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ABSTRACT

The escalating prices of commercial fertiliser among other reasons have persuaded many agricultural manufacturing companies in South Africa to commence producing organic and inorganic substances associated with improved growth and yields of crops without substantial agronomic information of their efficacy relating to crop performance. To mitigate these claims, replicated rainfed field trials were carried out at four localities with different soil and climate backgrounds to assess the effect of these substances on growth and yield of maize. Eleven treatments, including commercial N, P and K and an untreated control were included in the trials namely, Biozone, Gliogrow, Gromor, Promis and Growmax, Crop Care, K-humate, Lanbac and Montys. These were applied based on recommendation rates by manufactures, while NPK was applied depending on the optimum required rate at each site. Treatments were arranged in a randomized completely block design. A test crop maize cultivar (PAN 6479) was used at all the sites. The effect of biological products showed inconsistent effects on the growth and phenological characteristics of maize. Maize growth consistently showed a depressive effect with the amendment of Gliogrow during vegetative growth but had comparable results by the reproductive stage. The effect of biological substances on maize yield was more pronounced in soils with better production practices and higher nutrient residual effects. Maize grain yield constantly decreased with Gromor additions as manure based active ingredient amendment suggesting the fundamental role of enriching organic materials with inorganic fertiliser for their efficient use in farmlands. Biological products Gliogrow, Growmax, Crop care and K-humate appeared most promising for sustainable crop production.

Key words: biological substances, maize phenology, maize grain yield

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INTRODUCTION

Maize ranks third in world recognition among cereals after wheat and rice. It is grown in almost all the Provinces of South Africa, but Free State, Gauteng, Mpumalanga and North West provinces are the main areas of production. It represents not only the most widely cultivated crop in South Africa but plays a key role in many household diets. Its production requirements include high fertilizer usage, particularly nitrogen derived from either chemical or organic materials for optimal production (Awotundum *et al*., 1994). The major maize producing regions of South Africa are characterized by soils that are highly subjected to occasional N leaching due to their sandy nature rendering them deficient in major plant nutrients. Poor soil fertility status had forced grain producers to annually increase inorganic fertilizer rates to meet higher crop yields, but is often outside the reach of millions of resource-poor farmers (Pocock, 2007). Fertilizer addition to crops on agricultural land has always been through mostly inorganic fertilization, the importance of which had increased over the years (Teichert-Coddington & Green, 1993).

The continuous use of chemical fertilizers also disturbs the equilibrium of agro-ecosystems and occasionally pollutes the environment (Peoples *et al*., 1995; Wagner, 1997). Improper fertilizer management under continuous crop production may result in reduced soil nutrient reserves and the consequent compromising of crop growth and productivity (Gruhn *et al*., 2000). Most of the nitrogenous chemical fertilizers are produced by industrial N fixation with each unit of N fertilizer produced requiring two units of petroleum (Hamdi, 1982; Wagner, 1997). This is expensive; especially for the producers in the less developed countries as petroleum-based inputs are derived from non-renewable resources. For higher crop yield, nutrients should be applied in balanced proportion so that nutrients removed from soil due to cropping are adequately replenished (Potash & Phosphate Institute, 2007) by applying materials that replenish soil nutrients (Peoples *et al*., 1995; Wagner, 1997). Application of either organic or inorganic fertilizers could assist, maintain and/or enhance soil nutrient reserves (Gruhn *et al*., 2000; Mohamed *et al*., 2008). Gruhn *et al*. (2000) pointed out that where fertilizer usage is low, greater application of organic fertilizer would assist to improve crop production and benefit the environment by limiting soil mining and reduce land degradation.

The rising concern on the yearly hike in prices of commercial NPK fertilizers among other reasons has persuaded many South African agricultural manufacturing companies to commence with the production of biological amendment substances. Manufacturers and/or suppliers of these substances claim that they could bring about increased crop growth and yield and make it not only productive but also sustainable (Viljoen, 2006). These materials are been registered and marketed often without substantial agronomic information of their effectiveness relating to crop performance. Since most of these substances appear to be supplements of commercial NPK fertilizer rather than complete replacements of NPK would mean an extra cost to producers. Therefore, usage of invalidated materials as crop growth and yield promoters either as soil or foliar amendments could negatively affect producers that are already under financial constraint. It is therefore an obligation of science to apply the "law of the consumer jungle", *caveat emptor* (let the buyer beware). On the other hand, suppliers that sell effective substances backed by validated scientific information will have larger business opportunities. Besides, this will lead to a win-win situation for both suppliers and consumers. The integrated use of organic and inorganic plant nutrient sources may not only recycle organic wastes that could potentially cause environmental pollution, but could also conserve a rich pool of nutrient resources and hence reduce the sole dependence on chemical fertilizers (Ahmed *et al*., 2006). It also increases the potential of organic fertilizers and improves the efficiency of inorganic fertilizers (Heluf, 2002). The goal of this study was to evaluate biological substances from various groups of active ingredients and their effects on maize phenology and grain yield comparative to NPK fertilizer.

MATERIAL AND METHODS

Description of the field trial study sites

Initial field trials in this study were conducted during 2004/05 and 2005/06 and were simply assessed for the effect of biological substances on grain yield of maize. These only evaluated the wide range of biological substances with very diverse active ingredients available in the market. In some cases a product was not evaluated for the two seasons due to the reluctance of the producers to let their products be evaluated. By 2006/07 season, several biological substances representing many others available in the market were grouped into three groups of active ingredients for an in-depth study on their efficacy on maize phenology and grain yield. Rainfed field trials were carried out for four consecutive planting seasons (2006/07-2009/10) at Bethlehem, Ottosdal and Potchefstroom and for three consecutive years (2006/07-2008/09) at Bothaville (Nampo Park). The geographical description, climatic and soil characteristics of the different study sites are indicated in Table 1. Trials at Ottosdal and Potchefstroom were conducted on fields previously planted to cowpea while wheat and sunflower were previously grown at the Bethlehem and Bothaville sites. The soil compositions in three of the four localities were sandy whereas the fourth locality has a clay soil (34%). The Bethlehem and Potchefstroom study sites presented fairly stable and higher summer rainfall while rainfall is sometimes erratic at Bothaville and Ottosdal.

Prior to trial establishment, 10 soil cores were randomly collected at the surface (0-20 cm) using a soil auger (ARC-MIG, 2006). Sub-samples were bulked and mixed thoroughly to form a composite sample for each trial site. The soil samples were processed and analyzed by the ARC Institute for Industrial Crops in Rustenburg. Standard analysis procedures (The Non-affiliated Soil Analysis Work Committee, 1990) were used to determine particle size distribution (Hydrometer), pH (H_2O), mineral N (0.1 N K_2SO_4), extractable P (Bray 1), exchangeable Ca, Mg and K (1 N NH_4OAc) and organic C (Walkley-Black).

Experimental procedure and treatments evaluated

The treatments evaluated during the 2004/05 and 2005/06 seasons were Crop care (A and B), Hygrotech, MBF, Montys, Mitygro, Novon (A, B C and D), Organics and Soygro (A and B) (Table 2). All of the substances contain one or another biological stimulant and some additional chemical nutrients. Treatments studied in 2006/07 to 2009/10 were locally produced biological substances consisting of Biozone, Crop care, Gliogrow, Gromor, Growmax, K-humate, Lanbac, Montys and Promis. These were grouped and classified in terms of their active ingredients representing many other existing products in the market and were applied based on manufacturers' recommendation rates (Table 3). They were further selected based on factors such as affordability, availability, ultimately manufactured from renewable sources and readily accessible to both commercial and small-scale grain producers. Optimum recommended rates of commercial NPK fertilizer at each site and a control were included as standard checks. The NPK was applied using limestone ammonium nitrate (LAN 28% N), superphosphate (SSP 10.5% P) and potassium chloride (KCl 60% K). Treatments were arranged in a randomized complete block design with four replications.

All field trial sites were planted to one maize cultivar PAN 6479 at 0.3 and 1.5 m intra and inter-row spacing's, respectively, with 10 m row lengths to obtain a calculated plant population of 22 222 plants ha^{-1}. Each treatment was assigned in a 6 m x 10 m block. The different treatments were applied on the same plots throughout the period of study at all trial sites. As presented in Table 4, soil K levels at Bethlehem and Bothaville were below the lower recommended threshold value of 125 mg kg^{-1} (Van Biljon *et al*., 2008) and were supplemented using KCl at 55 kg K ha^{-1}. The required P and K fertilizers were applied at planting with 30% of the total required N amount. The remaining 70% of N was band placed as top dressing 5 to 10 cm away from the row four weeks after emergence. Application of substances occurred at different times and growth stages as prescribed by either manufacturer or supplier (Tables 2 & 3). All the soil-applied biological substances were uniformly broadcasted over the experimental plots and lightly worked into the soil with a hand hoe. Liquid substances were foliar applied at various growth stages using a CP15 knapsack sprayer.

Crop husbandry

Seedbed preparation was done across all trial sites through mould-board ploughing, disking and harrowing. Primary tillage commenced two months prior to planting with secondary tillage carried out just before planting. All trials were manually planted using hand planters designed to sow seeds at an intra-row spacing of 30 cm. Two uniform seeds were planted per hole in order to cater for seedling survival rate. Maize plants were subsequently thinned to one per hill four weeks after emergence. After each planting, experimental plots were sprayed with 2 l ha^{-1} of S-metolachlor to destroy existing and emerging weeds. During the planting season, trials were kept weed free by means of mechanical weeding when necessary. At six to eight weeks after planting, all trials were treated with Combat stalk borer pesticide at 4 kg ha^{-1} for the control of maize stalk borer.

Data collection

Measurement of maize growth and phenological traits

The growth and phenological characteristics of maize were only determined during the 2006/07 to 2008/09 seasons. Percent plant emergence was determined four weeks after planting across all trial sites. Growth and phenological parameters such as plant height, biomass production and leaf area index were determined at the ninth leaf (V9) and silking growth stages (R1), respectively. Plant height and leaf area measurements were obtained from five randomly selected tagged plants in the two central rows of each plot. Leaf area (LA) was determined from intact leaves by multiplying leaf length and breadth by a factor of 0.75 as described by Saxena and Singh (1965). From the leaf area measured, leaf area index (LAI) was calculated by dividing the LA with the unit ground surface area. Biomass production was determined through destructive sampling of all plants within a 1 m^2 area at the outer four rows of each plot. All plant samples were immediately weighed at the relevant site and later oven-dried at 65°C for 48 hours in order to obtain dry weights.

