature so far, except for a news report published very recently indicating its abundance off shore along the Pakistani coast. To date, ten species of scyphozoan jellyfish have been reported plus, three species were recorded based upon damaged specimens from FAO collections (Morandini and Gul, 2016; Gul and Osmany, 2017). Jellyfish *C. orsini* presented here is the first record from Pakistani coast.

CONCLUSION: The species reported here is the first record for the coast of Pakistan thus, an addition to the scyphozoan fauna of the region. The abundance of this jellyfish along the coast may generate its fishery.

CONFLICT OF INTEREST: Author has no conflict of interest.

ACKNOWLEDGEMENTS: Thanks to Nasir Rahimmon (Dua Sea Foods) for providing the specimens and necessary information, and André C. Morandini (Universidade De São Paulo, Brazil) for comments upon species identification and corrections/suggestions in the manuscript.

REFERENCES: Daryanabard, R. and M. N. Dawson, 2008. Jellyfish blooms: *Crambionella orsini* (Scyphozoa: Rhizostomeae) in the gulf of Oman, Iran, 2002–2003. Journal of the marine biological association of the United Kingdom, 88(3): 477-483.

Gul, S., S. Jahangir and A. Schiariti, 2015. Jellyfish fishery in Pakistan. Plankton benthos research, 10(4): 220-224.

Gul, S. and H. B. Osmany, 2017. Additions to the scyphomedusae

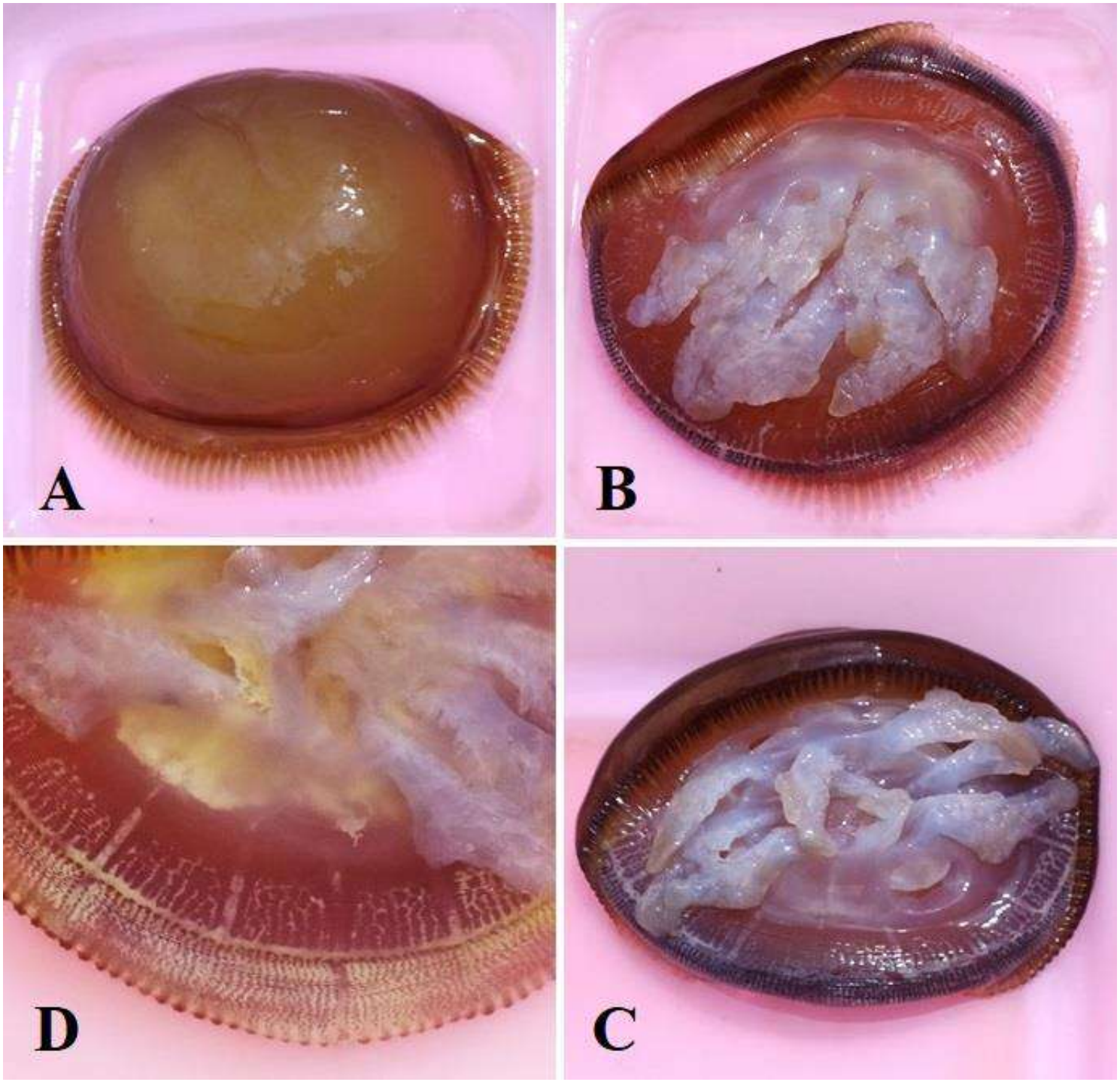

Figure 1. Jellyfish *Crambionella orsini* (Vanhöffen, 1888) from the coast of Pakistan. A, B) aboral and oral views of a specimen; C) aboral view of another specimen, note the crescent-shaped subgenital ostia; D) details of canal system.

(Cnidaria: Scyphozoa) of Pakistan. International journal of biology and biotechnology, 14(4): 665-667.

Morandini, A. C. and S. Gul, 2016. Rediscovery of *Sanderia malayensis* and remarks on *Rhopilema nomadica* record in Pakistan (Cnidaria: Scyphozoa). Papéis avulsos de zoologia, 56(15): 171-175.

Nishikawa, J., S. Ohtsuka, N. Mujiono, D. J. Lindsay, H. Miyamoto and S. Nishida, 2015. A new species of the commercially harvested jellyfish crambionella (scyphozoa) from Central Java, Indonesia with remarks on the fisheries. Journal of the marine biological association of the United Kingdom, 95(3): 471-481.

Omori, M. and E. Nakano, 2001. Jellyfish fisheries in Southeast Asia. Hydrobiologia, 451(1-3): 19-26.

Purcell, J. E., 2005. Climate effects on formation of jellyfish and ctenophore blooms: A review. Journal of the marine biological association of the United kingdom, 85(3): 461-476.

© 2020 by the authors. Licensee Scientific Platform. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Review Manuscript www.sciplatform.com

Food Poisoning: causes, precautions, diagnosis and treatment: A brief review

Anil Kumar
School of Biotechnology, Devi Ahilya University, Khandwa Road, Indore-452001, India

Authors' Contribution	**A. Kumar** planned the mini-review, conducted literature survey, designed the mini-review, wrote as well as finalized the manuscript
Article History	*Corresponding email address: ak_sbt@yahoo.com **Received: 19 February 2020, Revised: 08 March 2020, Accepted: 14 March 2020, Published Online: 05 April 2020**

ABSTRACT

Food poisoning is the pathological condition when a person gets sick after consuming a particular foodstuff which is mostly contaminated with either pathogenic bacteria or any other pathogen or virus. In food poisoning, person suffers from vomiting, abdominal cramps and diarrhea. Sometimes, under severe conditions, there may be fever and blood in feces etc. Body dehydration is also common due to loss of liquid from the body during diarrhea. If precautions are taken, food poisoning is not much life threatening. However, on prolonged and severe food poisoning, many times, persons also die. Here, in the present mini-review, a brief discussion on causes, diagnosis, symptoms and treatment about food poisoning has been done.

Key word: Pathogenic bacteria, *Escherichia coli, Salmonella typhimurium, Vibrio vulnificus,* allergens

INTRODUCTION: When a person gets sick after consuming a foodstuff, it is normally called as 'Food poisoning'. The most basic symptoms of food poisoning are vomiting, pain in stomach, diarrhea etc. It is considered that food poisoning is as a result of consuming toxic or contaminated food. As per available information; cases of food poisoning are more common in poor and developing countries; however, people from developed countries also suffer from food poisoning. To bring awareness about food poisoning, its causes and precautions to be taken, the present mini-review was planned. A brief discussion about food poisoning, its symptoms, and agents causing food poisoning, diagnosis and treatment has been carried out.

Symptoms: There is no definite time limit when symptoms of food poisoning are felt by the patient after consuming a toxic or contaminated food. It depends on the type of toxic or contaminated material present in the food and its quantity. It also depends on the body defense mechanism of the person. Symptoms may be visible within an hour of consuming contaminated food or sometimes even after many days or weeks. The common symptoms of food poisoning are abdominal pain (cramps), nausea, vomiting, diarrhea, fever, headache etc. The symptoms must not be ignored if persist for a longer time. It has been reported that sometimes, food poisoning may be life threatening. In fact, many deaths occur annually due to food poisoning. As per recent data released by the World Health Organization (WHO), on an average, one in ten people falls sick due to food poisoning every year and there are nearly 420,000 deaths due to food poisoning every year. Out of these, more than 50% deaths are due to diarrhea. If any person suffers any of the following symptoms, it is recommended that person must consult the doctor as soon as possible:

- Dehydration in the body which may be indicative by dry mouth, problem in drinking liquids, no or little excretion of urine.
- Having problem in speaking or eye sight.
- High fever (more than 102°F).
- Severe diarrhea persisting more than a couple of days
- Visibility of blood in the urine.

Agents causing food poisoning: There may be two different types of contaminants in the food responsible for causing food poisoning. These are living and non-living agents. The living agents include bacteria, other pathogens and viruses, and the non-living agents may be certain toxins secreted by the bacteria or other pathogens, certain poisonous chemicals which sometimes are added as preservatives or certain physical agents.

