The Use of Vitormone (Azotobacter chroococcum) A Liquid Bio-Fertilizer Along with Chemical Fertilizer on Crop Growth and Yield of Wheat (Triticum aestivum L)

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ABSTRACT

Wheat (Triticum aestivum L) is widely cultivated as a staple food crop of the world as well as in India. However, due to continuous use of chemical fertilizers the productivity is decreasing. Hence an experiment was conducted to study the effect of Vitormone (Azotobacter chroococcum) along with RDF at Agronomy Division, I.A.R.I., New Delhi. Results compiled indicated that application of Vitormone @ 2.0 ml/L along with RDF significantly increase tillers/m² (49.17%), productive tillers/m² (56.50%), ear length (32.18%), spikelets/ear (25.86%), grains/spikelet (29.72%), straw yield (53.77%), 1000 grain weight (21.80%), protein content (20.6%), spikelets/ear (25.86%), grains/spikelet (29.72%), straw yield (53.77%), 1000 grain weight (21.80%), protein content (20.6%) and yield (78.10%) over control and on par results with RDF. Vitormone did not show any phyto-toxic effect on wheat plant.

Keywords- Wheat, Triticum aestivum, Liquid Bio-fertilizer, Vitormone, Azotobacter chroococcum, Phytoxicity, Wheat yield.

I. INTRODUCTION

Wheat (Triticum aestivum) is an annual crop plant belonging to Gramineae family. It is widely cultivated as a staple food crop of the world as well as in India. It is mostly cultivated in north western and central zone. North west India along with Afghanistan probably forms the centre of origin of bread wheat and India is one of the ancestral land of this essential food crop. USA, Russia, China, Australia, Germany, France, Argentina and India are the main wheat producing countries in the world. The main species of wheat are Common Wheat (Triticum aestivum), Durum wheat (Triticum durum) and Emmer wheat (Triticum dicoccum). It is grown across the wide range of environments around the world which has the highest adaption among all the crop species. Worldwide maximum land is used for the production of wheat than any other crop. In India, more than 80% of the total area of wheat is under Triticum aestivum. However, the area under T. durum and T. dicoccum is only 10% and 1.0% respectively. In India wheat is second important food crop next to rice. It was the crop which has brought in the green revolution and shown the way for the food security in India. It contributes about 25% of the total food grain production in India.

Azotobacter spp. is Gram negative, free living, aerobic soil dwelling (Gandore et. al., 1995), oval or spherical bacteria that form thick walled cysts which are a sexual reproduction under favorable condition (Salhia, 2013). They are typically polymorphic and their size ranges from 2-10 µm long and 1-2 µm wide. The Azotobacter genus was discovered in 1901 by Dutch microbiologist and Botanist Beijerinck et. al. (1901) who is the founder of environmental microbiology. Azotobacter chroococcum is the first aerobic free-living nitrogen fixer. These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. This cell protein is then mineralized in soil after the death of Azotobacter cells thereby contributing towards the nitrogen availability of the crop plants. Azobacter spp. is sensitive to acidic pH, high salts and temperature (Tchan, 1989). Azotobacter has beneficial effects on crop growth and yield through biosynthesis of biologically active substances, stimulation of rhizospheric microbes, producing phytopathogenic inhibitors (Chen, 2006 and Lenart, 2012).

Inoculation effect of free living Azotobacter species are largely associated with nitrogen fixation (Lakshminarayana 1993), formation of various physiologically active growth hormones (Gonzalez et at. 1986), protection against root pathogens (Verma et.al. 2001). Stimulation of beneficial rhizospheric microorganisms and enhancement of plant yield (Sindhu et. al. 1994, Lakshminarayana et. al. 2000, Kanitkar and Kanitkar, 2004, Kanitkar et. al. 2013, Raut et. al. 2014a, 2014b, 2016). Alikhan et. al. (2007) reported that inoculation of three salt tolerant bacterial strains i.e. Azotobacter chroococcum, A. vinelandii and...
A. beijerinckia enhance 75.8%, and 56.12% root and shoot dry biomass in Ceriops decandra and Avicennia marina respectively. Similarly, inoculation of Azotobacter in Brassica campestris var. italica and wheat resulted in greater plant growth stimulation (Egamberdieva et al. 2008).