Determination of maize grain yield and yield components data

All the field trials were manually harvested in an area of 21 m^2 (7m row length x 1.5 inter-row) within the two central rows of each plot at physiological maturity. Cobs were weighed prior to shelling for cob mass and subsequently grain yield determined after shelling. Final maize grain yield was adjusted to a standard moisture content of 12.5%.

Statistical analysis and presentation of data

Data were subjected to analysis of variance (ANOVA) as a randomized complete block design (RCBD) using Statistix version 8.1. Differences in treatment means were separated using Tukey's honestly significant difference (HSD) post-hoc test at 5% probability level. Tables 3.2 – 3.5 showing grand means across the treatments and seasons, coefficient of variation (CV %) and Tukey's least significance difference (LSD_T) to indicate differences in results obtained in the different experiments.

RESULTS AND DISCUSSION

Effect of biological substances on maize phenology

Effect of biological substances on plant emergence

In the 2006/07-production season, all treatments emerged exceptionally well at all localities except for the Gliogrow treated plots. Severe poor stand establishment was obtained at Potchefstroom and improved in the order of Bethlehem> Ottosdal > Bothaville. Levels of low plant stand strongly correlated with the clay content at the different localities and the poorest stand was obtained with a clay content of 34% while soils with lower clay content had satisfactory stands. Comparable results on maize stand was observed in 2007/08 but slightly improved for the 2007/08 season. Plots treated with Gliogrow consistently had severe poor plant stand establishment in the 2006/07 and 2007/08 seasons. Kruger (2009) indicated that seeds inoculated with Gliogrow form strong root systems prior to emergence and then emerge quickly followed by vigorous seedling growth. This whole mechanism could last about 10 to 14 days (Pers.comm), whereas in other treatments where soil moisture was not limiting, plants emerged from 6 days after planting in some localities (Máthé-Gáspár & Rátonyi, 2008). Conversely, in 2008/09 the percentage plant emergence steadily improved across treatments. Plots that were treated with Gliogrow showed a higher percentage plant emergence relative to the previous two seasons of the study. This could partly be due to the reduced rate of Gliogrow application that encouraged better seedling emergence following previous results from a glasshouse trial that assessed three rates (50, 75 and 100% recommended rates) of the biological substances (Baloyi *et al*., 2010).

Interaction effects of season x locality on the performance of the biological substances on plant height, biomass production and leaf area index

Table 5 presents a synopsis on the analysis of variance illustrating the effects of the treatments on plant height, biomass production and leaf area index at the 9th leaf and silking growth stages. The results showed that all the growth and phenological parameters determined were not significantly influenced by the interaction of season x locality x treatment, but were largely limited to the significant interactions of season x locality. This indicated that genotype x environment interaction effects amongst seasons in various localities had profound effects on the performance of the various biological substances assessed.

Effect of biological substances on plant height

The interaction of season x locality was significant on plant height at both sampling intervals (Figure 1). At the ninth leaf stage, plants were significantly shorter in 2006/07 at Bothaville compared to the height of plants in 2007/08 and 2008/09 seasons. Similar results were obtained during silking at Bothaville and Potchefstroom. The height of plants as measured at the ninth leaf stage across seasons was generally comparable, though being higher in 2008/09 (Figure 1). During silking, plant height recorded during 2008/09 showed a 13% increase compared the height of plants recorded in 2006/07; however it increased marginally in 2007/08. In all cases, plants obtained from the Bethlehem locality were significantly shorter for both sampling intervals during the 2006/07 season. This reduction in plant growth could be associated with limited soil moisture prevailing during the most critical stages of plant growth at the site. In 2006/07 approximately 26 mm of rain was recorded during November as compared to 97 and 150 mm of rain recorded during the same growth stage in 2007/08 and 2008/09.

The consistent reduction in plant growth obtained at Bethlehem during the 2006/07 could also partly be due to annual rainfall variability at the site since 321 mm of rain was recorded in 2006/07 compared to the 694 and 1132 mm rainfall recorded in 2007/08 and 2008/09. Annual rainfall at the four localities is in many instances very variable. During the study period, annual rainfall from July to June ranged from 321 to 1132 mm at Bethlehem, 490 to 580 mm at Bothaville, 333 to 486 mm at Ottosdal and 547 to 643 mm at Potchefstroom. During the trial period the daily mean minimum and maximum temperatures were almost similar to the long-term measurements. Thus, the daily mean evaporation recorded during the trial period at the four localities corresponds well with the long-term daily mean evaporation.

The height of plants was significantly reduced by Gliogrow during the vegetative stage but comparable results were obtained at silking (Table 6). Across seasons at Bethlehem, plots treated with Lanbac consistently gave taller plants than the NPK check at the ninth leaf stage. At Bothaville, the height of plants in plots treated with Crop care at both the ninth leaf and silking growth stages was consistently taller plants than NPK check. Again, plots treated with Gromor consistently gave shorter plants at both periods of sampling. At Ottosdal, the different biological substances consistently gave taller plants across the seasons at both periods of sampling, despite that they were constantly shorter in Promis treated plots at the ninth leaf and with Gliogrow, Gromor and Growmax during the silking stage. The different biological substances applied showed inconsistent positive effects on plant height at both sampling intervals at Potchefstroom. Plots treated with Gliogrow consistently had shorter maize plants during silking compared to the NPK standard.

In this study, biological substances applied as total replacement of NPK (without application of NPK) gave improved plant height during the ninth leaf stage but consistently exerted reduced plant height during the silking stage relatively to optimum NPK standards, at each site. These findings concur with earlier findings by Ayoola and Makinde (2009) who reported that the application of poultry manure gave significantly taller maize plants during earlier growth stages than those treated with inorganic fertilizer. The results of their study during the later growth stages were also comparable to the findings in this study. A similar report by Obi and Ebo (1995) noted significant improvement in average maize plant height during earlier maize growth stages upon application of poultry manure on a severely degraded Ultisol soil in southern Nigeria. These results may be associated with increased soil microbial activities following application of organic materials as opposed to inorganic fertilizer (Goshal & Singh, 1995).

Effect of biological substances on maize biomass

The interaction of season x locality on maize biomass yield at both sampling growth stages is shown in Figure 2. Biomass yield recorded in 2006/07 at Bethlehem by the ninth leaf growth stage increased than that recorded during 2007/08 and 2008/09 seasons. Biomass yield recorded during 2008/09 at Bothaville was greater compared to all the other seasons, although the differences was not significant (Figure 2). Similar results were recorded at Potchefstroom, except that yield of biomass recorded in 2006/07 season had comparable results to that recorded in 2008/09. Biomass yield produced during silking in 2006/07 at Bothaville decrease compared to values recorded in 2007/08 and 2008/09 seasons. On the contrary, the yield of biomass recorded during silking in 2008/09 at Ottosdal showed a depressive effect compared to values recorded in 2007/08, although the difference was not significant. Biomass yield variability in the different localities could as well partly be associated with rainfall variability in the various seasons. Annual rainfall at the four localities is in many instances very variable.

At Bethlehem, biomass yield was reduced at the ninth leaf stage in most plots that were treated with biological substances compared to the NPK standard, though biomass increased above the NPK control at silking (Table 7). During silking stage, maize biomass treated with biological substances increased on average by approximately 44% in 2006/07 and 56% in 2007/08 and 2008/09. At Bothaville, however, the different biological substances resulted in higher maize biomass at the ninth leaf stage in 2006/07 and 2008/09 compared to the NPK standard. Biomass however increased only marginally in K-humate plots in 2007/08. At silking stage, biomass yield substantially increased following the amendment of biological substances in 2006/07 but consistently decreased in the 2007/08 and 2008/09 seasons compared to NPK standard.

At Ottosdal, the different biological substances showed fairly higher biomass yield increases compared to the NPK standard at the ninth leaf stage in 2006/07 and 2008/09 seasons (Table 7). However, in 2007/08, biomass yield decreased from all the biological products and only marginally increased in Lanbac treated plots compared to NPK. During silking stage, the biological substances showed a depressive effect on biomass yield in most cases compared to the NPK standard, but was consistently higher compared to the control (Table 7). At Potchefstroom, biomass yield for all biological substance treatments were depressed compared to NPK standard at the ninth leaf growth stage in 2006/07. However, biomass yield constantly increased during the 2007/08 and 2008/09 seasons but decreased in a few cases in 2008/09 (Table 7). At the silking stage, the different biological substances showed fairly higher biomass increases in 2006/07 for most treatments, but constantly showed a depressive effect in 2007/08 and 2008/09 seasons. Of all the biological substances applied, Lanbac at Bethlehem and Growmax and Lanbac at Ottosdal resulted in increased biomass yield compared to the NPK standard at the 9th leaf stage. However, it was depressed in the Montys plots at Bethlehem as well as with Crop care, Montys and Promis at silking stage.

Regardless that Gliogrow received optimum inorganic NPK fertilizer, it resulted in consistently lower biomass yield across all the treatments during the ninth leaf stage, but comparable with all the other treatments during silking. This could be attributed to the relatively lower content of major plant nutrients (N, P, K) in the Gliogrow treatments coupled with lower organic carbon content. Comparable results during silking may be associated with the presence of living bacteria in Gliogrow that might have possibly induced microbial proliferation. This consequently promoted faster mineralization and an increased amount of plant available nutrients (Chen, 2006). These findings further revealed that the blending of biological substance with NPK and/or applying the optimum NPK rate was an effectual means to improve plant growth substantially. This is in agreement with observations by Ayoola and Makinde (2009) who stated that maize growth was flavoured by the application of enriched organic fertilizers and inorganic fertilization as from four weeks after planting. They further asserted that this is an indication that adequate nutrients required to support early growth can be attained from organic fertilization that is enrich with inorganic nutrients. These results agree well with observations on increased biomass production due to synergy between application of poultry manure and chemical fertilizer combinations, as reported by Boateng *et al*. (2006).

