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Research Article

Influence of Nodumax Inoculant on Nodulation, Root Growth Parameters of Soybean and Soil Physico-Chemical Properties

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Abstract

The field study was carried out at the Teaching and Research Farm of Faculty of Agriculture and Veterinary Medicine of Imo State University, Owerri to determine the influence of Nodumax inoculant on nodulation, root growth parameters of Soybean and Soil physico-chemical properties. The experimental design was Randomized Complete Block Design with five treatments and four replications. Treatments consist of gumarabic slurry, honey slurry, powdered milk, sugar (adhesive agents) and control. The results indicated that Nodumax inoculation, using gum arabic as adhesive agent produced significantly ($p<0.05$) higher numbers of Nodules (83), number of root (106.25), root length (35cm) and root dry weight (2.12g) than others. However it was observed that higher nodulation correspond to increase in soybean root growth characteristics which subsequently increased the yield and improved soil nutrients dynamics. There was significant increase in physicochemical properties of soil after inoculation although the changes observed were dependent on type of adhesive agents. This study have shown that the type of adhesive for coating of seed during rhizobium inoculation could impact positive change in root growth parameters, nodulation and soil nutrient status.

Keywords: *Adhesive Agents; Nodulation; Nodumax; Inoculants and Soybean*

Introduction

Soybean (*Glycine max* L. Merr) is one of the most important recognized oil seed and protein rich crops in the world [1]. Successful *Rhizobium*-legume symbiosis will definitely increase the incorporation of biological N_2- fixation into soil ecosystem [2]. On a global basis these symbiotic association between legume and *Rhizobium* may reduce about 70 million tons of atmospheric N_2 to ammonia per annum which amounts to about 40% of all biologically fixed N per year [1].

The root colonization efficiency of plant growth promoting bacteria (PGPB) such as *Rhizobium* spp. is closely associated with microbial competition and survival in the soil, as well as with the modulation of the expression of several genes and cell-cell communication via quorum sensing [3-6].

The rhizosphere is the soil region where processes mediated by micro-organisms are specifically influenced by the root systems. This area includes the soil connected to the plant roots and often extends a few millimeters off the root surface, being an important environment for the plant and microorganism interactions [7], because plant roots release a wide range of compounds involved in attracting organisms which may be beneficial, neutral or detrimental to plants [8]. Plant roots react to different environmental conditions through the secretion of a wide range of compounds which interfere with the plant-bacteria interaction, being considered as an important factor in the efficiency of the inoculants [9-11].

The soybean (*Glycine max*), is an annual summer legume native of South-eastern Asia, which is used as human food (Liu 1999) and livestock feed as well as for several industrial purposes [12]. Currently, this legume is one of the main crops cultivated for oil extraction (35.9 million tons oil and 57% global oilseed production), preceded only by the palm oil [13]. Hence there is need to improve soybean productivity in area with depleted soil nutrients due to excessive use of chemical fertilizers which are presently increasing environmental pollution. Nodumax technology is inexpensive and environmentally friendly [14].

Nodumax is a new legume inoculation technology developed at the IITA Business Incubation Platform that boosts the yield of Soybean by 30 - 40%.it also serves to replace nitrogen [14]. Nodumax is a biofertilizer, it contains 50% of culture (rhizobia) and 50% peat based carrier containing elite *Bradyrhizobi*a. It is packaged in 100g quantities intended for 10 to 20 kg of soybean seed along with gum Arabic for distribution to agro dealers and soybean production associations in Nigeria [14]. Nodumax is made to solve the issue of low crop productivity of most legume crops such as Beans and Soybeans The success and efficiency of Nodumax as an inoculants for agricultural crops are influenced by various factors, among which the ability of these bacteria to colonize plant roots, type of sticky agents, the exudation by plant roots and soil nutrient status.

Main objectives of this study is to influence of nodumax inoculant on nodulation, root growth parameters of soybean and soil physico-chemical properties.

Materials and Methods

Study location

The experiment was conducted at the Teaching and research Farm of Faculty of Agriculture and Veterinary Medicine, Imo State University, Owerri. Owerri lies between latitudes 5°20'N and 6°55'E and longitudes 6°35'E and 7°08'E on elevation of 71m above the sea level, within the South East Rain Forest Agricultural Zone of Nigeria. The area as reported by NIMET (2012) maintains an average annual rainfall of 2,500 mm, 27^{0}C temperature and Relative humidity of 85%.