Sustainable food production to ensure food and nutritional security is one of the most important challenge before the world including India. Thus to ensure the food and nutritional security in India, these issues require to be adequately addressed through various scientific, technological and policy interventions. Among plant nutrients, nitrogen is the most important and the availability or deficiency of which make the largest differences in yield of crops. Mostly crop needs for nitrogen are being largely met by chemical fertilizers like urea and ammonium sulphate. In view of the rising prices of fertilizers, environmental pollution and emission of greenhouse gases, some potential sources of nitrogen are to be explored to replace a part of fertilizer nitrogen need of the crop. Hence, Kan-biosys has developed new foliar nitrogen fixing bio-fertilizer like ‘Vitormone’ which is capable of supplying nitrogen to the crop plants (Kanitkar and Kanitkar, 2004). Biologically nitrogen fixation is important for maintaining and improving crop growth and yield. However, the long-term excessive use of chemical fertilizers in agriculture has unanticipated environmental impacts (Dacko et al. 2016), including soil fertility degradation, soil organic matter deterioration, and decreased water and nutrient holding capacities and nutrient use efficiency (Baligar et al 2001, He et al. 2005).

II. MATERIALS AND METHODS

A field investigation was conducted at Agronomy Division, I.A.R.I., New Delhi-110012 during Rabi season of 2013. The soil of experimental plot was clayey in texture, medium in organic carbon, moderate in available nitrogen, medium in available phosphorus and medium in potassium. The trial was conducted in a Randomised Block Design (RBD) with three replications. Wheat variety HD-2967 was used in all the three replications in a plot size of 3.6 m x 4.0 m with a row to row spacing of 22.5 cm. A basal fertilizer dose of NPK was applied at the time of sowing. Vitormone sprays were given at 30-35 days after sowing (DAS) and 50-60 DAS. Protective irrigations were given to all the treatment as and when needed. All the package of practices were followed to raise a good crop. The treatment details are given below:

| Tr. No. | Treatments                                      |
|--------|------------------------------------------------|
| T-1    | Recommended Dose of Fertilizer (RDF)            |
| T-2    | RDF + Vitormone @ 0.5 ml/L                     |
| T-3    | RDF + Vitormone @ 1.0 ml/L                     |
| T-4    | RDF + Vitormone @ 2.0 ml/L                     |
| T-5    | 75% RDF + Vitormone @ 0.5 ml/L                 |
| T-6    | 75% RDF + Vitormone @ 1.0 ml/L                 |
| T-7    | 75% RDF + Vitormone @ 2.0 ml/L                 |
| T-8    | Control (Water Spray)                          |

Vitormone is a foliar liquid bio-fertilizer which has boosting effect on crop growth with various nitrogen fixing microbes. It contains cysts of Azotobacter chroococcum bacteria which are in dormant form and are suspended in clay based suspension/formulation. These dormant forms of bacteria germinate in the presence of oxygen, water and carbon source to form colonies of Vitormone bacteria. The colonies of these microbes produce amino-acids, vitamins and PGPS which are absorbed immediately by the leaves. These beneficial bacteria increase the plant vegetative growth, healthy foliage, bumper flowering, stops flower drop, increases healthy spikelets/ear and finally increasing in total yield. (Inamdar et. al. 2000, Kanitkar and Kanitkar 2004). Similarly, it is residue free, non-toxic, organic certified and environmentally safe for spraying.

Data were recorded on five randomly selected plants from each plot. Observations were recorded on Tillers/m², Productive tillers/m², Ear length (cm), Spikelets/ear, Grains/spikelets, straw yield (T/ha), 1000 seed weight (g), protein content (%) and Grain yield (T/ha). All the data collected were statistically analysed (ANOVA) according to the method suggested by Panse and Sukhatme (1985).