Effect of the biological substances on leaf area index (LAI)

The interaction effect of season x locality on maize leaf area index at the ninth leaf and silking growth stages is displayed in Figure 3. Table 8 indicates the effect of biological substance application on LAI across seasons and localities at both ninth leaf and silking growth stages. Leaf area index values recorded on both growth stages varied substantially and ranged in the order of 0.89 to 1.52 and 1.70 to 2.81 (Table 8). Leaf area index recorded in 2008/09 at Bethlehem had bigger canopy architecture compared to values recorded during 2006/07 and 2007/08 seasons (Figure 3). The LAI values recorded at Bethlehem was depressed at both sampling intervals in 2006/07. This could be associated with the consistent reduced plant height and lower biomass production as a result of less annual rainfall (321 mm) compared to the long term average of 717 mm. Climate at the four localities is in many instances very variable. The long-term mean annual rainfall varies from 502 mm at Bothaville to 718 mm at Bethlehem. The long-term daily minimum temperature ranges from 8^{o}C at Bethlehem to 11^{o}C at Potchefstroom. The Bethlehem site has as expected the lowest long-term daily mean maximum temperature of 24^{o}C. The long-term daily mean maximum temperature at the other three localities is around 27^{o}C. The daily mean evaporation recorded during the trial period at the four localities corresponds well with the long-term daily mean evaporation.

Generally, all plots that were treated with biological substances at both sampling stages gave higher LAI's compared to that in the NPK and control plots (Table 8). The LAI values from Gromor were constantly lower at both sampling intervals. The LAI values in Gliogrow were significantly lower across the seasons and localities during the ninth leaf stage but gave comparable results during silking stage. Consistent reduced LAI values in Gliogrow in 2006/07 and 2007/08 seasons is attributed to poor plant growth and development, possibly due to late plant emergence as a result of applying the 100% recommended rate that inhibited proper plant emergence. The comparable LAI values obtained in 2008/09 with Gliogrow, on both growth stages, was the result of reducing the recommended dosage of the product by half. Subsequent findings from a glasshouse study showed reduced seedling emergence when 100% of the recommendation was applied (Baloyi *et al*., 2010). Higher coefficient of variation values across the localities at the ninth leaf stage in

2006/07 is due to differences in plant growth and development, possibly as the result of the invariable rainfall distribution patterns recorded during this growth stage.

A report of an earlier study (Shortall & Liebhardt, 1975) has revealed that LAI and grain yield are positively correlated as long as the LAI is below 5. Boateng *et al*. (2006) reported low LAI of maize variety Abeleehi that resulted from the relatively low rainfall (511 mm) compared to the long term average of 700–900 mm. The low LAI values due to low rainfall were also found in this study. The LAI values obtained from NPK and Promis plots in 2008/09 and Biozone in 2007/08 were higher during the ninth leaf stage compared to all the other treatments, although the differences was not significant. Boateng *et al*. (2006) reported that the application of poultry manure and mineral fertilizer influenced the LAI significantly during the earlier stages of growth.

Effect of biological substances on maize grain yield

Grain yield obtained with the different biological amendments across the localities and seasons were compared to the NPK check in 2004/05 and 2005/06 seasons (Figures 4 - 6). Grain yield across the localities has in many instances decreased during the 2005/06 as opposed to grain yield recorded during 2004/05. The different biological substances showed inconsistent yield increases relative to the NPK check in most cases across all trial sites. Plots treated with Novon D consistently gave higher grain yield than the NPK check across all trial sites, whilst Novon A showed grain yield increase only at Bethlehem and Ottosdal. Grain yield from plots treated with Hygrotech was consistently higher at Ottosdal, whereas Crop care B and Mitygrow showed consistent grain yield increases compared to the NPK check at Potchefstroom. Regardless of the consistent grain yield increase over the NPK check for some stimulants, at Bethlehem, the differences in grain yield was not significant, whilst it only differed significantly ($P = 0.05$) in two and three cases at Ottosdal and Potchefstroom, respectively.

Effect of beneficial microorganism based products

Grain yield obtained with the different biological amendments across localities and seasons were compared to that in the NPK standard during 2006/07 to 2009/10 (Figures 7 and 8).

As indicated in Figures 7 and 8, grain yield of plots treated with Biozone has increased in eight out of 15 cases compared to the yield obtained with NPK standards but was only statistical significant in two cases. This substance appeared to do well on heavy textural soil and average on sandy soils. The yield of grain increased in 13 out of 15 cases following application of Gliogrow compared to yield obtained in NPK checks but yield differences were only significant in eight cases. The latter substance consistently increased maize grain yield regardless of the soil types investigated.

Effect of manure based products

Figures 7 and 8 indicate maize grain yield following application of the various biological substances during the four year period of investigation across the different localities. Yield in plots treated with Gromor consistently decreased across the seasons and localities compared to yield in NPK checks. Unexpectedly, significantly higher grain yield was obtained on a sandy soil but only in one case. In plots amended with Promis, yields increased compared to NPK treatments in nine cases out of 15 but yield differences were only significant in four cases. This substance appeared to do well under higher summer rainfall conditions and on sandy soils such as at Bethlehem. It, however, did not perform well under condition at Bothaville which also consisted of a sandy soil. This might be attributed to lower rainfall and higher temperatures. Promis also appeared to exert average performance on clay soils since the yield of maize has increased in two cases out of four. The Growmax treatment was blended with commercial N fertilizer to supply the same optimum N rate at each site. Grain yield however, decreased compared to the NPK checks across the seasons and localities in 10 out of 15 cases. This substance did not perform well on soils with low clay content and erratic rainfall.

Effect of humic/fulvic acid based products

Yield in plots treated with Crop care increased in 10 out of 15 cases compared to NPK checks, but the yield difference was only significant in one case (Figures 7 and 8) . Besides the one significant case, Crop care appeared to do well under all climatic and soil conditions used in this study as it consistently increased yields on both clay and sandy soils. Grain yield in plots treated with K-humate has increased in 10 out of 15 cases compared to NPK checks across the seasons and localities and was significant in six cases, rendering it a better proposition than Crop care. This substance appeared to do well under higher summer rainfall and sandy soil conditions such as at Bethlehem but also showed grain yield increases at all the other localities. Maize grain yield treated with Lanbac and Montys increased in eight out of 15 cases compared to NPK checks across seasons and localities, but the yield differences were significant in six cases for Lanbac and only one case for Montys. Both of these substances, however, showed grain yield decreases at Bothaville across the seasons. Seven out of nine biological substances generally resulted in grain yield increases over the NPK check by an average of nine out of 15 cases, although the differences were not always significant.

Grain yield increases following amendment of different biological substances compared to the NPK check was inconsistent across the seasons and localities during the years of assessment. At Bethlehem, grain yield consistently increased over seasons when treated with Promis and K-humate with an average increase of 15 and 11%, respectively. Maize grain yield consistently increased following addition of Gliogrow over seasons at Ottosdal and Potchefstroom with an average increase of 12 and 21%, respectively. Four years of field trials by ARC-GCI revealed that grain yield decreased in most cases following amendment of the different active ingredients during the first season of application but gradually increase in the preceding years. Grain yield has consistently improved with humic/fulvic acid and beneficial microorganism based active ingredients, although the yield differences were only always significant. For example, grain yield recorded with the beneficial microorganisms was significant in 10 out of 21 cases, whereas grain yield showed significant increase with the humic/fulvic acid in 14 out of 36 cases. This was partly as a result of beneficial microorganisms and humic/fulvic acids that were supplements to already optimum NPK rates at each site.

Several reports have documented the higher yield of maize from the combined use of NPK fertilizer and poultry manure compared to sole application of either composition. Such studies revealed that organic fertilizers can be enriched with inorganic nitrogen to obtain maize yields similar to yields from plants fertilized with inorganic fertilizers (Makinde *et al*., 2001; Adeniyan & Ojeniyi, 2005; Boateng *et al*., 2006; Ayoola & Makinde, 2009). Moreover, Boateng *et al*. (2006) noted that there is a synergy between poultry manure and chemical fertilizer which resulted in improved maize yield.

CONCLUSIONS

Based on the results for the 2004/05 and 2005/06 season, there seems to be a tendency for the stimulants to be more effective at lower potential conditions. Because of the intensive treatments with certain biological substances (five sprayings per season plus a seed treatment) and the costs associated with it, some biological substances do not seem to be cost effective even where yields are higher compared to the NPK check. It is important to evaluate such substances over a longer period of time to make comprehensive conclusions. With every season the number of companies participating increased. However, certain companies are reluctant to let their products be tested.

The effects of the various biological substances were assessed on maize phenology and grain yield. The variable responses of plant phenological traits to the biological substances were also dependent on climate and soil characteristics such as nutrient holding capacity and/or fertilization history. Maize growth consistently showed a depressive effect with Gliogrow at vegetative growth stage but had comparable results with all the other treatments during the reproductive sampling intervals. Care should be exercise with the application of Gliogrow when planting in soils with high clay content of over 30%. The results of this study showed that the application of Gromor could partly increase crop yields in soils where P is limiting, such as the case in most South African soils. Soils used for the current studies were not P deficient possibly due to a long history of crop fertilization and fertilizer research. Regardless halving recommended fertilizer rates, Monty's treatments resulted in fairly vigorous plant growth and maize productivity. The inconsistent grain yield responses by the different biological substances amended suggest zonal specific effectiveness of the substances. The consistent reduced grain yield from manure based substances suggested it is not yet time to do away with the use of synthetic fertilizers but rather enrich them with inorganic fertilizer thereby enhance their efficiency. Crop care, Gliogrow, Growmax and K-humate treatments appeared most promising for sustainable crop production.

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LITERATURE CITED

ADENIYAN, O. N. & OJENIYI, S. O., 2005. Effect of poultry manure, NPK 15-15-15 and combination of their reduced levels on maize growth and soil chemical properties. *Nig. J. Soil Sci*.15, 34 - 41.

AHMED, R., NASEER, A., ZAHIR, Z. A., ARSHAD, M., SULTAN, T. & ULLAH, M. A., 2006. Integrated use of recycled organic waste and chemical fertilizers for improving maize yield. *Int. J. Agric. Biol*. 8, 840 - 843.

ARC-MIG, 2006. MAIZE INFORMATION GUIDE. Soil sampling methods. Pp 49 - 51.

AWOTUNDUM, J. S., BANDELE, M.T. & AMINU, A., 1994. Effect of different levels of nitrogen fertilizer on the yield of hybrid maize. *Nig. J. Bot* 7, 39 - 44.

AYOOLA, O. T. & MAKINDE, E. A., 2009. Maize growth, yield and soil nutrient changes with N-enriched organic fertilizers. *Afr. J. Food Agric. Nutr. & Environ*. 9 (1), 582 - 595.

BALOYI, T. C., KUTU, F. R. & DU PREEZ, C. C., 2010. Assessment of variable rates of industrially manufactured biological amendment substances on the establishment and growth characteristics of maize seedlings. Paper presented at the 2010 Combined Congress of SASSS, SASCP, SAWSS and SASHS held at the University of the Free State, Bloemfontein on the 21 - 24 January 2010.