Bacteria: Although, a large number of pathogenic bacteria are known, all do not contaminate foodstuff. There are only few pathogenic bacteria which contaminate the food and may cause food poisoning (Mayounga, 2018). There are many steps through which food has to pass starting from leaving the farm area and up to ready to eat food reaching on the dining table. All these steps together are called as 'food production chain'. In spite of taking care, probability always exists for the pathogenic microbes to contaminate the food (Lynch *et al.*, 2009; Kumar, 2019). The level of contamination and nature of the microbe also depends upon its animal or plant origin. The most common pathogenic bacteria which contaminate food are *Escherichia coli*, *Salmonella typhimurium* and *Vibrio vulnificus*. All these three bacteria are reported to be transferred in the body of humans through contaminated food and food is contaminated by water (Kumar, 2019). *Escherichia coli* and *Salmonella typhimurium* have also been reported to be present in manures and survive for a longer time. There are also reports that *E. coli* strain becomes resistant to acids and thereafter, survives in the stomach of humans and colon of many other animals especially which survive on grain feed (Okafo *et al.*, 2003). A particular pathogenic strain of *Escherichia coli* named as strain O157:H7 has been reported in lettuce where it reaches through irrigating water (Fonseca *et al.*, 2011). This strain has also been found in other vegetables and fruits. Not only plant products, this strain of *E. coli* has also been reported in various animal origin foods like pork, chicken, beef, milk and milk products. This strain of *E. coli* may contaminate water if fecal matter is in the surrounding.

This is deadly harmful bacteria which may cause diarrhea, abdominal cramps, vomiting, colitis or even kidney failure(Alum *et al.*, 2016).

In addition, *Campylobacte*r sp. is also much lethal bacteria which contaminates food and causes food poisoning. There are reports that wild animals in surroundings of farms are the carriers of *Campylobacte*r sp. These lethal bacteria may cause diarrhea, fever, vomiting and abdominal cramps etc. Food may also get contaminated with pathogenic bacteria during harvesting. The equipments, instruments and vehicles such as choppers, knives, boxes, trailers, truck beds etc. are the sources of pathogenic bacteria (Kumar, 2019). In case of animal derived food, unhygienic atmosphere at or near the slaughter house, poultry, feedlots during transport, lairage before and after slaughter may also add bacterial contamination in food (Miller and Griffin, 2012). If poultry animals are fed bacterial contaminated foodstuffs, poultry chicken and eggs may get contaminated with food poisoning bacteria. In poultry animals, drinking water, rodents, dogs, cats, birds, faeces and clothes via surrounding environment may infect poultry animals and their derived food. Packaging material used for packaging the food, if contaminated, will contaminate the food. Many other pathogenic bacteria such as *Clostridium perfringens*, *Staphylococcus aureus*, *Clostridium* sp., *Listeria monocytogenes*, *Bacillus cereus*, *Toxoplasma gondii*, *Shigella* sp., *Entamoeba hystolytica* and *Cyclospora* sp. have been reported to contaminate foodstuffs throughout the food production chain (Kumar, 2019). Bintsis (2018) in a review discussed the status of microbial pollution in foodstuffs. Zeighami *et al.* (2020) reported that *Bacillus cereus* is an important cause of food poisoning globally. They collected 200 different samples of meat from different retailer shops and restaurants in Zanjan, Iran and studied the presence of hemolysin BL and non-hemolytic enterotoxin genes. They found that 14.1 % raw meat samples and 15% cooked meat samples showed presence of *Bacillus cereus*. They also showed that 89.6% isolates had one or more enterotoxin genes. Hemolysin BL genes were comparatively in lower frequency than non-hemolytic enterotoxin genes. Presence of enterotoxigenic *Bacillus cereus* in meat samples is a probable risk for public health. They also recommended that routine testing of foods must be carried out for the presence of enterotoxigenic *Bacillus cereus*. As per estimate, alone in USA, on an average, annually nearly 1,00, 000 cases of food poisoning with 20,000 admissions in various hospitals are reported and in most cases, *Salmonella* infection is found.

Parasites: Although food poisoning due to parasites is not much frequent unlike bacterial infection, still a few dangerous parasites have been reported. *Toxoplasmais* has been reported responsible for food poisoning. It has been reported in cat litter boxes. It is also found that certain parasites may stay in the digestive tract for years and these may be dangerous for pregnant ladies and sick persons who got weakened immune system. It has been found that other food borne parasites are tapeworms, roundworms and protozoa which may cause various diseases. Chang *et al.* (2019) reported the presence of anisakid larvae in anchovies (*Engraulis japonica*) fish found in Korea. On the basis of their studies and results obtained, they suggested that anchovies could be a potential source of human anisakiasis in Korea.

Viruses- A number of viruses have been reported which enter in the body through food and cause various dreaded diseases. The norovirus has been shown to be responsible for food poisoning in majority of cases. Many other viruses such as rotavirus and astrovirus are also reported to enter in the body through food. Hepatitis viruses have also been reported to enter in the body via food (Kumar, 2019).

Toxins and contaminants- In some cases, food poisoning occurs due to the presence of some toxins in the food. These toxins may be natural toxins or added. Many times, food preservatives are added which if consumed in more quantity, act as toxic materials. The polluting substances present in the environment enter in the human body through ingestion, absorption, inhalation and injection etc and cause adverse reactions. These food borne diseases may also cause disability and another diseases. These diseases may be caused due to toxins produced by bacteria or other toxic substances present in the food. The diseases may be like diarrhea, toxic shock syndrome, debilitating infections such as meningitis and even death. Pathogenic bacteria present in food may have multiple factors of virulence responsible for infection. Some bacterial species may produce toxins directly in the food whereas some others may produce them after they get colonized in the intestine. They mentioned that main pathogenic bacteria are *Salmonella sp., Vibrio parahaemolyticus, Vibrio cholerae, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Bacillus cereus, Listeria monocytogenes*.

Allergens- Quite often, it has been observed that a substance present around or is consumed even in much smaller quantity, triggers defense system in the body of a particular person, then that substance is called as allergen for that person and the phenomena as allergy. It is pertinent to mention that for other persons, it is a normal substance and body behaves normally. There are certain foodstuffs like nuts, fish, egg, milk and cereals which have been observed to be allergens for certain individuals. Most common symptom visible on the body as a result of allergy is rashes on the skin. In certain cases, there may be difficulty in normal breathing, severe abdominal cramps or even problem in cardiovascular system. Sometimes, these symptoms may be so fatal that risk of life occurs. Kassahun and Wongiel (2019) studied food poisoning cases in *Dewachfa worecha* of Ethiopia. They found that 35 food poisoning cases with no death were reported during 2018 and overall attack rate was 25.58 out of 10,000. Eating raw meat, drinking raw milk, sex, no hand washing before eating and sources of drinking water were significantly associated with food poisoning.

Precautions to be taken to avoid food poisoning: It is always preferred to consume food after cooking at or more than 70^{o}C temperature. In general, most of the bacteria, parasite and viruses get killed on heating at higher temperature. It is also true that on heating at higher temperature, there is loss of many nutrients especially the heat labile vitamins. These heat labile vitamins may be taken as supplements in the diet. There are many foods especially fruits and some vegetables which are eaten in raw form (without heating). These foods are the

sources of microbial contamination leading to food poisoning. In these cases, it is always preferred to wash these foods thoroughly before eating to washout microbial contamination. Nowadays, it is also preferred to boil such vegetables or steam sterilized before eating. Many times, when person does not wash the hands thoroughly after using toilet and touch or eat the food using those hands, there is a probability of the food to get contaminated with the microbes present in the faecal matter. Therefore, it should be assured that person washes the hands after using toilet, before touching or cooking the food. Not only this, one should also wash the hands thoroughly before taking any meal. The milk and other dairy products, meat and eggs have been found to get contaminated much frequently. Therefore, it is necessary to heat or cook or sterilize these products before consumption. The most important is water which is drunken or used in preparation of many foods, get contaminated with pathogenic microbes. Many times, if water pipelines are very near to faecal matter or drainage lines, get contaminated with the microbes present in faecal matter or dirty water. Zyoud *et al*. (2019) interviewed four hundred and twelve parents and out of those 92.7% were mothers. The study was conducted in Nablus district of Palestine between May and July, 2015. In the study, data were collected on food safety knowledge, attitudes and practices along with socio-demographic characteristics. After doing analysis of the data they concluded that knowledge, attitude and practices regarding measures for avoiding food poisoning among their children are associated with each other and get influenced by the socio-economic variables. On the basis of data collected, they recommended the conduct of health education programs and general awareness programs in order to educate the parents to follow food safety measures strictly and to enhance their awareness level. If non-vegetarian diets are prepared, then in addition to coliforms, contamination of *Staphylococcus aureus* (MRSA) bacteria was also detected. Unhygienic conditions are responsible for such contaminations on towels which are strong source of these pathogenic bacteria in the food. Study showed that 37% of towels showed contamination of coliforms, 37% had Enterococcus bacteria whereas 14% developed *Staphylococcus aureus*. It has been recommended to use disposable, single use sterile paper towels in the kitchen. Ding *et al.* (2019) reported that food borne bacteria, *Bacillus cereus* is widely found in different environments and may also be present in fresh vegetables. Therefore, fresh vegetables especially which are consumed raw or processed minimally and are not sterilized by proper heat treatment, must be evaluated for the presence of *B. cereus*. They studied vegetables from different cities of China to analyze genetic polymorphism, presence of virulence genes and antimicrobial resistance. They found presence of *B. cereus* in nearly 50% samples of vegetables and out of those, nearly 10% had contamination level of more than 1100 MPN (most probable number)/g. They also detected the presence of virulence genes in more than 80% positive samples. Therefore, fresh vegetables must be thoroughly washed with clean water properly and it is preferable not to eat raw vegetables. Finger *et al.* (2019) assessed the food borne diseases (FBD) outbreaks reported between the year 2000 to 2018 in Brazil. According to official data of Brazilian Ministry of Health, 13,163 FBD outbreaks occurred in Brazil during this period and 247, 570 cases were reported and out of these 195 deaths occurred. In many outbreaks, *Salmonella* infection was detected. These data indicated alarming situation of food poisoning in Brazil and there is need to take full precautions and to educate the people.