III. RESULTS AND DISCUSSION

All the observations recorded on various morphological and yield contributing characters are presented in Table-1 and Figures 1 to 2. Data compiled in Table-1 indicated that all the parameters were highly influenced by application of Vitormone. Only significant findings are discussed below:-

i) Tillers/m² and productive tillers/m²

The effect of spraying of Vitormone was found to be significant in all the treatments over control. Tillers/m² ranged from 421 (T-8 control) to 628 (T-4 – RDF + Vitormone @ 2.0 ml/L). Application of Vitormone @ 2.0 ml/L along with RDF (T-4) influenced very well with maximum tillers/m² of 628 followed by T-3 (RDF + Vitormone @ 1.0 ml/L) with 624; T-2 (RDF + Vitormone @ 0.5 ml/L) with 618, T-7 (RDF – 75% + Vitormone @ 2.0 ml/L) with 611 and T-1 (RDF) with 606 tillers/m². Similar results were also found for productive tillers/m² in T-4 treatment (565) followed by T-3 (564), T-2 (556), T-6 (553) and T-7 and T-2 (552) which are statistically on par with each other. All these treatments gave more than 50% higher tillers than control treatment.
ii) Ear length (cm)

Application of RDF and Vitormone significantly increased the ear length. The combined application of RDF + Vitormone @ 2.0 ml/L (T-4) recorded significantly highest ear length (11.5 cm) followed by T-6 (RDF 75% + Vitormone @ 1.0 ml/L) with 11.3 cm, T-3 (RDF + Vitormone @ 1.0 ml/L) with 11.2 cm; and T-7 & T-5 (RDF 75% + Vitormone @ 2.0 ml/L & Vitormone @ 0.5 ml/L) with 11.0 cm ear length. Similarly, T-4 treatment gave 32.18% more ear length than control treatment which was followed by T-6 (29.88%), T-3 (28.74%), T-7 and T-5 (26.44%) and T-1 and T-2 (22.98%) treatments.

iii) Spikelets/ear

Normally wheat plants exposed to the high temperature reduced Spikelets/ear. Foliar sprays with plant growth promoters/NPK nutrients/bio-fertilizers increased the Spikelets/ear. Different treatments of RDF individually and along with different concentrations of Vitormone differ significantly for producing higher no. of Spikelets/ear. The significantly highest number of Spikelets/ear (21.9) was observed in T-4 (RDF + Vitormone @ 2.0 ml/L) followed by T-7 (21.4), T-3 (21.2) T-2 and T-6 (20.9) and T-1 (20.7) treatments. Similarly, T-4 treatment gave 21.9% higher spikelets/ear, than control treatments which was followed by T-7 (22.98%), T-3 (21.84%), T-2 and T-6 (20.11%) and T-1 (18.96) treatment.
Grains/spikelets

Application of RDF and Vitormone alone or in combination significantly influenced number of grains/spikelets over control treatment. Treatment No.4 (RDF + Vitormone @ 2.0 ml/L) produced maximum number of grains/spikelets (2.75) followed by T-3 (RDF + Vitormone @ 1.0 ml/L) with 2.68 grains/spikelets, T-7 (RDF – 75% + Vitormone @ 2.0 ml/L) with 2.64, T-2 (RDF + Vitormone @ 0.5 ml/L) with 2.61 and T-1 (RDF) with 2.57 grains/spikelets than control treatment. Likewise, T-4 treatment gave maximum 29.72% more grains/spikelets than control treatment followed by T-3 (26.42%) T-7 (24.53), T-2 (23.11%) and T-1 (21.23%) treatment.

Straw yield

Straw yield was significantly influenced due to application of RDF individually or in combination with various doses of Vitormone. Except for T-5 (RDF 75% + Vitormone @ 0.5 ml/L), all the treatments gave significantly higher straw yield than control treatment. Results obtained indicated that straw yield ranged from 6.62 T/ha (control – T-8 treatment) to 10.18 T/ha (T-4 RDF + Vitormone @ 2.0 ml/L). Similarly, T-4 treatment gave 53.77% higher straw yield/ha over control treatment which was followed by T-3 (44.10%) T-2 & 42.15%) and T-1 (37.31%) treatment.