BOATENG, S. A., ZICKERMANN, J. & KORNAHRENS, M., 2006. Poultry manure effect on growth and yield of maize. *West Afr. J. Appl. Ecol.* 9, 1 - 11.

CHEN, J-H., 2006. The combined use of chemical and organic fertilisers and/or biofertilisers for crop growth and soil fertility. International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertiliser use 16 - 20 October 2006. Bangkok, Thailand. Pp. 1 - 11.

GOSHAL, N. & SINGH, K. P., 1995. Effects of farmyard manure and inorganic fertiliser on dynamics of soil microbial biomass in a tropical dryland agroecosystem. *Biol. Fertil. Soils* 19, 232 - 238.

GRUHN, P., GOLETTI, F & YUDELMAN, M., 2000. Integrated nutrient management, soil fertility, and sustainable agriculture: current issues and future challenges. 2020 Brief 67. A 2020 Vision for Food, Agriculture, and the Environment. International Food Policy Research Institute, Washington, D.C., USA.

HAMDI, Y. A., 1982. Application of nitrogen-fixing systems in soil improvement and management. Food and Agriculture Organisation, Rome. FAO Soils Bull. 49.

HELUF, G., 2002. Soil and water management research program. Summary report of 2000/2001 research activities, Alemaya Research Centre, Alemaya University. 95pp.

MAKINDE, E. A., AGBOOLA, A. A. & OLUWATOYINBO, F. I., 2001. The effects of organic and inorganic fertilizers on the growth and yield of maize in a maize/melon intercrop. *Moor J. Agr. Res*. 2, 15 - 20.

MOHAMED, S. A., EWEES, S. A., EL-YAZAL, S. & EL-SOWFY, D. M., 2008. Improving maize grain yield and its quality grown on a newly reclaimed sandy soil by applying micronutrient, organic manure and biological inoculation. *Res. J. Agric. & Biol. Sci.* 4, 537 - 544.

OBI, M. E. & EBO P. O., 1995. The effects of different application rates of organic and inorganic fertilizers on soil physical properties and maize production in a severely degraded ultisol in southern Nigeria. *Bioresource Tech.* 51, 117 - 123.

POCOCK, J., 2007. Fertilizer prices flatten. Farm Industry News, Penton Media. http://farmindustrynews.com/

POTASH & PHOSPHATE INSTITUTE, 2007. Soil test based fertilizer application for yield improvement of maize, wheat and potato in Jharkhand. Norcross, Georgia, USA.

SAXEMA, M. C. & SINGH, Y. 1965. A note on leaf area estimation of intact maize leaves. *Indian J. Agron.* 10, 437 - 439.

SHORTALL, J. G. & LIEBHARD, T. W. C., 1975. Field and growth of corn as affected by poultry manure. *J. Environ. Qual.* 4 (2), 186 - 191.

TEICHERT-CODDINGTON, D. R. & GREEN, B. W., 1993. Usefulness of inorganic nitrogen in organically fertilized tilapia production ponds. European Aquaculture Society Special Publication No. 19. Oostende, Belgium. 273pp.

THE NON-AFFILIATED SOIL ANALYSIS WORK COMMITTEE, 1990. Handbook of Standard Soil Testing Methods for Advisory Purposes. Soil Sci. Soc. of South Africa, Pretoria.

VAN BILJON, J. J., FOUCHE, D. S. & BOTHA, A. D. P., 2008. The lower and upper threshold values, biological optimum and mineralization of N in the main maize producing soils of South Africa. *S. Afr. J. Plant Soil* 25, 8 - 13.

VILJOEN, B., 2006. An Africa Bio - Source Fertilizer Project: fulvic and humic formulations, scientific, process engineering and business development of organic commercial fertilizer in Southern Africa.

Table 1: Selected geographic description, climatic and soil characteristics of the different localities used during the four-year period of study

Characteristics	Bethlehem	Bothaville	Ottosdal	Potchefstroom
Climatic				
Latitude	28°23'	26°62'	26°08'	27°09'
Longitude	-28°23'	-27°38'	-26°81'	-27°7'
Altitude (m)	1850	1317	1587	1355
Ave. rainfall (mm)	716	544	426	614
Ave. long term rainfall (mm)	718	502	593	622
Ave. min.Temperature (°C)	7.1	8.1	9.0	10.2
Ave. max.Temperature (°C)	22.9	26.6	26.5	26.0
Ave. A-pan (mm)	4.4	5.0	4.5	4.8
Soils				
Sand (%)	74.6	91.1	80.9	48.7
Silt (%)	11.4	0.9	7.1	17.3
Clay (%)	14	8	12	34
Textural class	Sandy loam	Sand	Loam	Clay loam
pH (H_2O)	5.47	7.02	5.83	6.61
N (mg kg^{-1})	2.9	0.9	2.8	5.7
P (mg kg^{-1})	19	22	16	56
K (mg kg^{-1})	112	74	135	192
Ca (mg kg^{-1})	298	348	317	840
Mg (mg kg^{-1})	77	97	102	360
Na (mg kg^{-1})	11	15	13	32
Organic C (%)	0.43	0.20	0.38	0.82

Table 2: Biological substances evaluated during 2004/05 and 2005/06 at three field trial sites

Treatments	Application technique	Recommendations
Crop care	Seeds	**Crop care A and B**: 2 ml Agri-Zn + 20 ml water on 500 g seeds
	Soil	**Crop care A**: 5 l Agri-Balance ha^{-1}
	Foliar	**Crop care B**: Apply 5 l Agri-Zn ha^{-1} at 1 and 2 weeks after emergence + 3 l Agri Boost at 2, 3, 4, 5 and 9 weeks after emergence
	Foliar	**Crop care A and B**: Apply 5 l Agri-Zn ha^{-1} at 1 and 2 weeks after planting + 3 l Agri Boost at 2, 4 and 5 weeks after emergence
Novon	Seeds	**Novon A**: Maxiflo: 2 ml + 20 ml water on 500 g seeds + 4 ml Teprosyn Zn/P
		Novon B: Trykocide: 2 ml + 20 ml water on 500 g seeds + 4 ml Teprosyn Zn/P
		Novon C: Maxiflo + Trykocide: 2 ml + 20 ml water on 500 g seeds + 4 ml Teprosyn Zn/P
		Novon D: Maxiflo + Trykocide 2 ml + 20 ml water on 500 g seeds + 4 ml Teprosyn Zn/P
	Foliar	**Novon A**: 300 ml ha-1 Maxiflo with 200 l water + 100 ml wetting agent per 100 l water per hectare 4 weeks after emergence
		Novon B: 300 ml ha-1 Maxiflo + Trykocide with 200 l water + 100 ml wetting agent per 100 l water per hectare two weeks after emergence
		Novon C: 300 ml ha-1 Maxiflo with 200 l water + 100 ml wetting agent per 100 l water per hectare four weeks after emergence
		Novon D: 300 ml ha-1 Maxiflo with 200 l water + 100 ml wetting agent per 100 l water per hectare between flowering and silking
Soygro	Seeds	**Soygro A**: 2 ml elk van middels 1, 2 nd 3 + 4ml from middle on 20 ml water per 500 seeds
		Soygro B: 2 ml elk van middels 1, 2 nd 3 + 4ml from middle on 20 ml water per 500 seeds
	Foliar	**Soygro A**: 1.5 L ha-1 elk from Spoor and Boor + Groenwoema with 200 l water 4 weeks after emergence
		Soygro B: 1.5 l ha-1 elk from Spoor and Boor + Groenwoema with 200 l water + 1.5 l Patostop ha^{-1} 4 weeks after emergence
MBF	Foliar	500 ml INS with 180 l of water per hectare 1 week after emergence
Mitygrow	Soil +	150 ml in 8000 L water
	Foliar	150 ml in 8000 L water from 14 days after planting with intervals of 14 days until flowering
Montys	Seeds	5 ml 4-15-12 + 5 ml water per 350 g seeds
	Foliar	45 ml 8-16-8 + 5 l water per 360 m^2 two weeks after emergence
Hygrotech	Seeds	3 ml Arise Liquid per 500 g seeds
	Foliar	2.5 l Maize plus per ha (1% solution) at six and 10 leaf stage
Organics	Soil	10 l ha^{-1} MS Humate + 2 kg ha^{-1} Microboost + 2 l ha^{-1} Microbial inoculants

Table 3: Biological substances evaluated at four localities during 2006/07 – 2009/10

Treatment	Active ingredient	Application procedure	Recommendations
Biozone	BM	Soil	Optimum fertilizer rate (OFR) at the site + 10 l ha^{-1} at planting
Gliogrow	BM	Seed dressing + foliar	OFR at the site + Maxiflo and Trykocide (200 ml each) + 100 ml Teprosyn Zn/P per 25 kg seeds; 400 ml Maxiflo & Trykocide 4 weeks after emergence
Gromor	MN	Soil	2000 kg ha^{-1} at planting; complete replacement of NPK
Promis	MN	Soil	1000 kg ha^{-1} at planting; complete replacement of NPK
Growmax	MN + F	Soil	Rates should be equal to the OFR at the sites; applied to meet the requirement of P; Contain 3 kg ha^{-1} P and N. The N remaining is blended with N containing fertilizer
Crop care	H/F	Soil + foliar	400 kg ha^{-1} Growmax and 70% OFR + 5 l ha^{-1} Agri-balance at planting; 2.5 l ha^{-1} Agri-boost and 2.5 l Agri-Zinc at 4 weeks after planting; 2 l ha^{-1} Agri-fulbor at tasseling
K-humate	H/F	Soil	OFR at the site + 20 kg ha^{-1} a week prior to planting
Lanbac	BM + H/F	Soil	OFR at the site + 10 l ha^{-1} MS Humate + 2 kg ha^{-1} Microboost + 2 l ha^{-1} Microbial inoculants at planting
Montys	H/F	Soil	50% of OFR + 3 l ha^{-1} at planting
NPK		Soil	Depended on the required NPK rate at each site for optimum fertility conditions.
Control			No application of either NPK or biological substance

BM = Beneficial microorganism from bacterial and fungal sources based; **F** = commercial fertilizer; **H/F** = Humic/fulvic acids based and MN = Manure based substance.

Table 4: Details of NPK fertilization rates applied at the four sites for optimum fertility.