Persons prone to food poisoning: Almost every person is at risk for food poisoning. It is estimated that almost everyone suffers from food poisoning at least once in his or her life. Since animals including humans have defense systems to fight against the contaminations, those who suffer from auto-immune disease are more prone to food poisoning. It is also found that pregnant ladies are at more risk because their bodies cope up with changes in their metabolism and circulatory systems during pregnancy. Older people are also at more risk since their immune systems generally do not respond quickly to infectious microbes. Young children are also more prone to food poisoning since their immune system remains lesser developed compared to adults. Children also get dehydration more rapidly upon vomiting and diarrhea.

Diagnosis for food poisoning: Mostly doctors diagnose food poisoning on the basis of symptoms like abdominal cramps, vomiting and/or diarrhea. These symptoms mostly occur within few hours of consuming food. In some cases, symptoms may be visible after a day or two days of consuming contaminated food. Sometimes along with these symptoms, person may suffer from high fever, dehydration in the body, blood in faeces, dry throat and inability of engulfing any food or liquid down in the alimentary canal (there is immediate vomiting on keeping food or liquid in the buccal cavity and doing effort to swallow the same. Sometimes, doctors prefer to conduct certain pathological tests to confirm. There are large number of bacteria and other pathogens responsible for food poisoning. Stool culture is the most common pathological test where presence of pathogenic bacteria/ parasite is determined. Sometimes, DNA fingerprint of the pathogenic bacteria present in the stool is also carried out which help in prescribing the antibiotics.

Treatments for food poisoning: Most doctors prescribe antibiotics capable of killing the responsible bacteria or other pathogen. However, with awareness of ill-effects of antibiotics, nowadays, many people do not prefer to take antibiotics. In that case, doctors prescribe electrolytes to keep the body hydrated. There are many soft drinks which may also be taken for maintaining ionic balance in the body especially of sodium and potassium ions. Fruit juice and /or coconut water is also preferred to consume in order to regain body hydration. It is always advised not to consume any drink having caffeine since it irritates the digestive tract. In severe cases, it is preferred to give intravenous saline cum glucose for hydration purpose.

CONCLUSION: Food poisoning generally occurs mostly after consuming some contaminated foodstuff. The contamination may be of pathogenic bacteria, parasite or virus. Sometimes, allergen may also cause food poisoning. If proper care is taken and timely treatment is done, it is mostly curable. However, sometimes when it is severe and prolong for a longer time, it may be life threatening. People must take care of food safety before consuming it. It is also true that almost every person

globally becomes sufferer of food poisoning at least once in life time in spite of taking precautions. There may be more emphasis on food quality and food safety globally since food poisoning occurs in developed as well as developing and poor countries.

ACKNOWLEDGEMENTS

Author acknowledges the facilities of the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi (DBT) under the Bioinformatics Sub Centre as well as M.Sc. Biotechnology program used in the present work.

REFERENCES

Alum, E. A., S. Urom and C. M. A. Ben, 2016. Microbiological contamination of food: The mechanisms, impacts and prevention. International Journal of science and technological research, 5(3): 65-78.

Bintsis, T., 2018. Microbial pollution and food safety. Microbiology, 4(3): 377.

Chang, T., B.-K. Jung, S. Hong, H. Shin, J. Lee, L. Patarwut and J.-Y. Chai, 2019. *Anisakid larvae* from anchovies in the South Coast of Korea. The Korean journal of parasitology, 57(6): 699-704.

Ding, Y., P. Yu, Y. S. Bo, J. Wang, H. Guo, Y. Zhang, X. Liao, J. Zhang, S. Wu and Q. Gu, 2019. *Bacillus cereus* isolated from vegetables in china: Incidence, genetic diversity, virulence genes, and antimicrobial resistance. Frontiers in microbiology, 10: 948.

Finger, J. A., W. S. Baroni, D. F. Maffei, D. H. Bastos and U. M. Pinto, 2019. Overview of foodborne disease outbreaks in Brazil from 2000 to 2018. Foods, 8(10): 434.

Fonseca, J., S. Fallon, C. A. Sanchez and K. D. Nolte, 2011. Escherichia coli survival in lettuce fields following its introduction through different irrigation systems. Journal of applied microbiology, 110(4): 893-902.

Kassahun, M. and S. J. B. Wongiel, 2019. Food poisoning outbreak investigation in Dewachefa Woreda, Oromia Zone, Amhara Region, Ethiopia, 2018. BMC Research Notes 12(1): 377.

Kumar, A., 2019. Food quality: Hygiene, contaminations and quality testing. Journal of nutrition & food sciences 2(1): 100008.

Lynch, M. F., R. V. Tauxe and C. W. Hedberg, 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: Risks and opportunities. Epidemiology iniection, 137(3): 307-315.

Mayounga, A. T., 2018. Antecedents of recalls prevention: Analysis and synthesis of research on product recalls. In: Supply Chain Forum: An international journal Taylor & Francis: pp: 164-177.

Miller, J. M. and P. M. Griffin, 2012. One health through eyes of clinical and public health microbiology. Microbe, 7(1): 23-27.

Okafo, C. N., V. J. Umoh and M. Galadima, 2003. Occurrence of pathogens on vegetables harvested from soils irrigated with contaminated streams. Science of the total environment, 311(1-3): 49-56.

Zeighami, H., G. Nejad-dost, A. Parsadanians, S. Daneshamouz and F. Haghi, 2020. Frequency of hemolysin BL and non-hemolytic enterotoxin complex genes of bacillus cereus in raw and cooked meat samples in Zanjan, Iran. Toxicology reports, 7: 89-92.

Zyoud, S., J. Shalabi, K. Imran, L. Ayaseh, N. Radwany, R. Salameh, Z. Sa'dalden, L. Sharif, W. Sweileh, R. Awang and S. Al-Jabi, 2019. Knowledge, attitude and practices among parents regarding food poisoning: A cross-sectional study from Palestine. BMC public health, 19(1): 586.

© 2020 by the authors. Licensee Scientific Platform. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Research Manuscript www.sciplatform.com

Role of boric acid on economic seed production of alfalfa under climatic conditions of Sargodha

Asim Pervez, Abdul Razzaq, Muhammad Shoaib Farooq, Sikandar Hayat, Abdul Basit, Suleman Raza, Shoaib Anwar Kohli and Muhammad SaleemAkhtar

Fodder Research Institute, Sargodha, Pakistan.

Authors' Contribution	"**A. Pervez**, **A. Razzaq** & **M. S. Akhtar** developed the concepts and executed the experiments. **M. S. Farooq** & **S. Hayat** performed soil analysis and statistical analysis of recorded data. **A. Basit**, **S. Raza** & **Shoaib Anwar Kohli** compiled results and discussion".
Article History	*Corresponding email address: assipervez@gmail.com **Received: 13 March 2020 Revised: 05 April 2020, Accepted: 09 April 2020 Published Online: 13 April 2020**

ABSTRACT

ABSTRACT

Alfalfa is one of the most important forage crops worldwide because of its wide range of adaptability and good forage quality. Seed yield is generally considered to be of secondary importance and is characterized by often poor seed yield and seed quality. A field experiment was conducted to determine the impact of boric acid foliar applications along with a basal dose during anthesis to enhance the alfalfa seed yield in agro-ecological conditions of Sargodha. In foliar boric acid fertilization 0, 2, 4, 6, 8, 10 boric acid along with basal dose 23-80-50 (NPK kg ha^{-1}) was used. Phosphorous and potash were applied at the time of sowing while nitrogen will be applied in two split doses; half at sowing time and half of 1st irrigation. Two foliar applications, 1st at intensive plant growth stage and the 2ndat the beginning of the blossoming of the crop. The concentration of boric acid solutions used were 2 ,4 , 6 ,8 and 10 $glit^{-1}$ which produces a seed yield of 161.7, 167.9,171.3, 175.0,186.7 and 176.1 kg ha^{-1} in year 2015-16 while next year in 2016-17 produces a seed yield of 286.5, 299.2,304.6, 308.3, 312.1 and 310.1kg ha^{-1} respectively which is higher than year 2015-16. Foliar fertilization with boron influenced forming of slightly higher number of seeds in the pod. Boron influenced the average increase of yield, with a slightly greater difference compared to control. Number of seeds per pod in boric acid dose of 0, 2, 4, 6, 8 and 10 remained 6.1, 6.3, 6.4, 6.6, 6.8 and 6.7respectively in year 2016-17 which were higher than 4.3,4.4,4.5, 4.5,4.9 and 4.7 in year 2015-16. Overall 8 g $liter^{-1}$ boric acid foliar fertilization produces 8.9 % higher seed yield, 11.4 % higher no of seeds per pod compared to control in the year 2016-17 while produces 15.4% higher seed yield and 13.9 % higher no of seeds per pod as compared to control in the year 2015-16.

Key word: Alfalfa, boron, foliar application, seed, yield

INTRODUCTION: Alfalfa seed produced throughout the world is primarily used for forage production. Alfalfa is used for grazing, green chop, silage and hay to support the livestock industry, including dairy, beef, horses, and sheep. Most researchers have been unable to detect increases in seed yield as a result of soil or foliar applications of fertilizer containing both major and minor elements. Production practices are tailored to the specific climatic conditions of Sargodha region. Irrigation must be carefully controlled to stress the plants to encourage flowering and seed production. Insect pests, especially lygus bugs, are managed throughout the season, Crops with higher boron requirements such as alfalfa, sunflowers, rapeseed, cauliflower and apples are most likely to respond to boron. It is also needed for the growth of the pollen tube during flower pollination and is therefore important for good seed set and fruit development. Boron is thought to increase nectar production of flowers, and this attracts pollinating insects. There are several reports in a number of crops which demonstrate that boron can be deficient and has a significant effect on yield even when there are no vegetative signs of deficiency and even when B concentration is in adequate range (Nyomora *et al.*, 1999; Dordas, 2006). It was reported that there was an increase in alfalfa seed yield with boron foliar applications (Dordas, 2006).

OBJECTIVES: Objective of the study was to investigate the effect of boric acid foliar fertilization on seed yield of Alfalfa in agro-ecological conditions of Sargodha.