1000 grain weight (g)

Application of RDF 100% and 75% along with Vitormone influenced very well and statistically significant at different combinations (Table-1). Results compiled indicated that 1000 grain weight ranged from 37.69 (control T-8) to 45.8 g (T-4 – RDF + Vitormone @ 2.0 ml/L). Similarly, application of Vitormone @ 2.0 ml/L along with RDF (T-4) recorded highest 1000 grain weight (45.8 g) followed by T-7 (RDF 75% + Vitormone @ 2.0 ml/L) with 44.7g, T-3 (RDF + Vitormone @ 1.0 ml/L) with 44.4 g, T-2 (RDF + Vitormone @ 0.5 ml/L) with 43.3 g, T-6 (RDF 75% + Vitormone @ 1.0 ml/L)
with 43.3 g and T-1 (RDF) with 42.8 g. All the above six treatments gave significantly higher 1000 grain weight than control treatment. Likewise, all the above six treatments gave 21.80%, 18.88%, 18.08%, 15.16%, 14.36% and 13.82% higher 1000 grain weight respectively over control treatment.

**vii) Protein content (%)**

Data presented in Table-1 indicate that protein content (%) was significantly influenced due to application of RDF and Vitormone individually or in combination with each other. Similarly, protein content (%) ranges from 9.7% (control) to 12.0% RDF + Vitormone @ 1.0 ml/L. Treatment No.3 recorded highest protein content (12.0%) followed by T-4 (RDF + Vitormone @ 2.0 ml/L) with 11.7%, T-7 (RDF 75% + Vitormone @ 2.0 ml/L) with 11.4%, and T-2 (RDF + Vitormone @ 0.5 ml/L) with 11.3% protein content. Likewise, T-3 treatment gave 23.71% higher protein content than control treatment which is followed by T-4 (20.62%), T-7 (17.53%), T-2 (16.49%) and T-1, T-5 and T-6 (15.46%) treatments.

![Protein Content Graph](image1.png)

**viii) Grain yield (T/ha)**

It is revealed from Table-1 that wheat crop responded very well and gave significantly higher yield than control treatment due to application of Vitormone at various combinations along with RDF and 75% RDF. Grain yield ranges from 3.70 T/ha (T-8 control treatment to 6.59 T/ha (T-4 + RDF + Vitormone @ 2.0 ml/L) which is 78.10% higher than control treatment. Similarly T-4 treatment (RDF + Vitormone @ 2.0 ml/L) gave significantly higher yield of 6.59 T/ha followed by T-3 (RDF + Vitormone @ 1.0 ml/L) with 6.44 T/ha, T-2 (RDF + Vitormone @ 0.5 ml/L) with 6.26 T/ha and T-1 (RDF) with 6.07 T/ha. Similarly, T-4 treatment gave 78-10% higher yield over control treatment followed by T-3 (74.05%), T-2 (69.19%), T-1 (64.05%), T-7 (61.08%) over control treatment. Even T-4 and T-3 treatments gave 8.57% and 6.09% higher yield than RDF (T-1) treatment.

![Grain yield Graph](image2.png)
ix) Phyto-toxicity

The visual phyto-toxicity of wheat revealed that the plants sprayed with Vitormone (T-2 to T-7) have shown zero visual phyto-toxic effect on crop health viz. injury to leaf tip and surface, wilting, vein clearing, necrosis, epinasty and hyponasty were noticed.

Sustainable food production for nutritional security is one of the most important challenges before the world including India. Accordingly, when the actual development of agriculture is not possible, consequently the farmers will compensate for increasing their production by taking more types of inputs, especially mineral fertilizers, which is neither environmentally acceptable nor agriculturally sustainable. For example, nitrogen fertilizer is one of the main applied nutrients in agricultural production for its key role in the formation of proteins and nucleic acids in the plants. Thus, one of the basically alternative methods is the use of environmentally friendly biological fertilizers such as plant growth promoting rhizobacteria. Bio-fertilizers include mainly the nitrogen fixing and plant growth promoting micro-organisms. These promoting bio-fertilizers consist of Azotobacter, Azospirillum, blue green algae, Azolla, P-solubilizing micro-organisms, and mycorrhizae (Selvakumar et. al. 2009). Among them, Azotobacter is capable of fixing an average 20 kg nitrogen per hectare during the cropping season (Kizilkaya, 2009). Azotobacter can also produce thiamin, riboflavin, indole acetic acid and gibberellins.