	Optimum NPK fertilizer application rates (kg ha^{-1})			
Fertilizer source	Bethlehem	Bothaville	Ottosdal	Potchefstroom
N	40	100	70	80
P	69.5	73.7	73.7	43.5
K	55	55	0	0

Table 5: Variance ratio of testing differences on the effects of season, locality and treatment on plant height, biomass yield and leaf area index

	Plant height		Biomass yield		Leaf area index	
Factors	9th leaf	Silking	9th leaf	Silking	9th leaf	Silking
Season (S)	667***	3531***	8967***	6626***	2.18***	2.94***
Locality (L)	3213***	4449***	1067***	7102***	3.23***	18.4***
Treatment (T)	140***	634*	173*	993ns	0.45***	0.09ns
SxL	2412***	458***	1210***	9423***	0.53***	0.82***
SxT	95***	250ns	188ns	993ns	0.26***	0.10ns
LxT	44ns	309ns	134ns	447ns	0.13ns	0.07ns
SxLxT	49ns	310ns	156ns	580ns	0.10ns	0.13ns

ns = not significant; * = significant at P=0.05; *** = significant at P=0.001

Table 6: Plant height (cm) as influenced by application of biological substances

	Plant height (cm)															
	9th leaf growth stage				Silking growth stage				9th leaf growth stage				Silking growth stage			
Treatments	**2006/07**	**2007/08**	**2008/09**	**Mean**	**2006/07**	**2007/08**	**2008/09**	**Mean**	**2006/07**	**2007/08**	**2008/09**	**Mean**	**2006/07**	**2007/08**	**2008/09**	**Mean**
				Bethlehem								**Bothaville**				
Biozone	96.0	118.1	108.7	107.6	185.7	184.2	175.9	181.9	141.7	121.9	95.9	119.8	201.0	219.8	195.0	205.3
Gliogrow	82.7	110.2	97.3	96.7	194.7	183.8	178.9	185.8	120.9	131.4	94.1	115.5	206.7	223.8	178.8	203.1
Gromor	96.3	87.2	109.8	97.8	193.6	183.0	181.5	186.0	129.9	97.2	91.6	106.2	204.8	194.9	172.5	190.7
Promis	75.9	107.1	116.2	99.7	193.4	191.8	170.5	185.2	131.5	117.1	92.7	113.7	221.3	209.4	186.3	205.6
Growmax	89.9	108.0	102.3	100.1	182.0	199.2	173.6	184.9	128.3	118.0	94.8	113.7	213.3	205.3	186.3	201.6
Crop care	100.4	118.1	102.1	106.9	183.2	182.3	183.3	182.9	137.7	134.4	93.7	121.9	216.6	218.1	193.8	209.5
K-humate	104.1	126.1	99.1	109.8	177.4	186.6	180.0	181.3	134.2	136.1	95.0	121.7	207.7	207.5	185.0	200.1
Lanbac	83.9	121.4	112.2	105.8	198.6	189.7	179.1	189.1	136.1	120.2	97.7	118.0	219.4	214.2	176.3	203.3
Montys	88.9	105.1	86.4	93.5	179.5	201.0	182.6	187.7	135.8	115.1	92.6	114.5	210.8	210.0	186.3	202.4
NPK	106.1	116.6	106.7	109.8	186.5	187.0	171.8	181.7	134.6	126.6	92.0	117.7	207.4	212.1	180.0	199.8
Control	91.1	88.6	103.8	94.5	184.6	163.0	167.3	171.6	132.4	98.6	86.3	105.7	203.5	185.7	171.3	186.8
Mean	**92.3**	**109.7**	**104.1**	**102.0**	**187.2**	**186.5**	**176.8**	**183.5**	**133.0**	**119.7**	**93.3**	**115.3**	**210.2**	**209.2**	**182.8**	**200.7**
Cv (%)	**11.2**	**11.8**	**15.1**		**8.2**	**7.3**	**8.2**		**5.8**	**10.4**	**6.2**		**10.9**	**7.8**	**9.0**	
$LSD_{T(0.05)}$	**25.4**	**31.7**	**38.6**		**37.6**	**33.5**	**35.7**		**19.1**	**30.7**	**14.1**		**56.1**	**39.8**	**40.6**	
				Ottosdal								**Potchefstroom**				
Biozone	145.4	124.6	113.1	127.7	236.8	226.5	201.3	221.5	123.6	137.8	115.0	125.5	242.1	238.3	210.7	230.4
Gliogrow	129.2	103.9	152.0	128.4	237.0	202.5	211.3	216.9	83.5	132.4	143.4	119.8	223.2	238.6	187.0	216.2
Gromor	143.4	114.1	155.0	137.5	229.5	205.0	212.5	215.7	104.1	137.6	156.9	132.9	231.7	238.8	175.2	215.2
Promis	146.7	131.2	128.1	135.3	251.0	223.1	210.0	228.0	128.5	135.5	116.1	126.7	231.7	245.4	209.3	228.8
Growmax	149.4	139.1	144.4	144.3	236.4	210.6	196.3	214.4	120.2	128.9	143.6	130.9	232.4	251.5	191.9	225.2
Crop care	149.3	126.7	133.4	136.5	234.2	231.7	205.0	223.6	108.9	134.0	126.2	123.0	222.2	250.2	190.7	221.0
K-humate	152.4	138.3	161.8	150.8	230.4	225.8	213.8	223.3	121.8	129.0	156.1	135.6	220.5	250.6	189.4	220.1
Lanbac	146.3	141.3	162.5	150.0	237.6	224.2	202.5	221.4	130.6	138.6	162.4	143.8	234.0	249.9	197.0	226.9
Montys	149.9	134.5	138.1	140.8	235.2	222.9	215.0	224.4	132.4	137.4	140.6	136.8	237.2	248.2	211.8	232.4
NPK	148.9	137.1	135.2	140.4	246.5	219.0	213.8	226.4	122.3	132.7	141.6	132.2	230.1	240.8	212.5	227.8
Control	141.4	108.9	136.1	128.8	229.4	201.6	201.3	210.8	112.9	129.4	114.3	118.8	215.5	222.2	166.5	201.4
Mean	**145.7**	**127.2**	**141.8**	**138.2**	**236.7**	**217.5**	**207.5**	**220.6**	**117.1**	**133.9**	**137.8**	**129.6**	**229.1**	**243.1**	**194.7**	**222.3**
Cv (%)	**5.6**	**10.2**	**17.0**		**6.8**	**5.4**	**7.0**		**11.5**	**6.0**	**20.0**		**8.1**	**4.5**	**8.6**	
$LSD_{T(0.05)}$	**20.1**	**31.7**	**59.4**		**39.8**	**28.6**	**35.8**		**33.1**	**19.7**	**67.9**		**45.7**	**27.1**	**41.3**	

Table 7: Effect of biological substances on biomass yield at various stages of growth at four sites over three seasons

	Biomass yield (kg ha^{-1})															
	9th leaf growth stage				Silking growth stage				9th leaf growth stage				Silking growth stage			
Treatments	**2006/07**	**2007/08**	**2008/09**	**Mean**	**2006/07**	**2007/08**	**2008/09**	**Mean**	**2006/07**	**2007/08**	**2008/09**	**Mean**	**2006/07**	**2007/08**	**2008/09**	**Mean**
	Bethlehem								**Bothaville**							
Biozone	1099	475	720	765	3402	2635	3390	3142	646	644	1468	919	2360	3806	3446	3204
Crop care	998	602	603	734	2723	3128	3300	3050	824	734	1660	1072	2433	3959	3411	3268
Gliogrow	839	408	533	593	3393	2604	3300	3099	815	683	1213	904	2407	3625	3301	3111
Gromor	836	193	477	502	3164	2564	3608	3112	911	343	1272	842	2191	2394	3249	2611
Growmax	1209	396	512	706	2794	3076	2629	2833	838	546	1771	1052	2132	3417	3677	3075
K-humate	887	663	742	764	2419	3323	3506	3083	844	813	1414	1023	2121	3957	3501	3193
Lanbac	1217	533	537	762	3675	3236	3822	3578	796	558	1530	961	2736	3791	3248	3259
Montys	1119	470	532	707	2906	2901	3208	3005	900	620	1349	956	2031	3321	3470	2940
Promis	1116	373	355	615	3562	3214	3293	3356	713	523	1229	822	1903	3091	3472	2822
NPK	1517	584	771	957	3294	3033	3302	3210	629	734	1217	860	1765	4517	3699	3327
Control	799	345	334	493	2360	1583	2437	2126	404	495	1098	666	1599	1766	2269	1878
Mean	**1058**	**458**	**556**	**691**	**3063**	**2845**	**3254**	**3054**	**756**	**608**	**1384**	**916**	**2152**	**3422**	**3340**	**2972**
Cv (%)	**39.3**	**42.6**	**40.9**		**26.1**	**17.7**	**15.7**		**33.6**	**32.3**	**33.1**		**19.7**	**22.0**	**9.3**	
$LSD_{T(0.05)}$	**1023**	**480**	**560**		**1962**	**1241**	**1258**		**624**	**484**	**1127**		**1035**	**1848**	**761**	
	Ottosdal								**Potchefstroom**							
Biozone	1060	1095	1506	1220	3952	3794	3604	3783	1197	1012	1668	1292	4122	3330	3568	3673
Crop care	1101	1082	1351	1178	3266	3914	3705	3628	1145	792	1270	1069	4060	3340	3315	3572
Gliogrow	1070	776	1277	1041	3271	3232	3529	3344	1149	873	1595	1205	3613	2991	3472	3359
Gromor	1124	743	1479	1115	2745	3140	4028	3304	1407	787	1149	1114	4325	3078	2934	3446
Growmax	1119	1177	1618	1305	3709	4084	3431	3741	1344	794	1300	1146	3882	3149	3225	3419
K-humate	1350	1023	1340	1238	3921	3865	2905	3564	1242	889	1365	1165	3305	3378	3547	3410
Lanbac	1103	1197	1166	1155	3674	4030	3457	3720	852	931	1688	1157	3745	3495	3717	3652
Montys	1211	947	1458	1205	2929	4045	3602	3525	1261	870	1853	1328	3842	3197	3872	3637
Promis	1158	1030	1214	1134	3189	4053	3258	3500	996	877	1492	1122	3704	3158	3931	3598
NPK	1074	1121	1126	1107	3301	4069	3752	3707	1527	750	1536	1271	3809	3437	3823	3690
Control	876	836	783	831	2390	2222	2884	2498	846	729	971	849	2371	2570	2555	2499
Mean	**1113**	**1002**	**1302**	**1139**	**3304**	**3677**	**3469**	**3483**	**1179**	**846**	**1444**	**1156**	**3707**	**3193**	**3451**	**3450**
Cv (%)	**21.3**	**18.1**	**40.0**		**19.5**	**15.7**	**11.6**		**38.4**	**20.9**	**25.8**		**14.0**	**12.5**	**19.1**	
$LSD_{T(0.05)}$	**583**	**447**	**1279**		**1586**	**1415**	**988**		**1112**	**434**	**916**		**1273**	**979**	**1619**	