MATERIALS AND METHODS: A field experiment was conducted during 2015-17 at Fodder Research Institute, Sargodha. The trial was laid out in randomized complete block design with three repeats having a plot size of $18m^2$. The soil characteristics were given in the table 1.

Soil characteristics	Year 2015-16	2016-17	Mean
Soil texture	Loam	Loam	Loam
pH	7.9	8.07	7.99
EC($mScm^{-1}$)	0.62	0.64	0.63
O.M. %	0.67	0.69	0.68
Available K mg kg^{-1}	125	129	127
B mg kg^{-1}	0.54	0.55	0.55

Table 1: Pre-sowing physico-chemical analysis of experimental soil.

The basal dose of fertilizer was applied as 23-80-50 (NPK kg ha^{-1}) at the time of sowing to all the treatments later on the foliar treatments were applied as 0,2,4,6,8 and 10 g lit^{-1} boric acid solutions. Alfalfa variety sgd. Lucerne was sown and seed was applied @ 2 kg per acre. Seed was sown by hand drill. After the final cut of alfalfa for green fodder yield the trial was left in the field for seed production. Two foliar applications, 1st at intensive plant growth stage and the2nd application at the beginning of blossoming of crops. Dilute solutions of boric acid as per treatment were applied at 100 litter of water per acre as a foliar spray at each stage. The data were significantly analyzed using analysis of variance at LSD at 5% level of significance was worked out

RESULT AND DISCUSSION: Looking at the average seed yield per year in table 2, large differences in yield depending on

Treatments	Number seeds per pod		No of branches per plant		1000 grain wt (g)		Seed yield (kg ha^{-1})	
	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
T_1 23-80-50 (NPK kg ha^{-1}basal dose)	4.3 D	6.1 D	21.3 C	22.2 A	0.47 E	0.53 D	161.7 E	286.5 F
T_2 2glit^{-1} boric acid foliar spray + NPK basal dose	4.4 C	6.3 C	21.4 B	22.3 A	0.53 D	0.57 D	167.9 D	299.2 E
$T_3$4 g lit $^{-1}$ boric acid foliar spray+ NPK basal dose	4.5 BC	6.4 C	21.5 ABC	22.3 A	0.57 C	0.67 C	171.3 C	304.6 D
$T_4$6 g lit $^{-1}$ boric acid foliar spray+ NPK basal dose	4.5 BC	6.6 B	21.5 ABC	22.5 A	0.57 BC	0.70 BC	175.0 B	308.3 C
T_5 8g lit $^{-1}$ boric acid foliar spray+ NPK basal dose	4.9 A	6.8 A	21.7 A	22.5 A	0.63 A	0.77 A	186.7 A	312.1 A
T_6 10 g lit $^{-1}$ boric acid foliar spray+ NPK basal dose	4.7 B	6.7 B	21.6 AB	22.4 A	0.60 B	0.73 AB	176.1 B	310.1 B
LSD at 5%	0.23	0.19	0.26	2.47	0.029	0.047	3.10	1.36

Table 2: Yield components and seed yield of alfalfa.

Treatments	Average Seed yield (kg ha^{-1})	Increase yield over control (kg ha^{-1})	Net return of increased yield over control (Rs.)	Additional cost (Rs./ha)	Benefit cost ratio
T_1 23-80-50 (NPK kg ha^{-1}basal dose)	224.1	-		-	-
T_2 2glit^{-1} boric acid foliar spray + NPK basal dose	233.5	9.4	9400	845	11.12
T_3 4 g lit $^{-1}$ boric acid foliar spray + NPK basal dose	237.95	13.85	12465	1004	12.42
T_4 6 g lit $^{-1}$ boric acid foliar spray + NPK basal dose	241.65	17.55	15795	1162	13.59
T_5 8g lit $^{-1}$ boric acid foliar spray + NPK basal dose	249.4	25.3	22770	1321	17.24
T_6 10 g lit $^{-1}$ boric acid foliar spray + NPK basal dose	243.1	19.0	17100	1479	11.56

Table 3: Economic analysis on per hectare basis for each treatment for alfalfa seed yield as affected by boric acid foliar spray.

the year can be observed. The lowest average yield was recorded in 2015-16 (108.9 and 186.7 kg ha^{-1}) while in year 2016-17 (286.5 and 312.1 kg ha^{-1}). Climatic conditions have had a major impact on seed yield. In 2016-17 during February, March, April and May precipitation dropped to 94.49 mm. In year 2015-16, in February, March, April and May it was 160.58mm. Similarly Mueller (2008) also reported that Alfalfa seed production is well adapted to the arid climates of the western United States. A warm, dry production and harvest season is important to maximize seed yield and quality. It suggests that precipitation in March, April and May one of the most important parameters for the pollination of flowers and seed setting. Large variations in the climatic factors in investigation years have contributed to large differences in yield. Bolanos-Aguilar *et al.* (2002) was also agreed in their assessment that the large variation in seed yield among different cultivars of alfalfa in different climatic conditions was mostly influenced by environmental conditions during the year.

Some yield components and seed yield. Foliar boric acid fertilization produces higher number of seeds per pod in T5 which were 6.5 and 7.1 compared to controls 4.0 and 4.9 respectively. Similar results about the effects of boron fertilization on the number of seeds per pod are reported by Du *et al.* (2009). One of the main problems in the production of alfalfa is pod abortion. This is caused by the distribution of the assimilative, but the real cause is not known (Genter *et al.*, 1997). Dordas (2006) stated that B applied foliar may affect fertilization, development of seeds and pods and increase the seed yield. The author suggests that B may play a significant role in abortion of pods. Boron content in the soil in our studies 0.54 mg kg^{-1} which are considered adequate (Rashid, 2005). The T5 achieved a higher 1000 grain weight as compared to control in both years, in 2015-16 (34.04%) compared to 2016-17 (45.28%) higher 1000 grain weight in treatments with boron is in agreement with the results obtained by Dordas (2006) and Du *et al.* (2009). Higher seed yield in T5 is due to the number of seeds per pod and higher grain weight, which is consistent with the view that boron has a special significance in flowering and pollination. Boron has a direct role in flowering, pollen germination, and seed formation. There are many issues on which there is no answer to how boron affects the yield and how it moves in the process of development of flowers and seeds. The main limitation of movement of boron taken from the root is under developed xylem connections between seed and maternal tissues.

Since flowers and seeds do not transpire as leaves, they are not able to adopt boron directly from the soil. This is one of the reasons why many studies show a significant effect of foliar B

application on seed yield (Goldbach *et al.*, 2002). Roy *et al.* (2006) suggested that the increase in pH reduces the availability of boron and the depressing effect is more noticeable in soils with a pH greater than 6. The impact of drought on boron deficit is particularly stated by Terzić *et al.* (2012). The results of our research are consistent with the opinion of different authors (Dordas, 2006; Terzić *et al.*, 2012), who point out that boron may be in deficit and have a significant impact on the yield, even when there are no symptoms in the vegetative parts of the plant and when the concentration is in the appropriate range. Yield components such as number of plants m^2 had consistent values with no major deviations and statistically significant differences between both years. In both years the number of branches per plant had consistent values with no major deviations and statistically significant differences.

Economic analysis: Economic analysis as shown in table 3 was performed for each treatment combination. Treatment T_5 8gLit^{-1} boric acid foliar spray with NPK basal dose exhibited maximum benefit-cost ratio (17.24) with a net benefit of Rs. 22770, followed by treatment T_4 6 g lit $^{-1}$ boric acid foliar spray With NPK basal dose (13.59) with a net benefit of Rs. 15795. While minimum benefit-cost ratio of 11.12 was computed in treatment T_2 2glit^{-1} boric acid foliar spray with NPK basal dose with a net benefit of Rs.9400.

CONCLUSION: Climatic conditions, among which the most important were the amount and distribution of rainfall, had the most prominent influence on the yield and the seed yield components of alfalfa. 8g lit -1 boric acid foliar application influenced the formation of higher number of seeds in a pod that's why also showed a higher seed yield in both years as compared to control.

CONFLICT OF INTEREST: Author has no conflict of interest.

REFERENCES:

Bolanos-Aguilar, E.-D., C. Huyghe, C. Ecalle, J. Hacquet and B. Julier, 2002. Effect of cultivar and environment on seed yield in alfalfa. Crop science, 42(1): 45-50.

Dordas, C., 2006. Foliar boron application improves seed set, seed yield, and seed quality of alfalfa. Agronomy journal, 98(4): 907-913.

Du, W. H., X. H. Tian, Z. Z. Cao and A. Humphries, 2009. Effects of micronutrients on seed yield and yield components of alfalfa. Journal of plant nutrition, 32(5): 809-820.

Genter, T., E. Deléens and A. Fleury, 1997. Influence of photosynthetic restriction due to defoliation at flowering on seed abortion in lucerne (*Medicago sativa* L.). Journal of experimental botany, 48(10): 1815-1823.

Goldbach, H., B. Rerkasem, M. A. Wimmer, P. H. Brown, M. Thellier and R. W. Bell, 2002. Boron in plant and animal nutrition. Springer science & business media.

Mueller, S. C., 2008. Cooperative extension, plant sciences department. University of California, Davis, CA 95616.

Nyomora, A. M., P. H. Brown and B. Krueger, 1999. Rate and time of boron application increase almond productivity and tissue boron concentration. HortScience, 34(2): 242-245.

Rashid, A., 2005. Establishment and management of micronutrient deficiencies in soils of Pakistan: A review. Soil and envoronment, 24(1): 1-22.

Roy, R. N., A. Finck, G. Blair and H. Tandon, 2006. Plant nutrition for food security. Plant nutrition bulletin, 16: 368.

Terzić, D., R. Stanisavljević, B. Dinić, S. Vučković, D. Đokić and J. Marković, 2012. The effect of foliar application of boron on alfalfa seed yield. In: Proceedings of International Conference on BioScience: Biotechnology and Biodiversity. pp: 68-71.