Experimental materials

Seeds of soybean (TGX 1951 - 3f) and nodumax inoculants were procured from IITA Ibadan, Oyo State. Sugar, honey powdered milk were purchased from Ekeonunwa market in Owerri.

Adhesives (stickers) as treatments

Sugar (10% in water), honey (10% in water) and powdered milk (10% in water), to achieved the above treatment options, Mix 10g of each of the stickers in 100 ml of cold water. Stir until they are dissolved. The rate of sticker solution mixed with the inoculants depends on the type of seed used. Smaller seeds require more sticker solution per seed weight than larger seeds because they have a larger surface area to be coated.

Experiment design and layout

The experiment was laid out in Randomized Block Complete Design (RBCD) with four replicates. 10% sugar, 10% of honey, 10% gum arabic and powdered milk formed the treatment and it was replicated four times.

Planting and agronomic operations

Soil samples were collected randomly at 0 - 15 cm depth from different beds in the site and bulked together for physico-chemical analysis. Soybean seeds were planted at a depth of 2 - 3 cm in the beds with two seeds per hole at a planting distance of 50 x 50 cm.

Thinning was done at 14 days after planting to reduce the plant stand to one per hole. Weeding was done by hand picking throughout the period of research to keep the beds weed free.

Data collection

The following parameters were collected:

1. **Root length:** It was measured using ruler (graduated in centimeter).
2. **Number of root:** This was done by visual counting of the roots using the hand lens.
3. **Root dry weight:** It was obtained when the root has been dried up with weighing balance after at temperature of about 80°C for 24 hours.
4. **Number of nodules:** visual counting of Nodules were done after uprooting the plants
5. **Determination of plant-available nutrients in the soil:** Total N was determined by the method of micro-Kjeldahl as described by Bremner [15]. Phosphorus was determined by the molybdenum blue method [16]. Concentrations of elements such as Ca, Mg, K and Na were determined by method described in Hesse [17]. The trace elements such as Cu, Zn, Fe and Mn were extracted by diethylenetriamine penta acetic acid (DTPA) [18] and determined by an atomic absorption spectrophotometer. The rhizosphere soil pH was analysed in 1: 2.5 (soil:water) suspension, by the electrometric method [19], the organic carbon was determined by the Walkley and Black method [20].

Data analysis

Data collected were statistically analyzed using analysis of variance (ANOVA) to examine the treatment effects and the mean differences were adjudged by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez 1984) and ranking was indicated by letters.

Results

Effect of treatment on nodulation of soybean

The treatments were observed to significantly influence the number of nodules recorded throughout the period of this study. At 4 and 6WAP, T_2 recorded the highest mean number of nodules (5.25 and 22.5 respectively) which was significantly different from the lowest (0.500 and 0.00) recorded from control as showed in table 1. Similarly the same trend was observed at 8 and 10WAP at where T_2 have the highest number of nodules (57.50 and 83.75) which was significantly different ($P < 0.05$) from other treated plots and control. However at 8WAP T_5 recorded (26.00) which was not significant compare to the control (0.75), also whereas at 10Wap it was T_3 that followed T_2 with record of 46.25 number of nodules which was not statically different from the lowest (20.00) from T_4. It was observed at 10WAP, that the trend of nodulation as impacted by treatment is as follows $T_2 > T_3 > T_5 > T_4$ towards the maturity age.

Treatment	2WAP	4WAP	6WAP	8WAP	10WAP
T1 - Control	0	0.500^{c}	0.00^{b}	0.75^{b}	25.00^{b}
T2 - Gum arabic	0	5.25^{a}	22.500^{a}	57.50^{a}	83.75^{a}
T3 - Honey	0	1.00^{bc}	3.750^{b}	15.75^{b}	46.75^{ab}
T4 - Powder Milk	0	0.750^{bc}	4.00^{b}	10.25^{b}	20.00^{b}
T5 - Sugar	0	3.75^{ab}	16.500^{a}	26.00^{ab}	31.25^{b}

Table 1: *Effect of treatment on nodulation of soybean.*

Mean number of nodules.