Table 8: Effect of biological substances on leaf area index at two growth stages and at four sites over three seasons

	Leaf area index (LAI)															
	9th leaf growth stage				**Silking growth stage**				**9th leaf growth stage**				**Silking growth stage**			
Treatments	**2006/07**	**2007/08**	**2008/09**	**Mean**	**2006/07**	**2007/08**	**2008/09**	**Mean**	**2006/07**	**2007/08**	**2008/09**	**Mean**	**2006/07**	**2007/08**	**2008/09**	**Mean**
				Bethlehem								**Bothaville**				
Biozone	1.05	1.14	1.45	1.21	1.66	1.80	1.75	1.73	1.00	1.38	1.17	1.18	2.11	2.55	2.94	2.53
Crop care	1.01	1.30	1.40	1.24	1.72	1.69	2.14	1.85	1.32	1.48	1.18	1.32	2.17	2.47	2.61	2.41
Gliogrow	0.50	0.76	1.38	0.88	1.91	1.67	1.73	1.77	1.17	1.51	1.22	1.30	2.07	2.59	2.55	2.40
Gromor	0.90	0.40	1.32	0.87	1.85	1.69	1.86	1.80	1.12	0.61	1.16	0.96	2.24	2.45	2.42	2.37
Growmax	0.98	0.99	1.20	1.06	1.52	1.93	1.63	1.69	1.42	1.20	1.32	1.31	2.13	2.39	2.67	2.40
K-humate	1.11	1.24	1.37	1.24	1.51	1.73	1.74	1.66	1.35	1.45	1.22	1.34	2.26	2.28	3.04	2.53
Lanbac	0.93	1.30	1.43	1.22	1.82	1.79	1.70	1.77	1.25	0.97	1.17	1.13	2.20	2.52	2.60	2.44
Montys	0.81	0.87	1.19	0.96	1.49	1.84	1.64	1.65	1.06	1.08	1.25	1.13	2.14	2.54	2.54	2.40
Promis	0.44	0.86	1.59	0.96	1.98	1.70	1.93	1.87	1.04	1.06	1.26	1.12	2.03	2.34	2.63	2.33
NPK	1.31	1.29	1.59	1.40	1.90	1.70	1.71	1.77	1.03	1.50	1.20	1.24	2.07	2.32	2.66	2.35
Control	0.81	0.49	1.30	0.87	1.39	1.66	1.54	1.53	0.94	0.69	1.08	0.90	2.01	2.20	2.12	2.11
Mean	**0.89**	**0.97**	**1.38**	**1.08**	**1.70**	**1.74**	**1.76**	**1.74**	**1.15**	**1.17**	**1.20**	**1.18**	**2.13**	**2.42**	**2.61**	**2.39**
Cv (%)	**41.9**	**27.8**	**21.8**		**16.5**	**11.6**	**17.4**		**26.9**	**23.2**	**8.9**		**10.5**	**13.3**	**15.8**	
LSD$_{T(0.05)}$	**0.92**	**0.66**	**0.74**		**0.69**	**0.50**	**0.75**		**0.76**	**0.67**	**0.26**		**0.55**	**0.79**	**1.01**	
				Ottosdal								**Potchefstroom**				
Biozone	1.21	1.28	1.34	1.27	2.42	2.55	2.90	2.63	1.39	1.45	1.49	1.44	2.60	2.73	2.72	2.68
Crop care	1.27	1.57	1.31	1.38	2.35	2.41	2.93	2.56	1.27	1.57	1.46	1.43	2.41	2.90	2.19	2.50
Gliogrow	0.92	1.10	1.38	1.13	2.33	2.84	2.79	2.66	0.90	1.38	1.54	1.27	2.53	2.46	2.31	2.43
Gromor	1.31	1.05	1.35	1.24	2.19	2.36	3.01	2.52	1.45	1.47	1.49	1.47	2.44	2.57	2.20	2.41
Growmax	1.52	1.52	1.41	1.48	2.31	2.63	2.73	2.56	1.50	1.39	1.56	1.48	2.39	2.70	2.42	2.51
K-humate	1.28	1.23	1.33	1.28	2.33	2.50	2.80	2.54	1.38	1.40	1.48	1.42	2.43	2.40	2.74	2.52
Lanbac	1.09	1.68	1.40	1.39	2.29	2.41	2.93	2.54	1.69	1.49	1.56	1.58	2.63	2.46	2.56	2.55
Montys	1.33	1.50	1.30	1.38	2.35	2.68	2.68	2.57	1.68	1.55	1.61	1.62	2.52	2.29	2.65	2.48
Promis	1.32	1.38	1.39	1.36	2.28	2.42	2.71	2.47	1.43	1.38	1.53	1.45	2.51	2.37	2.65	2.51
NPK	1.01	1.56	1.50	1.36	2.48	2.71	3.01	2.73	1.40	1.33	1.66	1.46	2.35	2.40	2.61	2.45
Control	0.92	0.90	1.46	1.09	2.18	2.18	2.44	2.27	0.95	1.30	1.45	1.23	2.32	2.26	2.02	2.20
Mean	**1.20**	**1.34**	**1.38**	**1.31**	**2.32**	**2.52**	**2.81**	**2.55**	**1.37**	**1.43**	**1.53**	**1.44**	**2.47**	**2.50**	**2.46**	**2.48**
Cv (%)	**31.0**	**20.7**	**13.5**		**6.1**	**11.9**	**11.6**		**30.4**	**19.9**	**12.8**		**6.6**	**23.0**	**16.2**	
LSD$_{T(0.05)}$	**0.91**	**0.68**	**0.46**		**0.35**	**0.72**	**0.80**		**1.02**	**0.70**	**0.48**		**0.40**	**1.42**	**0.98**	

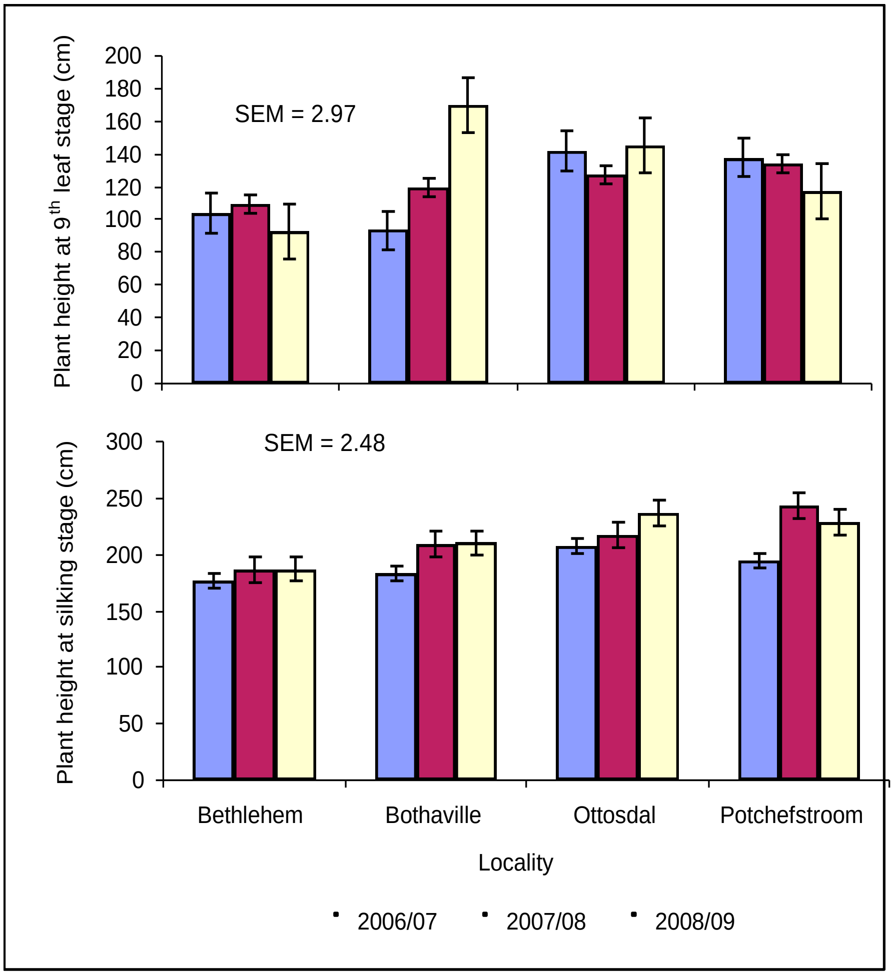

Figure 1: Season x locality interaction effect on plant height as influenced by biological substances during the ninth leaf and silking growth stages. **SEM** = Standard error of the mean