© 2020 by the authors. Licensee Scientific Platform. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Review Manuscript www.sciplatform.com

Gene silencing by double stranded Ribonucleic acid (RNA)

[a] **Hafiz Ghulm Muhu-Din Ahmed***, [a] **Amna,** [b] **Shadab Shaukat,** [c] **Iqra Kousar,** [a] **Maria Rafiq,** [a] **Maria Ilyas,** [b] **Aziz Ullah and** [a] **Khuda Buksh**
[a] University of Central Punjab, Department of Botany, Punjab Group of Colleges, Bahawalpur, Pakistan,
[b] Department of Plant Breeding and Genetics, University of Sargodha, Pakistan,
[c] Department of Botany, University of Gujrat, Pakistan,

Authors' Contribution: All authors are equally contributed in the literature review and manuscript writing.

***Corresponding email address**: ahmedbreeder@gmail.com
Received: 20 March 2020, Revised: 09 April 2020, Accepted: 10 April 2020, Published Online: 11 April 2020

ABSTRACT

Ribonucleic acid (RNA) silencing, RNA interference (RNAi) or post-transcriptional gene silencing takes place in a variety of eukaryotes and it was discovered firstly in the plants. The RNA silencing process is activated by a trigger from dsRNA predecessor. A very important step in the silencing pathways the conversion of dsRNA into small duplexes of RNA of the representative length and arrangement. Then these small dsRNA monitor RNA silencing by different mechanisms. Post transcriptional gene silencing mechanisms were initially identified as an anti-viral process that give protection to the organisms from the viruses or which inhibit the unsystematic incorporation of transposable components. The basic aim of this review article is to study the mechanism of gene silencing by dsRNA and the roles of certain proteins in cellular post transcriptional RNA silencing machinery and finally we also discuss the RNA silencing as an anti-viral defense mechanism in the plants.

Key word: RNA, gene, silencing, pathway, plant

INTRODUCTION: The appliance of gene silencing was initially learned in plants. Also termed as silencing of post-transcriptional genes. RNA silencing is prompted by the originators of double stranded ribo-nucleic acids. These double stranded or two pleated ribo-nucleic acids are commonly processed into the small ribo-nucleic acid duplexes and its length vary from 21-28 nucleotides nearly and which then direct recognition". It is worth notice that silencing ribo-nucleic acid is double stranded or two crumpled strands. First one is sense strand and the other one is anti-sense strand. The sense strand is of sequenced as a base sequence of messenger ribo-nucleic acid. And the further one, anti-sense RNA have the opposite base sequences (Bartel, 2004). Silencing of double stranded or two folded strand RNA (ribo-nucleic acid) have the two phases/stages. First one is the initial or starting or beginning phase / stage and the second one is the effective phase also called as a successful and viable stage or phase. In the preparatory stage, the long stranded ribo-nucleic acids are divided into the less interfering ribo-nucleic acid with the help of an enzyme that we called DICOR (Mello and Conte, 2004). The additional one is the effective stage, in which the parting of two-folded silencing RNAs strands and the conveyance of anti-sense strand occurs into a proteins group." (Hori *et al.*, 2014). Until that time, the mechanism of silencing ribo-nucleic acid can be conducted as the mechanism of anti-viral that gave protection to the living organisms from RNA viruses. Application of the transgene is the most increasing technology in plant growth and now days it becomes the most important point. The presence of duplicated homologous sequences causes the inactivation of transgenes (Mok *et al.*, 2010). Apparently, homology can trigger gene inactivation and serves as single. In transcriptional process it also triggers gene inactivation. RNA silencing is the term which is generally use to describe post-transcriptional gene silencing in plant (Tinoco *et al.*, 2010). Basic procedure of RNA silencing is simple and straight forward. In this process double stranded RNA or hairpin RNA is cleaved by Dicer RNase enzyme interfere RNA, which consist of 21-26 nucleotides, these nucleotides guides silencing complex of induced RNA to destroy single stranded RNA (Fire, 2015). RNA-RNA, RNA-DNA, RNA-protein and protein-protein interactions, all these processes involve RNA silencing which is a complex process (Aregger *et al.*, 2012). Eukaryotes conserved ancient RNA surveillance system. This mechanism act against invasive nucleic acids, including viruses and other highly repetitive genomic sequences (Bora *et al.*, 2012). In various organisms double stranded RNA shows gene expression in a specific sequence manner. This biological process termed as RNA interference or (RNAi) (Fire, 2007). RNAi is a potent method which only few molecules of dsRNA per cell to silence expression. Silencing is not only spread from digestive tract of worms but it can also be transmitted through the germ line for several generations (Christiaens *et al.*, 2014).

RNA silencing system: RNA silencing mechanisms have formerly been boosted as antiviral mechanisms that secure organisms from RNA-viruses, or that preclude random integration of transient elements but the general role of silence in the regulation of gene expression only becomes seeming when it is manipulated that definite genes in plants and animals encode the short form of fold-back dsRNA (Liu *et al.*, 2012). Many of these miRNA genes are evolutionarily conserved, in plants miRNA acts primarily as siRNAs that guide the cleavage of sequence complementary mRNAs (Garbutt *et al.*, 2013). In animals such as nematodes, miRNAs appear to inhibit translation by targeting partially complimentary sequences located primarily within the 3 'untranslated region (UTR) of mRNAs (Wynant *et al.*, 2014).

Gene silencing in c-RNA gene: Small interfering RNA is the finest approach to effectively influence gene expression to study protein function in a wide range of cell types (Garbutt *et al.*, 2013). The si-RNA in the prevention of cancerous cells,

including the corporation and enquiry of cancer-related siRNA archives and their submissions in anticancer drug target detection and cancer therapy The detection of the RNA interface is an operative mode to circumvent the expression of separable genes by double-stranded RNA (Luo *et al.*, 2013). Sooner or later, it will be thinkable to custom gene-specific remedy to act towards human's syndromes as well as cancer (Bolognesi *et al.*, 2012).

Small interfering RNAs [siRNAs], **ressed siRNAs** have not been existed in the category of mammals, but they can be turn out from dsRNA as well as a minute hairpin (shRNA) by dicer cleavage or RNase-3 nuclease activities or chemical synthesis (Woppmann *et al.*, 2010). RNA (ADAR), an adenosine deaminaminase effecting on an RNA-editing enzyme, has been revealed to contend for dsRNA so that it is uncomplimentary as a substrate for dicer and thus hinders cRNA formation (Hori *et al.*, 2014). correspondingly acknowledged as short inhibiting RNAs or else suppressing RNAs, to be precise as actually passionate technology that can be effortlessly permits the silencing of the genes of living creatures such as mammals or animals by means of extraordinary level of potential capacity in addition to it also be made up of twenty to twenty five base pairs (Mok *et al.*, 2010).

Endogenously (RISC): Dicer distributes sRNA to a group of proteins called RNA-inducing silencing complex (RISC), where the catalytic component argonaute (Ago) is capable to fix siRNA to the equivalent strand to drag the consequential mRNA and additional, harm the mRNA, resulting in gene silencing (Ota *et al.*, 2013). ATP is mandatory throughout the unwinding of the siRNA duplex (Allen and Walker III, 2012).

The Molecular mechanism of RNA that triggered Gene Silencing: RNA triggers of gene silencing is precise ancient as well as developmentally trained and far accomplishment phenomenon (Thakur *et al.*, 2014). This performance is furthermore acknowledged as RNA interference. It takes place at what time dual stranded RNA helices prompt cleavage of their correlative mRNAs (Kreutzer and Limmer, 2012; Thakur *et al.*, 2014). Since these RNA subdivisions can be presented exogenously as little intrusive RNAs for instance siRNAs. RNA interference has become an ordinary investigative scheme in laboratory look (Rossi *et al.*, 2011). Also, the quantity, of RNA-based therapeutics that are as of now in clinical preliminaries for an assortment of human toxicities spectacle the remedial capability of RNA interference (Masliah *et al.*, 2013). Like so, we center all over the place our present appreciative of the conformation and magnitudes of not the same programmes of RNA interference triggers and how this evidence has been added to our conception of the biogenesis and synergist essentials of siRNA and micro-RNA in mammalian cells (Komor *et al.*, 2016). Nevertheless of their reputation in science and drugs, the atomic and cell-constituents of micro RNA biogenesis and capability are not absolutely comprehended. Captivating inquiries stay both for accepting the impressions of alterations and altering on micro RNAs and the suggestions among micro RNAs and the other cell RNAs, as we give an instance such as, long non-coding RNAs (Komor *et al.*, 2016).

Pathways of RNA silencing: It can be classified into four functional based pathways in plants. These are as follows: (Liu and Paroo, 2010; Bologna and Voinnet, 2014; Borges and Martienssen, 2015; D'Ario *et al.*, 2017).

(1)mi-RNA pathway ___ microRNA pathway**:** MicroRNA can be generate by the transcription of an enzyme RNA-Polymerase-ll

(2)Trans-RNA pathway___trans-acting-small-interfering RNA: The Trans-acting small interfering RNA (tasiRNA) can be initiated by the specific types of miRNA (micro RNA).

(3)RNA directed DNA methylation Pathway.