Nitrogen is the component of protein and nucleic acids and chlorophyll. Thus, nitrogen supply to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Therefore, adequate supply of nitrogen is necessary to achieve high yield potential in crop. The atmosphere comprises of 78% nitrogen as an inert, in unavailable form. In order to be converted to available form it needs to be fixed through either the industrial process or through Biological Nitrogen Fixation (BNF). Without these nitrogen fixers, life on this planet may be difficult. Nitrogen (N) deficiency is frequently a major limiting factor for crops production (Dong et. al. 2012). Nitrogen is an essential plant nutrient, widely applied as N-fertilizer to improve yield of almost all the crops. Azotobacter, are non-symbiotic hetero-trophic bacteria capable of fixing an average 20 kg/ha/year (Kizilkaya, 2009). Bacterization helps to improve plant growth and to increase soil nitrogen through nitrogen fixation by utilizing carbon for its metabolism.

In the present study, it is significant to note that treatment No.4 (RDF + Vitormone @ 2.0 ml/L) to be more promising since they increase tillers/m² (49.17%), productive tillers m² (56.50%), ear length (32.18%), spikelets/ear (25.86%), grains/spikelets (29.72%), straw yield (53.77%), 1000 grain weight (21.80%), protein content (20.62%) and yield (78.10%). These results are agreeing with those obtained by Kanitkar et. al. (2013) by using Vitormone (Azotobacter chroococcum) in cucumber, Raut et. al. (2014a, 2014b, 2016) in soybean French bean and tomato, Ramteke et. al. (2016) in grapes. Earlier, in a detailed study on commercializing a new liquid bio-fertilizer technology first time in India, Kanitkar and Kanitkar (2004) reported that by using Vitormone (Azotobacter chroococcum) resulted in increase yield of various crops viz. wheat by 12.5 to 67.17%, Paddy by 3.8 to 7.8%, sorghum by 43.56%, potato by 20.6%, sesame by 22.5%, maize by 20.8%, gram by 18.41%, sugarcane by 10.75%, groundnut by 13.73% and Barley by 35.0%.

Kanitkar et. al. (2013) reported that the effect of Vitormone on cucumber suggest three sprayings of 1.0 to 2.0 ml/L along with RDF effectively increase no. of fruits, fruit length, fruit diameter, 100 fruit weight and yield by 23.5 to 30.75% over control. They also reported that foliar nitrogen nutrition by Vitormone (Azotobacter) can reduce 25% nitrogen dose and increase yield by 13.47% to 15.83%. Similarly, Raut et. al. (2014a) reported that three sprayings of Vitormone @ 1.0 to 2.0 ml/L along with RDF significantly increase pods/plant, pod length, pod weight, no. of seeds/pod, 100 seed weight, seed protein content and yield by 14.05% over RDF and 17.93% over control. Further Raut et. al. (2014b) studied the performance of Vitormone alone and in combination with 75% RDF and reported that soybean yield was increased by 10.67% when applied alone and 12.66% when applied with 75% RDF. Similarly, effect of Vitormone was studied by Raut et. al. (2016) on tomato and reported that three sprayings of Vitormone @ 2.0 ml/L at 30, 60 and 90 days after transplanting along with RDF significantly increases plant height (16.42%), no. of branches/plant (44.0%), days of flowering (26.83%) days of maturity (28.86%), fruits/plant (54.22%), fruit diameter (21.99%), 100 fruit weight (27.12%) and yield by 55.66% over control and 12.82% over RDF.

Foliar application of bio-fertilizers has an advantage over soil application in many ways (i) the nitrogen is being fixed at the site of its utilization (Sen, 1998), (ii) nitrogen fixers encounter less competition from other micro-organism and environmental factors on the phylloplane compared to the rhizosphere (Rangaswami, 1998, Saxena and Tilak 1994) and (iii) nitrogen fixers can reduce foliar diseases by antagonizing the pathogens (Sudhakar et. al. 2000). Earlier, Chandra et at (1979) have also reported that the nitrogen fixers are potential antifungal micro-organisms of the phylloplane of certain crops such as paddy and jute. Even the soil application of bio-fertilizers has been reported to control foliar diseases (Sharma et. al. 1994) and Pests (Mohan et. al. 1987). Azotobacter species are known for the use of non-symbiotic nitrogen fixation, antifungal metabolites, and secretion of growth promoting substances, vitamins and phosphate solubilization. Bio-fertilizers are microbial inoculants for increasing the growth and acts as a biocatalyst in providing valuable nutrients to the plants. (Ruinen 1979, Kanitkar 2006). Likewise, bio-fertilizers are cost effective and efficient source of nitrogen and
phosphorus for the plants. Inamdar (2000) reported that the traditional nitrogen fixing bio-fertilizer have suffered from problems of short life, instability to ambient temperatures and laborious large-scale application or production. However, the cysts of Azotobacter in liquid formation survive and retain the nitrogen fixing ability over two years.