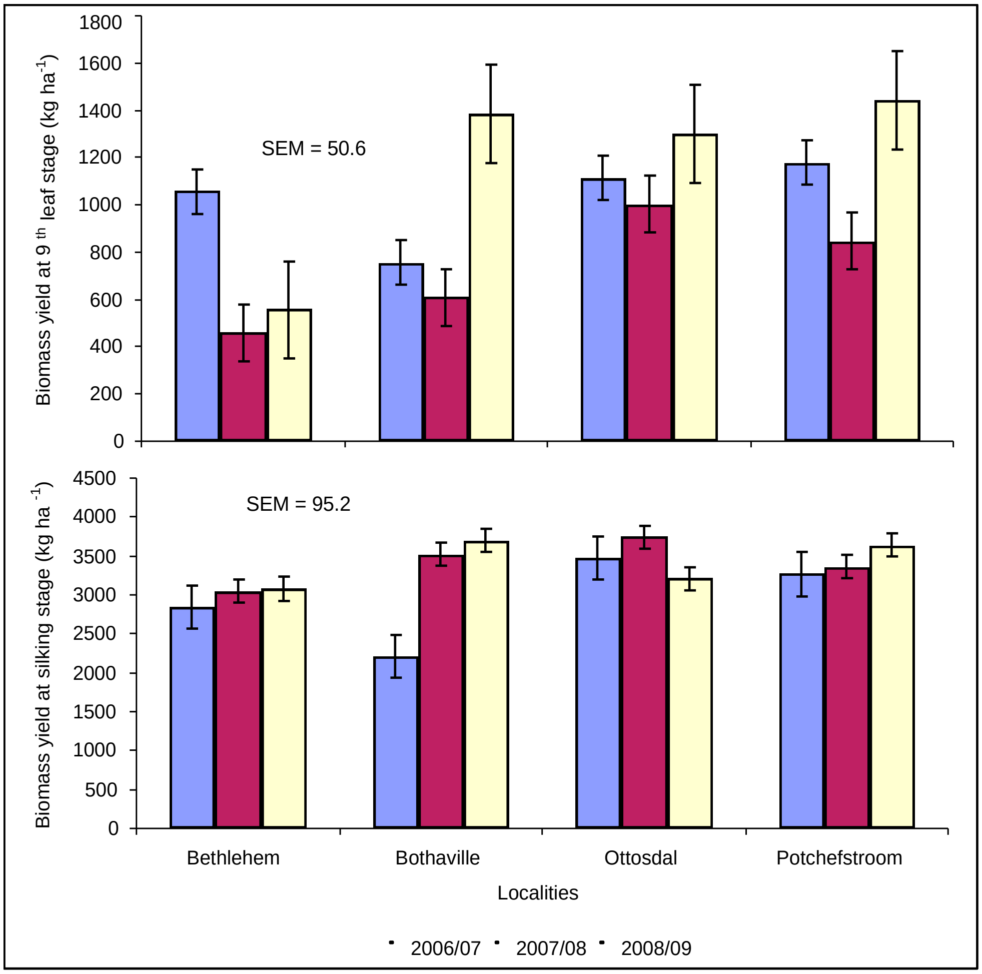

Figure 2: Season x locality interaction effect on biomass yield as influenced by application of biological substance at ninth leaf and silking growth stages. **SEM** = Standard error of the mean

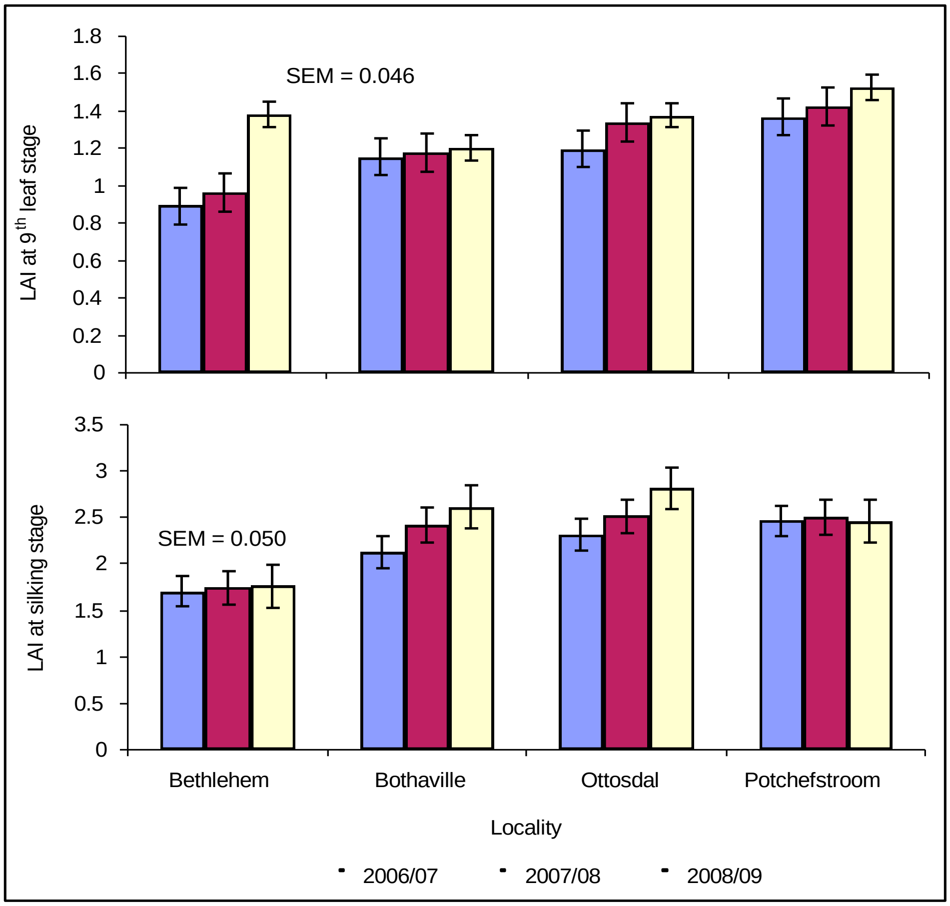

Figure 3: Interaction effects of season x locality on leaf area index (LAI) as affected by biological substances. **SEM** = Standard error of the mean.

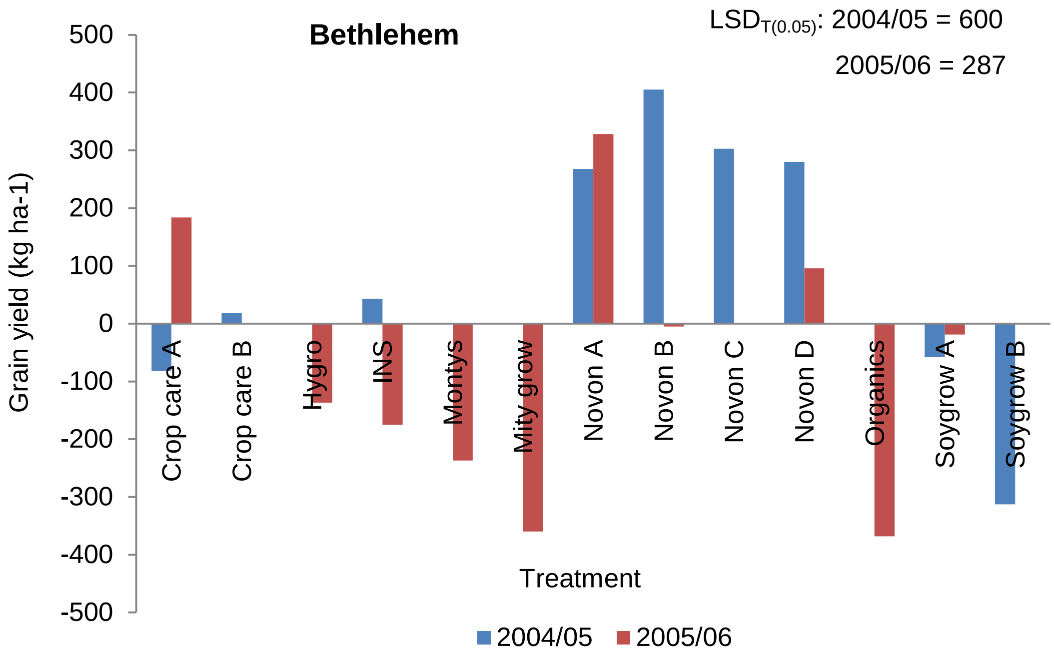

Figure 4: Effect of biological substances on grain yield at Bethlehem in 2004/05 and 2005/06 seasons. The bars above the zero (0) x - axis indicate grain yield increase to the NPK check and values below indicate grain yield decreases. **In cases where one bar is presented on the graph it indicates that the substance was evaluated for one season**.

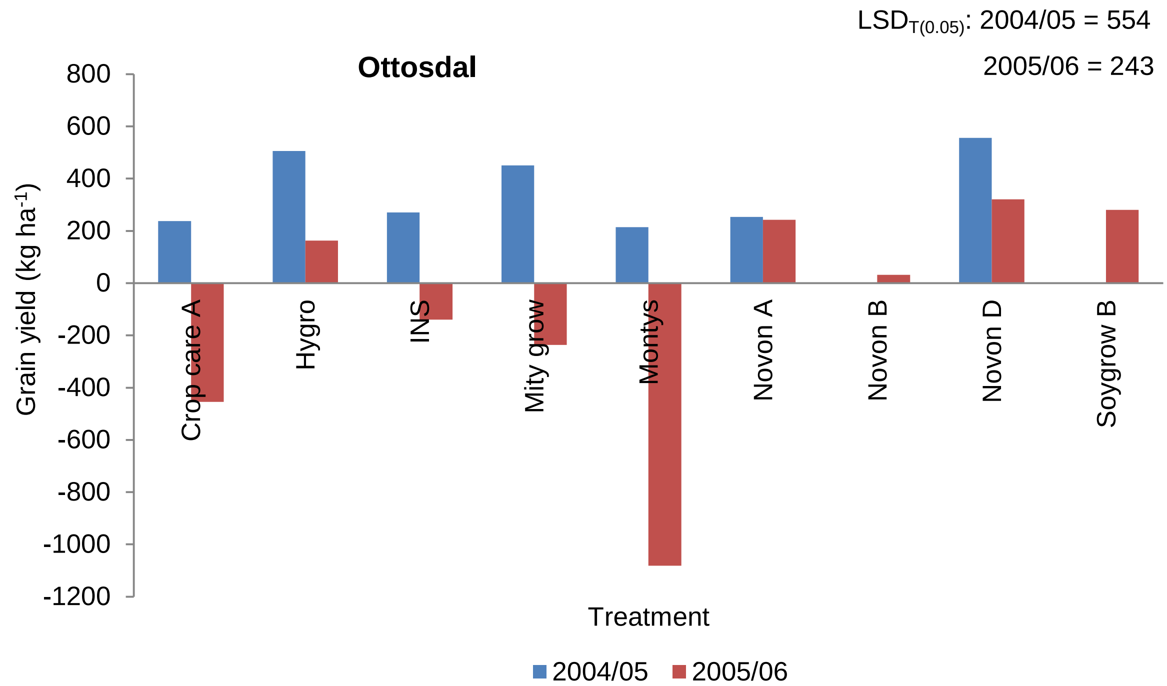

Figure 5: Effect of biological substances on grain yield at Ottosdal in 2004/05 and 2005/06 seasons. The bars above the zero (0) x - axis indicate grain yield increases compared to the control and values below indicate grain yield decreases.