(4)Exogenic RNA silencing pathway: The RNA-silencing induced by the viruses and trans-genes, is termed as Exogenic RNA

RNA silencing and its role in plant with virus interactions: During the plant transgenic studies the result of the RNA silencing was unexpected. This was observed in 1990 into a diverse of molecular biology to now recent research is also observed (Ghoshal and Sanfaçon, 2015). In biological processes and the other scientific mechanisms of RNA silencing, viruses have been a critical tool in unveiling. In fact, the study in 1990s on the viruses' resistance and the effect of the pathogens provided the first evidence of introducing the gene silencing (Wang *et al.*, 2012). The phenomenon of RNA- directing DNA methylation was firstly introduced in the tobacco plants. The basic processes of the RNA silencing process in plant is now well understood (Bivalkar-Mehla *et al.*, 2011; Burgyán and Havelda, 2011; Jaubert *et al.*, 2011; Incarbone and Dunoyer, 2013). Double stranded RNA is promoted by the Dicer-like (DCL) proteins into 21-24 nucleotides small RNAs which are loaded to member of Argonatue (AGO) family to from an RNA silencing complex (RISC). These RISC along with sRNAs which are guide to direct RNA degradation, translational repression or DNA methylation of homologous target genes (Redfern *et al.*, 2013). Mostly there are three basic RNA-silencing pathway in the plants, the first one is micro (mi) RNA pathway, the second is small interfering (si) RNA-directed RNA degradation pathway and the last but not the least is RdDM (RNA directed DNA Methylation) pathway (Yoo *et al.*, 2011). In miRNA pathway, imperfect short hpRNA (hairpin RNA) are formed between the complementary regions of a primary miRNA transcript is processed in the mucleus by the DCL1, one of these four DCL proteins in Arabidopsis, into a single 21 -24 nt miRNA. miRNA play critical role in the control of the development of the plant by repressing the expression of regulatory genes such as the transcription factors, although recent research suggested that plants miRNA can also perform translational repression (Kim and Kim, 2012). The RdDM pathway is very unique in plants and play important role in silencing transposons and the repetitive DNA elements to maintain genome stability and integrity (Sand *et al.*, 2012). The RdDM is directed by 24-nt siRNAs that are processed by DCL3 from dsRNA synthesized by the plant-specific DNA dependent RNA polymerase IV and RDR2. The molecular details of the RdDM is not fully described yet (Yoo *et al.*, 2011).

RNA silencing as an antiviral defense mechanism in plants: Positive-sense RNA viruses are mostly include in the majority of the plants viruses. Which has either ssRNA or dsRNA genome which mostly depends on the viral RNA dependent DNA polymerase (replicase) for the process of multiplication (Agius *et al.*, 2012). Like fungal and the bacterial pathogens, the viruses can also be subject of innate immune responses conferred by host encoded disease resistance gene. Resistance gene of natural virus are continuing to be sought for use in breeding of durable virus resistance in crop plant (Nuss, 2011).

The tobacco *N* gene against *Tobacco mosaic virus* (TMV) was firstly identified *R* gene conferring the resistance to a virus (Scholthof *et al.*, 2011). *Turnip mosaic virus* (TuMV) induced lethal necrosis in some crucifer plants such as *Arabidopsis* (Ying *et al.*, 2010; Wang *et al.*, 2012) and *Brassica napus* in response to the corresponding *R* genes.

Applications of RNA-silencing in plants: The applications of RNA-Silencing in Plants Biotechnology are as follows; (Simón-Mateo and García, 2011; Ma *et al.*, 2015; Guo *et al.*, 2016). (1) The RNA silencing can be used for the development of seedless fruits. (2) It plays a vital role in alteration of plants architecture and also involved in flowering time. (3) It can enhance the resistance to biotic stresses of plants (Ahmed *et al.*, 2017). (4)RNA silencing functioning as up or down in the regulation of genes in plants. (5)RNA silencing is the method that provide immunity against viruses or transposons.

CONCLUSION: This review assertively supports the fact that the RNA silencing play an actual significant part in the development of plants and animals by giving an efficient system of gene controlling. RNA silencing may also prove beneficial for human disease controlling because many linkages between the inherited or acquired genetic disorders and RNA silencing factors have identified. There is much more to study about the biological roles and the molecular process of the gene silencing in plants. Most likely, there may be more than one mechanism of gene silencing to exist. The understanding of gene silencing and the molecular basis of the gene silencing would enhance the application of transgene technology, and will also reveal new mechanisms which are involved in regulating the development of plants.

CONFLICT OF INTEREST: Author has no conflict of interest

REFERENCES

Agius, C., A. L. Eamens, A. A. Millar, J. M. Watson and M.-B. Wang, 2012. RNA silencing and antiviral defense in plants. In: antiviral resistance in plants. Springer: pp: 17-38.

Ahmed, H.., A. S. Khan, S. H. Khan and M. Kashif, 2017. Genome wide allelic pattern and genetic diversity of spring wheat genotypes through SSR markers. International journal of agriculture and Biology, 19: 1559-1565.

Allen, M. L. and W. B. Walker III, 2012. Saliva of lygus lineolaris digests double stranded ribonucleic acids. Journal of insect physiology, 58(3): 391-396.

Aregger, M., B. K. Borah, J. Seguin, R. Rajeswaran, E. G. Gubaeva, A. S. Zvereva, D. Windels, F. Vazquez, T. Blevins and L. Farinelli, 2012. Primary and secondary siRNAs in Geminivirus-induced gene silencing. PLoS pathogens, 8(9).

Bartel, D. P., 2004. Micrornas: Genomics, biogenesis, mechanism, and function. Cell, 116(2): 281-297.

Bivalkar-Mehla, S., J. Vakharia, R. Mehla, M. Abreha, J. R. Kanwar, A. Tikoo and A. Chauhan, 2011. Viral RNA silencing suppressors (RSS): Novel strategy of viruses to ablate the host RNA interference (RNAI) defense system. Virus research, 155(1): 1-9.

Bologna, N. G. and O. Voinnet, 2014. The diversity, biogenesis, and activities of endogenous silencing small RNAs in arabidopsis. Annual review of plant biology, 65: 473-503.

Bolognesi, R., P. Ramaseshadri, J. Anderson, P. Bachman, W. Clinton, R. Flannagan, O. Ilagan, C. Lawrence, S. Levine and W. Moar, 2012. Characterizing the mechanism of action of double-stranded RNA activity against western corn rootworm (diabrotica virgifera virgifera leconte). PloS one, 7(10).

Bora, R. S., D. Gupta, T. K. S. Mukkur and K. S. Saini, 2012. RNA interference therapeutics for cancer: Challenges and opportunities. Molecular medicine reports, 6(1): 9-15.

Borges, F. and R. A. Martienssen, 2015. The expanding world of small RNAs in plants. Nature reviews molecular cell biology, 16(12): 727-741.

Burgyán, J. and Z. Havelda, 2011. Viral suppressors of RNA silencing. Trends in plant science, 16(5): 265-272.

Christiaens, O., L. Swevers and G. Smagghe, 2014. DsRNA degradation in the pea aphid (*Acyrthosiphon pisum)* associated with lack of response in RNAi feeding and injection assay. Peptides, 53: 307-314.

D'Ario, M., S. Griffiths-Jones and M. Kim, 2017. Small RNAs: Big impact on plant development. Trends in plant science, 22(12): 1056-1068.

Fire, A. Z., 2007. Gene silencing by double-stranded RNA (nobel lecture). Angewandte chemie international edition, 46(37): 6966-6984.

Fire, A. Z., 2015. Gene silencing by double stranded RNA. Nobel lectures in physiology or medicine (2006-2010): 8.

Garbutt, J. S., X. Bellés, E. H. Richards and S. E. Reynolds, 2013. Persistence of double-stranded RNA in insect hemolymph as a potential determiner of RNA interference success: Evidence from *Manduca sexta* and *Blattella germanica*. Journal of insect physiology, 59(2): 171-178.

Ghoshal, B. and H. Sanfaçon, 2015. Symptom recovery in virus-infected plants: Revisiting the role of RNA silencing mechanisms. Virology, 479: 167-179.

Guo, Q., Q. Liu, N. A Smith, G. Liang and M.-B. Wang, 2016. RNA silencing in plants: Mechanisms, technologies and applications in horticultural crops. Current genomics, 17(6): 476-489.

Hori, Y., K. Kataoka, T. Fujita, H. Shimizu, S. Kaname, S. Matsumoto, N. Nishiyama, K. Miyata and M. Oba, 2014. Polyion complex of double-stranded ribonucleic acid. Google patents.

Incarbone, M. and P. Dunoyer, 2013. RNA silencing and its suppression: Novel insights from in planta analyses. Trends in plant science, 18(7): 382-392.

Jaubert, M., S. Bhattacharjee, A. F. Mello, K. L. Perry and P. Moffett, 2011. Argonaute2 mediates RNA-silencing antiviral defenses against potato virus x in arabidopsis. Plant physiology, 156(3): 1556-1564.

Kim, Y. and V. N. Kim, 2012. Micro RNA factory: Risc assembly from precursor micro RNAs. Molecular cell, 46(4): 384-386.

Komor, A. C., Y. B. Kim, M. S. Packer, J. A. Zuris and D. R. Liu, 2016. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature, 533(7603): 420-424.

Kreutzer, R. and S. Limmer, 2012. Method for making a 21 nucleotide double stranded RNA chemically linked at one end. Google patents.

Liu, J., L. Swevers, K. Iatrou, H. Huvenne and G. Smagghe, 2012. *Bombyx mori* DNA/RNA non-specific nuclease: Expression of isoforms in insect culture cells, subcellular localization and functional assays. Journal of insect physiology, 58(8): 1166-1176.

Liu, Q. and Z. Paroo, 2010. Biochemical principles of small RNA pathways. Annual review of biochemistry, 79: 295-319.

Luo, Y., X. Wang, X. Wang, D. Yu, B. Chen and L. Kang, 2013. Differential responses of migratory locusts to systemic RNA interference via double-stranded RNA injection and feeding. Insect molecular biology, 22(5): 574-583.

Ma, X., M.-C. Nicole, L.-V. Meteignier, N. Hong, G. Wang and P. Moffett, 2015. Different roles for RNA silencing and RNA processing components in virus recovery and virus-induced gene silencing in plants. Journal of experimental botany, 66(3): 919-932.

Masliah, G., P. Barraud and F. H.-T. Allain, 2013. RNA recognition by double-stranded RNA binding domains: A matter of shape and sequence. Cellular and molecular life sciences, 70(11): 1875-1895.

Mello, C. C. and D. Conte, 2004. Revealing the world of RNA interference. Nature publishing group.

Mok, H., S. H. Lee, J. W. Park and T. G. Park, 2010. Multimeric small interfering ribonucleic acid for highly efficient sequence-specific gene silencing. Nature materials, 9(3): 272-278.

Nuss, D. L., 2011. Mycoviruses, RNA silencing, and viral RNA recombination. In: Advances in virus research. Elsevier: pp: 25-48.