Means the same column with the same letters are not significantly different (P < 0.05).

Effect of treatment on the number of roots of soybean

The Analysis made on the number of root showed significant different (P < 0.05) among treatments. At 2 and 4WAP, the T_5 produced significantly (P < 0.05) higher number of roots (37.00 and 60.25) than T_2 (2400) and non-inoculated (39.25). Similarly T_2 at 6, 8 and 10WAP produced significantly higher number of roots (73.75, 77.50 and 106.25 respectively) which were significantly different (P < 0.05) compared to other treated plots as indicated in table 2. It was observed that T_2 and T_5 were better in terms of number of roots towards the maturity stage compared to control and other treated plots.

Treatment	2WAP	4WAP	6WAP	8WAP	10WAP
T1 - Control	35.750^{a}	39.250^{b}	36.75^{b}	52.25^{C}	68.77^{b}
T2 – Gum arabic	24.750^{b}	50.00^{ab}	73.75^{a}	77.50^{a}	106.25^{a}
T3 - Honey	32.250^{ab}	56.750^{ab}	35.00^{b}	42.500^{c}	65.25^{b}
T4 - Powder Milk	29.00^{ab}	45.500^{ab}	47.25^{b}	$60.75ab^{c}$	74.50^{b}
T5 - Sugar	37.500^{a}	60.250^{a}	51.25^{ab}	75.50^{ab}	80.00^{b}

Table 2: *Effect of treatment on the number of root.*

Mean number of root.

Means on the same column with same letters are not significantly different (P < 0.05).

Effect of treatment on root length (cm) of soybean

The results recorded for Root Length Sampling is shown in table 2. The length of the sample roots were significantly different (P < 0.05) at 2, 4 and 6 WAP (Seedling Stages).

It was observed that at 2 and 4WAP, T_3 significantly (P < 0.05) recorded higher root lengths (12.375 cm and 25.875 cm respectively), than the mean root lengths recorded from control (8.900 cm and 15 cm) as shown in table 3. While at 6WAP T_2 produced significantly (P < 0.05) root length (26.500 cm) than control (17.125 cm). Also T_3, T_2 and T_5 were found to recorded longer root lengths which were not statistically different when compare with control as shown in table 3. Similarly, at 8 and 10WAP T_2 produced longer root length of 28.625 cm and 35.2 cm respectively which were not significantly different from control and other treated plots. It was observed that T_2 followed by T_5 performed better than control and other treated plot towards the maturity stage.

Treatment	2WAP	4WAP	6WAP	8WAP	10WAP
T1 - Control	8.900^{b}	15.000^{b}	17.125^{b}	23.625^{a}	27.300^{a}
T2 - Gum arabic	9.675^{ab}	15.125^{b}	26.500^{a}	28.625^{a}	35.200^{a}
T3 - Honey	12.375^{a}	25.875^{a}	22.375^{ab}	19.375^{a}	25.975^{a}
T4 - Powder Milk	9.650^{ab}	15.500^{b}	22.600^{ab}	22.125^{a}	25.500^{a}
T5 - Sugar	9.000^{b}	20.875^{ab}	18.625^{b}	27.750^{a}	29.150^{a}

Table 3: *Effect of treatment on root length of soybean.*

Mean root length (cm).

Means in the same column with the same letters are not significantly different (P < 0.05).

Effect of treatment on root dry weight (g)

The result of root weight revealed a marked different among the treatments, however it was statistically different at early growth stages but not significant ($P < 0.05$) at maturity stage. At 2WAP, the highest root weight (0.067750g) was observed in treatment$_3$ which was statically different ($P < 0.05$) from control (0.0470g). The same trend was noticed at 4WAP, where T_3 recorded the highest root dry weight of 0.36175g which was significantly different from root dry weight from control (0.12625g) as shown in table 4. At 6WAP, T_3 recorded the highest root dry weight (0.3193g) followed by T_2 (0.3065g) which was not significantly different ($P < 0.05$) from control (0.2175g). T_5 recorded lowest mean root dry weight (0.1683g) which was statistically different from control (0.2175g). At 8 and 10WAP (maturity stages). T_5 and T_2 recorded the highest mean root dry weights (1.2475g and 2.120g respectively) which were not significantly different from control and other treated plots.