Table 1: Effect of Vitormone on morphological and yield attributes in wheat

| Tr. No. | Treatments                        | Tillers/m² | Productive Tillers/m² | Ear length (cm) | Spikelets/ear | Grain s/spikelets | Straw yield (T/ha) | 1000 grain weight (g) | Protein content % | Grain yield T/ha | % increase in yield over RDF |
|---------|----------------------------------|------------|------------------------|-----------------|---------------|-------------------|-------------------|----------------------|------------------|----------------|--------------------------|
| sT-1    | Recommended Dose of Fertilizer (RDF) | 606* (43.94) | 552* (52.90) | 10.7* (22.98) | 20.7* (18.56) | 2.57* (21.23) | 9.09* (37.31) | 42.8* (13.82) | 11.2* (15.46) | 6.07* (64.05) | -                        |
| T-2     | RDF + Vitormone @ 0.5 ml/L        | 618* (46.79) | 556* (54.01) | 10.7* (22.98) | 20.9* (20.11) | 2.61* (23.11) | 9.41* (42.15) | 43.3* (15.16) | 11.3* (16.49) | 6.26* (69.19) | 3.13                     |
| T-3     | RDF + Vitormone @ 1.0 ml/L        | 624* (48.22) | 564* (56.23) | 11.2* (28.74) | 21.2* (21.84) | 2.68* (26.42) | 9.54* (44.10) | 44.4* (18.08) | 12.0* (23.71) | 6.44* (74.05) | 6.09                     |
| T-4     | RDF + Vitormone @ 2.0 ml/L        | 628* (49.17) | 565* (56.50) | 11.5* (32.18) | 21.9* (25.86) | 2.75* (29.72) | 10.18* (53.77) | 45.8* (21.80) | 11.7* (20.62) | 6.59* (78.10) | 8.57                     |
| T-5     | 75% RDF + Vitormone @ 0.5 ml/L    | 597* (41.81) | 546* (51.25) | 11.0* (26.44) | 19.6* (12.64) | 2.52* (18.86) | 7.94* (19.94) | 41.8* (11.17) | 11.2* (15.46) | 5.44* (47.02) | -                        |
| T-6     | 75% RDF + Vitormone @ 1.0 ml/L    | 602* (42.99) | 553* (53.18) | 11.3* (29.88) | 20.9* (20.11) | 2.55* (20.28) | 8.94* (35.05) | 43.0* (14.36) | 11.2* (15.46) | 5.70* (54.05) | -                        |
| T-7     | 75% RDF + Vitormone @ 2.0 ml/L    | 611* (45.13) | 552* (52.90) | 11.0* (26.44) | 21.4* (22.98) | 2.64* (24.53) | 9.04* (36.56) | 44.7* (18.88) | 11.4* (17.53) | 5.96* (61.08) | -                        |
| T-8     | Control (Water Spray)             | 421         | 361                    | 8.7             | 17.4          | 2.12           | 6.62           | 37.6           | 9.7           | 3.70          | -                        |
|         | C.D. at 5%                         | 44          | 85                     | 1.7            | 1.5           | 0.31           | 2.18           | 4.2            | 1.1           | 1.11          | -                        |

IV. CONCLUSION

From the present study it may be concluded that two spraying of Vitormone @ 2.0 ml/L at 30-35 and 50-55 days after sowing along with RDF significantly increase tillers/m², productive tillers/m², ear length, spikelets/ear, grains/spikelet, straw yield, 1000 grain weight, protein content and seed yield by 78.10% and 8.57% over control and RDF respectively. Similarly, Vitormone (Azotobacter chroococcum) can reduce 25% nitrogen dose and increase yield by 47.2 % to 61.08 % over control and on par with RDF for yield. Similarly, spraying of Vitormone @ 2.0 ml/L did not show any phytotoxic effect on wheat crop.

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