Ota, H., M. Sakurai, R. Gupta, L. Valente, B.-E. Wulff, K. Ariyoshi, H. Iizasa, R. V. Davuluri and K. Nishikura, 2013. Adar1 forms a complex with dicer to promote micro RNA processing and RNA-induced gene silencing. Cell, 153(3): 575-589.

Redfern, A. D., S. M. Colley, D. J. Beveridge, N. Ikeda, M. R. Epis, X. Li, C. E. Foulds, L. M. Stuart, A. Barker and V. J. Russell, 2013. RNA-induced silencing complex (RISC) proteins pact, trbp, and dicer are SRA binding nuclear receptor coregulators. Proceedings of the national academy of sciences, 110(16): 6536-6541.

Rossi, J. J., M. A. Behlke and D. Kim, 2011. Methods and compositions for the specific inhibition of gene expression by double-stranded RNA. Google patents.

Sand, M., M. Skrygan, D. Georgas, C. Arenz, T. Gambichler, D. Sand, P. Altmeyer and F. G. Bechara, 2012. Expression levels of the micro RNA maturing microprocessor complex component dgcr8 and the RNA-induced silencing complex (RISC) components argonaute-1, argonaute-2, pact, tarbp1, and tarbp2 in epithelial skin cancer. Molecular carcinogenesis, 51(11): 916-922.

Scholthof, H. B., V. Y. Alvarado, J. C. Vega-Arreguin, J. Ciomperlik, D. Odokonyero, C. Brosseau, M. Jaubert, A. Zamora and P. Moffett, 2011. Identification of an argonaute for antiviral RNA silencing in *Nicotiana benthamiana.* Plant physiology, 156(3): 1548-1555.

Simón-Mateo, C. and J. A. García, 2011. Antiviral strategies in plants based on RNA silencing. Biochimica et biophysica acta (BBA)-gene regulatory mechanisms, 1809(11-12): 722-731.

Thakur, B. K., H. Zhang, A. Becker, I. Matei, Y. Huang, B. Costa-Silva, Y. Zheng, A. Hoshino, H. Brazier and J. Xiang, 2014. Double-stranded DNA in exosomes: A novel biomarker in cancer detection. Cell research, 24(6): 766-769.

Tinoco, M. L. P., B. B. Dias, R. C. Dall'Astta, J. A. Pamphile and F. J. Aragão, 2010. In vivo trans-specific gene silencing in fungal cells by in planta expression of a double-stranded RNA. BMC biology, 8(1): 27.

Wang, M.-B., C. Masuta, N. A. Smith and H. Shimura, 2012. RNA silencing and plant viral diseases. Molecular plant-microbe interactions, 25(10): 1275-1285.

Woppmann, C., H.-P. Vornlocher, P. Hadwiger, M. John and S. Limmer, 2010. Double-stranded ribonucleic acid with increased effectiveness in an organism. Google patents.

Wynant, N., D. Santos, R. Verdonck, J. Spit, P. Van Wielendaele and J. V. Broeck, 2014. Identification, functional characterization and phylogenetic analysis of double stranded RNA degrading enzymes present in the gut of the desert locust, *Schistocerca gregaria*. Insect biochemistry and molecular biology, 46: 1-8.

Ying, X.-B., L. Dong, H. Zhu, C.-G. Duan, Q.-S. Du, D.-Q. Lv, Y.-Y. Fang, J. A. Garcia, R.-X. Fang and H.-S. Guo, 2010. RNA-dependent RNA polymerase 1 from *Nicotiana tabacum* suppresses RNA silencing and enhances viral infection in *Nicotiana benthamiana*. The plant cell, 22(4): 1358-1372.

Yoo, B. K., P. K. Santhekadur, R. Gredler, D. Chen, L. Emdad, S. Bhutia, L. Pannell, P. B. Fisher and D. Sarkar, 2011. Increased RNA-induced silencing complex (RISC) activity contributes to *Hepatocellular carcinoma*. Hepatology, 53(5): 1538-1548.

© 2020 by the authors. Licensee Scientific Platform. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

AUTHOR GUIDELINES

Focus and Scope

The **World Journal of Biology and Biotechnology** (Abbreviation is **World J. Biol. Biotechnol.**) **Print ISSN: 2522-6746; Online ISSN: 2522-6754** is using **Open Journal System** (**www.sciplatform.com**) and it will accept research/ review manuscripts and shorts communication ranging from, biology, nucleic acids/molecular biology, microbiology, physiology/biochemistry, medical biotechnology, agricultural and food biotechnology, genomics, bioinformatics, environmental biotechnology, botany, biology, animal husbandry, agricultural sciences such as soil science, plant pathology, entomology, agronomy, plant breeding and genetics, forestry and horticulture.

Publication Frequency

Thrice in a Year (15 April, 15 August and 15 December) and started from April, 2016.

Open Access Policy

The World Journal of Biology and Biotechnology provides immediate open access to its content, on the principle that making research freely available to the public support a great global exchange of knowledge.

Journal Archiving

World Journal of Biology and Biotechnology supports the **LOCKSS (Lots of Copies Keep Stuff Safe)** system to ensure secure and permanent preservation of the journal. The previous issues are available https://www.sciplatform.com/index.php/wjb/issue/archive

Publishing Ethics and Malpractice Statement

World Journal of Biology and Biotechnology is based on the Committee on Publication Ethics (COPE) Code (https://publicationethics.org/)

Authors' Responsibilities

The submission has not been previously published, nor is it before another journal for consideration. The author will provide all the information (complete name, department and contact information) about the co-author. The corresponding author is responsible to make sure that each author has seen and approved the content of the submitted manuscript.

Author contributions must be provided

Conflict of interest must be clearly acknowledged

Acknowledgements must be provided, if any

Editors' Responsibilities

- The editor will maintain all the submission record of the journal.
- The editor will email (provided by reviewer) to all co-authors for the confirmation of authorship.
- The editor will assign reviewers to the manuscript based on the scientific content of the work.
- The editor is responsible to maintain the confidentiality of the complete review process
- The editor is responsible to make sure double blind peer reviewed of the manuscript and all efforts will be made to prevent the identities of the authors and reviewers from being known to each other.
- The editor is responsible to maintain the transparency of the academic research.
- The editor will not provide any information of the manuscript before publication of the manuscript.

Reviewers' Responsibilities

- The reviewer will evaluate the originality and scientific accuracy of the manuscript and reviewer will provide unbiased timely evaluation and decision on the paper
- The reviewer will decline to review the manuscript if a personal or financial conflict of interest was observed.
- The reviewer will inform any ethical concerns in the manuscript

Publishers' Responsibilities

- Scientific Platform will work with an editor and monitor the role to make sure the accurate procedure and transparency of editorial decisions.
- Scientific platform will make sure timely publication of research findings with confidential procedure, publication ethics and rectitude, authorship and conflict of interest if any
- Once the copyrights are transferred to Scientific Platform, the published matter will be the sole property of Scientific Platform, and exclusive permission will be conducted for reproduction (partial or full data).
- However, the author(s) will retain the intellectual property rights of the publication.
- The published manuscript will be freely available in the PDF format.

Sanctions

- If there is any violations of the above mentioned policies, the following sanctions will be applied:
- Rejection of the infringing manuscript.
- Rejection of every other manuscript submitted to this journal by any of the authors of the infringing manuscript and prohibition for 3 years against all of the authors
- Prohibition against all of the authors from serving on the Editorial Board.

- In cases where the violations of the above policies are found to be particularly flagrant, the Scientific Platform reserves the right to impose additional sanctions beyond those described above.

Editorial Policy

1. **World Journal of Biology and Biotechnology (Print ISSN: 2522-6746; Online ISSN: 2522-6754)** is using **Open Journal Systems** (www.sciplatform.com) and published research/review manuscript which has not been published elsewhere (only in the form of abstract, lecture and thesis) and are not currently under consideration elsewhere.
2. Corresponding author has responsibility for ensuring that all the other co-authors and a particular institution have approved the research.

Section Policies

Research Manuscripts, Review Manuscripts and Short Communication

Submissions, Indexed and Peer Review

Format or Structure of Manuscript

1. The manuscript must be according to the format of the Journal. The manuscripts should be written in English **(Cambria 10)** on letter paper **(width 8.5" and height 11")** with 1" margin on top and bottom and 0.5" on left and right. **Single spaced** and one column should be used in all manuscripts.
2. **The Tittle** of the manuscript should be in **capital** and **bold.**
3. **Full names of authors** (do not use abbreviations) and **their affiliation** should be written after tittle. The **email address** of each author is required while only email of the corresponding author will be used in final publication.
4. The importance of the particular studies, reliable results and the conclusion should be added in **200 to 250** words **abstract**.
5. Only **6 to 10 keywords** are required at the end of the abstract.
6. The background of study or problem, important and recent literature must be included in the **introduction**.
7. **Objectives or hypothesis** must be added at the end of the introduction
8. **Materials and Methods** should be accurate. **Results** (to the point) and **Discussions** (logical about findings and most recent) may or may not combine.
9. **International System of Units** (SI units) should be used to abbreviate the units. Tables and figures should be in editable form where applicable.
10. **References** must be at the end of the manuscript according to the format of the journal.

References (EndNote references are highly recommended.)

1. In running text or parenthesis, single author should be cited in the literature, e.g Abbas (2016) studied that or (Abbas, 2016), two authors should be separated from and not & e.g Abbas and Muhammad (2016) studied that or (Abbas and Muhammad, 2016) and in case of more than two authors, *et al*., should be used e.g Abbas *et al*. (2016) studied that or (Abbas *et al*., 2016). Chronological order should be used for two or more citations in the text (Abbas *et al*., 2013; Muhammad *et al*., 2014; Ali *et al*., 2015 and Fatima *et al*., 2016).

Journal

1. Abbas, M. F., S. Hameed and C. A. Rauf, 2014. Presence of new strain of potato virus y in Pakistan. International journal of agriculture and biology, 16(5): 941-945.

Book

1. Agrios, G., 2005. Plant pathology 5th edition: Elsevier academic press. Burlington, Ma. USA.

Website

1. IEA, 2014. International energy agency. World Energy Outlook. http://www.worldenergyoutlook.org/publications/weo-2014/.