Treatment	2WAP	4WAP	6WAP	8WAP	10WAP
T1 - Control	0.04700^{b}	0.12625^{b}	0.2175^{a}	0.9010^{a}	1.3188^{a}
T2 - Gum arabic	0.05500^{ab}	0.30950^{ab}	0.3065^{a}	0.9313^{a}	2.1200^{a}
T3 - Honey	0.67750^{a}	0.36175^{a}	0.3193^{a}	0.5263^{a}	1.3683^{a}
T4 - Powder Milk	0.5000^{ab}	0.16375^{ab}	0.2125^{a}	1.0718^{a}	1.9490^{a}
T5 - Sugar	0.051750^{ab}	0.30950^{ab}	0.1683^{a}	1.2475^{a}	1.9028^{a}

Table 4: *Effect of treatment on root dry weight (g) of soybean.*

Means in the same column with the same letter are not significantly different ($P < 0.05$).

Physical chemical properties of the soil before experiment

The result of Soil analysis (Table 1) showed that soil was slightly alkaline with pH value of 7.00 at the onset of the experiment with sand percentage of 81.60, 8.00% silt and 10.4% of clay.

The soil recorded 0.9% of organic Carbon (0C) and 169g of organic matter (0m). Total exchangeable acid (TEA) was at 1.8% and Aluminium ion (Al^{3+}) was 0.5% level while hydrogen ion H^+ stood at 1.3% having a total Nitrogen (TN) level of 0.8%.

The soil caution levels were as follows Calcium (2.05 meg/100g), Magnesium (1.56 meg/100g), Potassium (K) (0.32 meg/100g) and Sodium (0.19 meg/100g. the caution exchange capacity of the soil was 5.9 meg/100g with base saturation (BS) stood at 69.50% and available phosphorous of 3.20 ppm.

Physical and chemical properties of soil after experiment

In control plots, PH value recorded was 6.47 while sand; silt and clay levels recorded were 84.60%, 8.00% and 7.40% respectively as shown in table 5. Also organic Carbon (0C) and organic matter (0M) recorded value of 1.84% and 3.17% respectively. It was observed that after harvest, TEA, Al^{3+} and H^+ values were 1.30; 0.50 and 0.80% respectively. While total Nitrogen (TN) was 0.0160%, Ca^{2+} has 2.70%, meg/1005 mg^{2+} was 3.10 meg/100g whereas K^+ Na^+ and were recorded as 0.260, 0.210% and 7.770 meg/100g soil respectively while phosphorous and BS were recorded as 6.03 ppm and 83.27 respectively as indicated table 1.

In the gum Arabic (T_2) treated plots, the soil pH increased slightly to 6.55, which was significantly difference ($p < 0.05$) from PH from control. The value of sand 84.60% was at per with that of control and statistically not different while silt was lowered to 6.00% which was significantly different ($p < 0.05$) from the control (8.00%). Also clay increased to 9.40% which was statistically different ($p < 0.05$) from control (7.40%). Organic carbon (OC) and organic matter (OM) were 1.980% and 3.410% respectively which were significantly higher than those of control (1.854% and 3.170% respectively) the TEA was 1.30% while Al^{3+} increased to 0.70% and H^+ was lowered to 0.60% which was statistically different at $P < 0.05$ to that obtained from control as indicated table 1. The TN and Ca^{2+} increased slightly to 0.170% and 3.85% which were statistically different from those recorded from control. Mg^{2+}, K^+ and Na^+ were recorded as 2.82%, 0.290 meg/100g and 0.250 meg/i00g respectively. They were not statically different from the those of the control the CEC was lowered to 5.843g while available phosphorous stood at 8.85 ppm which was statistically different ($P < 0.05$) to 6.03 of phosphorous recorder in control.