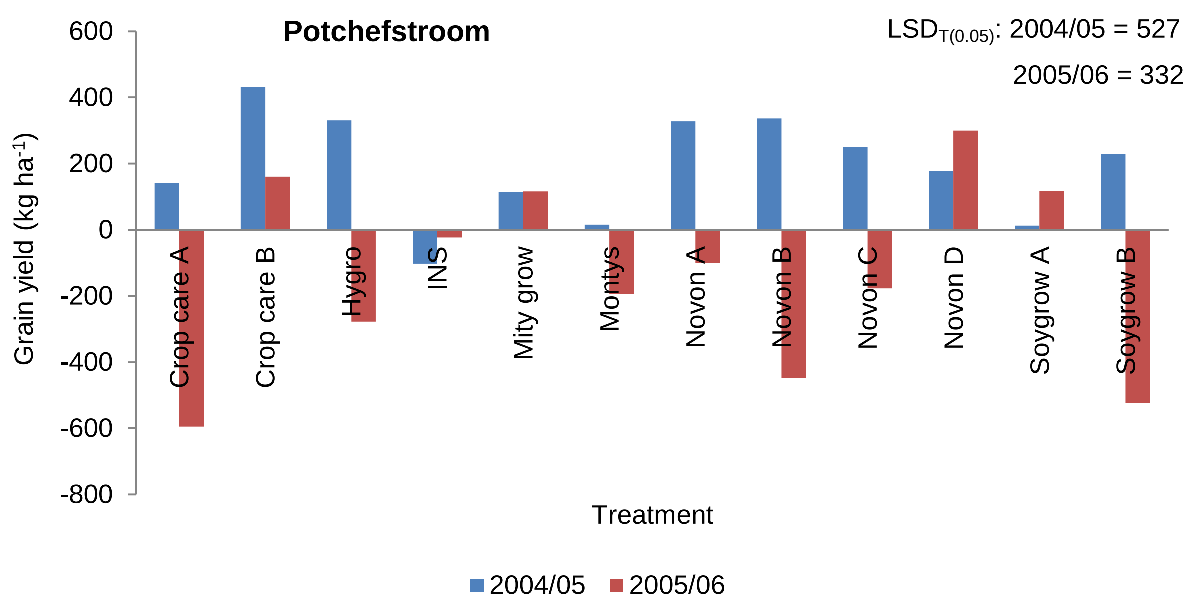

Figure 6: Effect of biological substances on grain yield at Potchefstroom in 2004/05 and 2005/06 seasons. The bars above the zero (0) x - axis indicate grain yield increases compared to the control and values below indicate grain yield decreases.

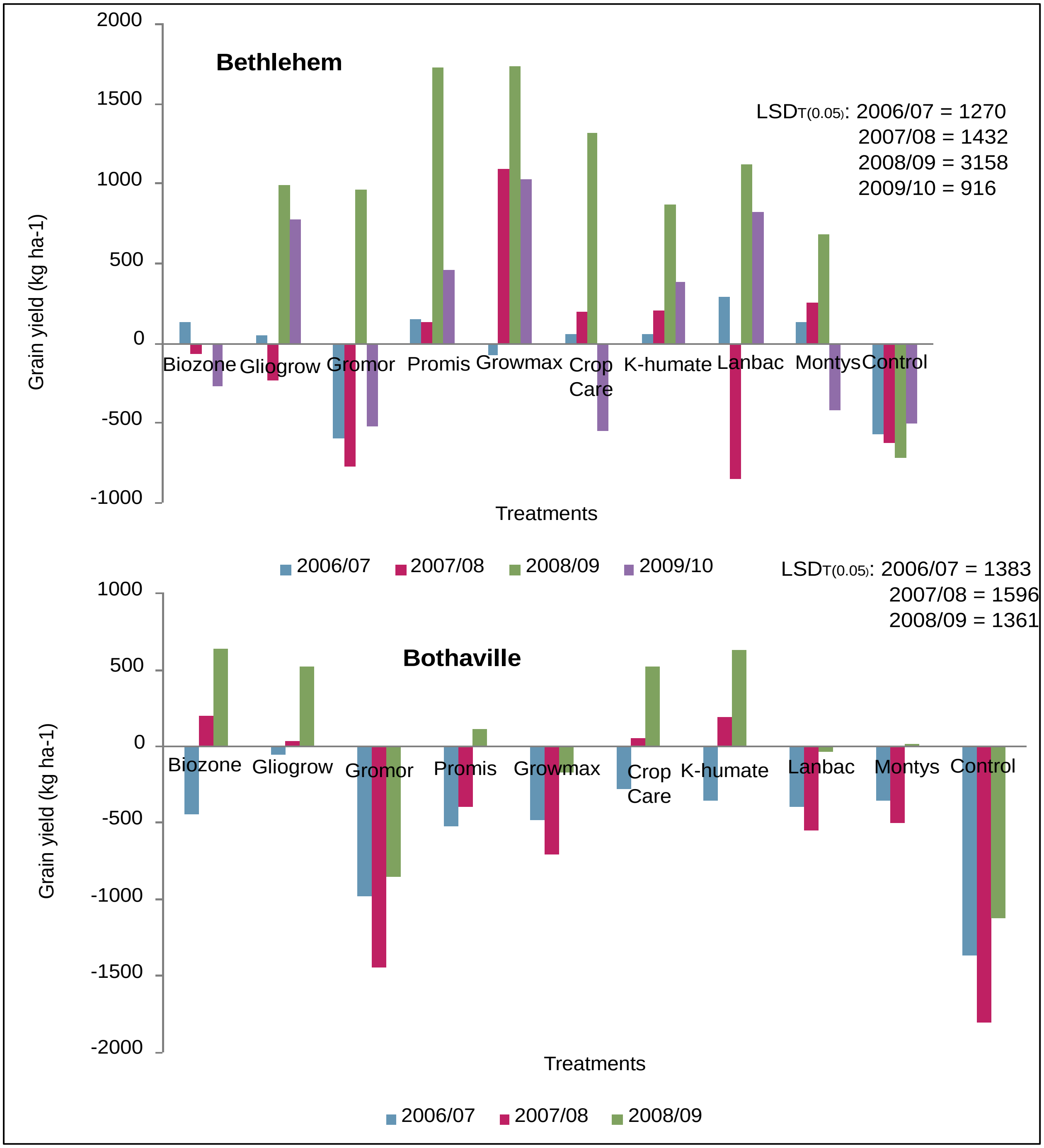

Figure 7: Effect of biological substances on grain yield at Bethlehem and Bothaville. The bars above the zero (0) x - axis indicate grain yield increases compared to the NPK checks and values below indicate grain yield decreases.

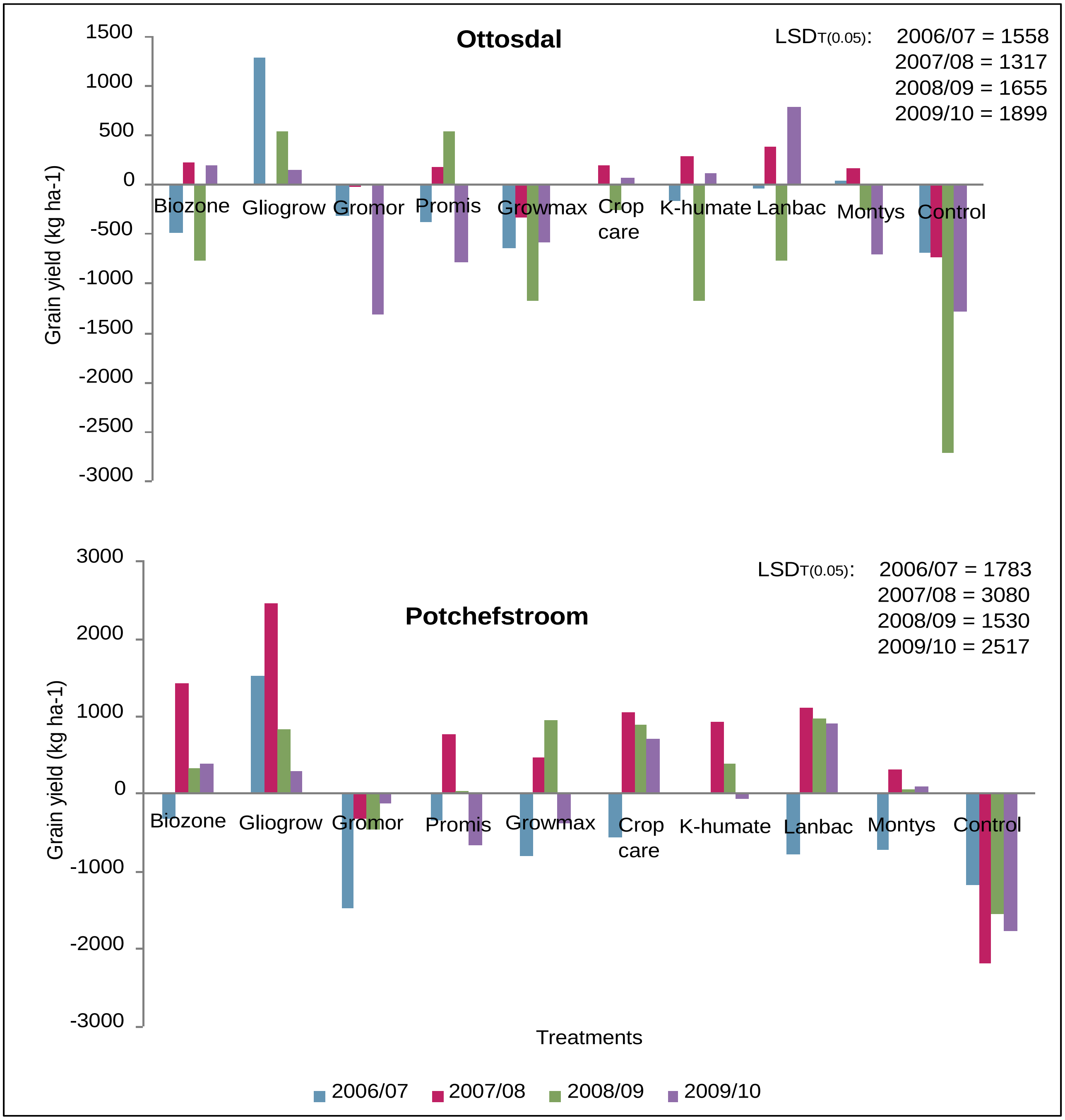

Figure 8: Effect of biological substances on maize grain yield at Ottosdal and Potchefstroom. The bars above the zero (0) x - axis indicate grain yield increases compared to the NPK checks and values below indicate grain yield decreases.