Plagiarism

1. Plagiarism of manuscript will be checked (**must be less or equal than 19%**) before sending for the review process.
2. If plagiarism will be found after publication, the Scientific Platform will contact the author's institute and funding agencies for necessary actions and plagiarism will be mentioned on published manuscript.

Submission of Manuscripts

1. The manuscripts (written in English) should be according to format of World Journal of Biology and Biotechnology and should be submitted online or email to wjb@sciplatform.com.
2. For online submission, authors must register with the World Journal of Biology and Biotechnology.

Online registration

1. Login is required to submit your manuscript.

Double Blind Peer Review Process

1. Research and review manuscript will undergo the **double blind peer review process.**
2. The manuscripts will be reviewed **(blind peer review)** from **two different** reviewers (**not provided by the author**) to ensure the integrity of the double blind peer-review for submission to World Journal of Biology and

Biotechnology, every effort should be made to prevent the identities of the authors and reviewers from being known to each other.

About Peer-Review

1. **World Journal of Biology and Biotechnology** aims at rapid publication of high quality research while maintaining rigorous but sympathetic peer review process.
2. Manuscripts (other than those that are of insufficient quality or unlikely to be competitive enough for publication) will be peer-reviewed by **two or more experts** in the fields, and a decision is returned to the authors in about **two months**.
3. If due to special circumstance, the review process takes more time, authors will be informed by email.
4. Manuscripts with significant results will be reviewed and published at the highest priority and speed. Possible decisions on a manuscript are:
    - **The manuscript is accepted as it is**
    - **The manuscript is accepted after minor revision**
    - **The manuscript is accepted after major revision**
    - **The manuscript is rejected due to plagiarism policy (Similarity Index) or other factors**

Review Process Time

1. The review process time will be **6 to 8 weeks**.
2. Please do expect a slight delay if the review period **overlaps with a long holiday or Summer/Winter break.**

The Decisions

1. The decision about manuscript will be provided to the corresponding author.
2. After acceptance, correction of proofs and transfer of copyrights to Scientific Platform, a paper will be published on its turn.
3. Reviews: World Journal of Biology and Biotechnology encourages the submission of reviews by the authors who have considerable attainment in their fields of specialization.
4. Before submission of reviews, the authors are encouraged to contact the Editor-in-Chief at wjb@sciplatform.com

Proof Read Version and Copyrights.

1. After satisfactory revision and acceptance, transfer of copyrights and an the electronic proof read version of the final manuscript will be sent to corresponding authors and only **minor corrections** in the text (5%) are allowed before publication.
2. The final manuscripts will be available online, but it will be published be printed on its turn.

About Authorship

1. The **corresponding (submitting) author** is responsible for having ensured that this agreement has been attained and for managing all communication between the journal and all co-authors, before and after publication.
2. A confirmation email will be sent to each listed co-author (email provided by corresponding author).
3. If co-author has any objections, co-author will contact the editorial office as soon as possible.
4. **If we do not hear back from co-author within five working days (one week), we will assume co-author is agreed with co-authorship**.
5. Any change to the authors list after submission, such as a change in the order of the authors or the deletion of authors needs to be approved by a signed letter from every author.
6. **You cannot add the author's name at the final stage of publication.**
7. If you want to add the name of an author, you should provide all the information why the author was skipped in the manuscript before? **and why it is necessary now?**

Minimum Requirements for Authorship

1. All persons designated as authors should qualify for authorship, and all those who qualify should be listed.
2. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.
3. One or more author should take responsibility for the integrity of the work as a whole, from inception to published article.

Authorship Credit

1. Substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data and b) Final approval of the version to be published.
2. Acquisition of funding, the collection of data, or general supervision of the research group, by themselves, do not justify authorship.
3. Authors should provide a description of what each contributed, and editors should publish that information.
4. All others who contributed to the work who are not authors should be named in the Acknowledgments, and what they did should be described.

Minimum Requirements for Acknowledgments

1. List all contributors who do not meet the criteria for authorship, such as a person who has provided purely technical help, writing assistance, or a department chair who provided only general support.

2. **Financial and material support should also be acknowledged.**
3. Groups of persons who have contributed materially to the paper but whose contributions do not justify authorship may be listed under a heading such as "clinical investigators" or "participating investigators," and their function or contribution should be described, e.g., "served as scientific advisors," "critically reviewed the study proposal," "collected data," or "provided and cared for study".
4. Because readers may infer their endorsement of the data and conclusions, all persons must have given written permission to be acknowledged.

Co-author Contributions

1. Authors are strongly encouraged to include a statement to specify the actual contribution of each co-author to the completed work.
2. World Journal of Biology and Biotechnology allows two co-authors to be specified as having contributed equally to the work being described. The author contribution statement should be clear like the following example:

Example

1. Author A, B., C. and D developed the concepts and also designed the experiments.
2. Author A, and B performed *P. infestans* transformations and plant inoculations.
3. Author C carried out confocal microscopy and advised on cell biology.
4. Author D performed GUS assays and light microscopy.
5. Author E and F quantified gene expression.
6. Antibody detection of tagged transform was performed by A, B, C and D.
7. Author F carried out experiments with *P. atrosepticum*.

1. If **minor revision** is required, authors should return a revised version as soon as possible within **5 working days**.
2. If **major revision** is required, authors should return a revised version within **15 working days**.

About Plagiarism

1. Plagiarism is the use or close imitation of the language and ideas of another author and representation of them as one's own original work. Duplicate publication, sometimes called self-plagiarism, occurs when an author reuses substantial parts of his or her own published work without providing the appropriate references.
2. This can range from getting an identical paper published in multiple journals, where authors add small amounts of new data to a previous paper.
3. Plagiarism can be said to have clearly occurred when large chunks of text have been copied and pasted.
4. Such manuscripts would not be considered for publication in the Journals. But minor plagiarism without dishonest intent is relatively frequent, for example, when an author reuses parts of an introduction of an earlier paper.
5. The editors will judge any case of which they become aware (either by their own knowledge of and reading about the literature, or when alerted by referees) on its own merits.

About Duplicate Publication

1. The material must be original and **not published** or **submitted for publication** elsewhere.
2. **Author is responsible to get permission** from previous publisher or copyright holder if an author is re-using any part of paper, e.g., figure(s), published elsewhere, or that is copyrighted.
3. The editors consider all the materials in good faith that their journals have full permission to publish every part of the submitted material including illustrations.

Self-citation/ Falsification

1. **Self-citations** are highly restricted for publication and **manuscript will be rejected immediately**
2. The scientific Platform has the right to impose an additional action, not mentioned above for the falsified data.

Author Fees

Handling Charges payment methods: 100.00 (USD)

INTERNATIONAL

WIRE INSTRUCTION: Routing / ABA No (073000545)
Account No (196475568396), Swift Code (USBKUS44IMT), US Bank, University Office, Ames, Iowa

NATIONAL

Amount 6,000/= PKR
Account Name (Scientific Platform), Account No (1005062604), Bank Name (Bank Alfalah), Branch Code(0481)
Branch Location Ghauri Town Phase-IV Branch, Islamabad

Partial Wavier Policy

If you do not have funds to pay such fees, you will have an opportunity to waive each fee. We do not want fees to prevent the publication of worthy work.
The published manuscript will be freely available in the PDF format.

Indexing

1. Academic Resource Index
2. Cite Factor Index = 26
3. IPI Indexing (Indexed - 2018 : 2.36)
4. Google Scholar
5. International Standard Serial Number (ISSN)
6. Journal Factor
7. Journal Impact Factor
8. PKP Index
9. Acdamia

EDITORIAL TEAM

Editor in Chief

Dr. Muhammad Fahim Abbas, World Journal of Biology and Biotechnology, Pakistan

Managing Editor

Mr Abdul Ghani, International Islamic University, Islamabad, Pakistan

EDITORIAL BOARD

1. Prof. Dr. Fakhru'l-Razi Ahmadun, HADR Research Centre and Civil Engineering Department, National Defense University of Malaysia (NDUM), Malaysia
2. Prof. Dr. Adel E. A Hegazy, Professor of plant biotechnology Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt
3. Prof. Dr. Ali G. A. Al-kaf, Sana'a University-Faculty of pharmacy-Medicinal Chemistry Department, Yemen
4. Prof. Dr. Said Elshahat Abdallah, Department of Agricultural Engineering, Faculty of Agriculture, Kafrelsheikh University, Egypt
5. Prof. Dr. Aïssa Abdelguerfi, National Agronomic School, Algeria
6. Prof. Dr. Idress H. Attitalla, Department of Botany, Faculty of Science Omar Al-Mukhtar University, Libya
7. Prof. Dr. Armen Trchounian, Faculty of Biology, Yerevan State University, Armenia
8. Prof. Dr. Paolo Cortesi, University of Milan, Department of Food, Environmental and Nutritional Sciences, Italy
9. Prof. Dr. Nevin Çankaya, Department of Chemistry, Usak University, Turkey
10. Prof. Dr. Nagwa Mohamed Aref, King Saud University, Saudi Arabia
11. Prof. Dr. Yousif Abd El-Aziz Elhassaneen, Minoufiya University, Egypt
12. Prof. Dr. Cafer Eken, Department of Agricultural Biotechnology Süleyman Demirel University, Isparta, Turkey
13. Prof. Dr. Soheil Eagderi, Department of Fisheries, University of Tehran, Karaj, Iran, Islamic Republic of
14. Prof. Dr. Giorgi Tawia Odamtten, Plant and Environmental Biology Accra, Ghana

Principal Contact

Dr. Muhammad Fahim Abbas
Editor in Chief
World Journal of Biology and Biotechnology
250/4-A, Ghouri Town Phase 5 Islamabad, Pakistan
Phone: 0923334186020
Email: wjb@sciplatform.com

Support Contact

Dr. Muhammad Fahim Abbas
Phone: 0923334186020
Email: info@sciplatform.com

Mailing Address

250/4-A, Ghauri Town Phase 5 Islamabad, Pakistan