PH	KCL	Sand	Silt	Clay	Texture Class	O.C (g)	O.M (g)	TEA	AL_3^+	H^+	T. N	Ca^{++}	Mg^{++}	K^+	Na^+	CEC	P^+	BS
7.13	5.74	81.60	8.00	10.4		0.98	1.69	1.8	0.5	1.3	0.08	2.05	1.56	0.32	0.19	5.9	69.50	3.20

Table 5: *Soil Physical Chemical Parameters before Planting.*

The soil analysis in this study showed that honey slurry sticker (T_3) influence soil properties significantly at $P < 0.05$. In the T_3 treated plots, pH was 6.57 which were slightly higher than control (6.47) as shown in table 5. Sand was higher at 86.60%, which was statistically different from control (84.60%). While silt was 6.00% and clay stood at 7.40%. However, OC and OM were recorded as 1.80% and 3.10% respectively which were significantly different from those recorded in control plots and T_2 plots as shown in table 5. While TEA, AL^{3+} and H^+ levels were, 1.40% 0.80% and 0.16% respectively with TN stood at 0.160%. The cations levels were recorded as Ca^{2+} (3.48 meg/100g), Mg^{2+} (3.00 meg/100g), 12^+ (0.300meg/100g) and Na^+ (0.190 meg/100g), CEC was slightly higher 8.370 Mg/100g than control (7.770 meg/100g). Available phosphorous was 9.00pp which was statistically different from the control (6.05 ppm). While the BS stood as 83.27%.

In T_4 plots (milk slurry sticker), pH level was 6.29 which was statistically lowered than those of control, T_2 and T_3 as shown in table 1, with sand silt and clay levels at 84.60%, 6.00% and 9.40% respectively. Similarly, OC, OM and TEA were recorded as 2.470% 4.28% and 1.40% respectively which were statically different from those value (1.84, 3.170 and 1.30%) respectively recorded from control. Al^{3+} was 0.05%, H^+ has 0.90% while TN stood at 0.210%. However, $Ca^{2+,}$ $Mg^{2+,}$ K^+ and Na^+ were recorded as 4.96, 3.15, 0.35 and 0.220 meg/100g respectively. Also CEC was recorded as 10.80 meg/100g which was not statistically different from that of control (7.770 meg/100g). While available phosphorous and BS were recorded as 5.95 ppm and 85.92% respectively.

In the sugar slurry sticker (T_5) treated plots, PH level was the lowest (6.27) which was significant different from the control (6.47) and the highest (6.57) from T3 treated plots, with sand silt and clay recorded as 86.60, 6.00 and 7.40% organic carbon and organic matter were recorded as highest with 3.21% and 5.60% respectively when compared to control and other treated plot as shown in table 1. Whereas TEA, AL^{3+}, H^+ and TN were recorded as 1.30%, 1.00%, 0.300% and 0.280% respectively.

Similarly, the cation (Ca^2, Mg^{2+}, K^+ and Na^+) were recorded as 4.25, 3.23, 0.360 and 0.20 meg/100g respectively as while CEC, available phosphorous and BS recorded as 9.340 meg/100g, 5.98 ppm and 86.08%.

It was observed that treated plots significantly ($P < 0.05$) influence the soil physical and chemical properties when compared to control plots and properties before planting.

	PH	KCL	Sand	Silt	Clay	Texture class	O.C (g)	O.M (g)	TEA	AL_3^+	H^+	T. N	Ca^{++}	Mg^{++}	K^+	Na^+	CEC	P^+	BS
T1	6.47^c	4.18^b	84.60^b	8.00^a	7.40^b	LS	1.84^d	3.170^d	1.30^b	0.50^a	0.80^b	0.160^d	2.70^e	3.10^a	0.260^b	0.2109	7.770^{ab}	6.03^b	83.27^d
T2	6.55^b	4.15^c	84.60^b	6.00^b	9.40^a	LS	1.980^c	3.410^c	1.30^b	0.70^a	0.60^c	0.170^c	3.85^c	2.820^a	0.290^b	0.2509	5.843	8.85^a	84.72^c
T3	6.57^a	4.19^b	86.60^a	6.00^b	7.40^b	LS	1.80^d	3.10^d	1.40^a	0.60^a	0.80	0.160^d	3.48^d	3.00^a	0.300^{ab}	0.190^a	8.370^{ab}	9.00^a	83.27^d
T4	6.29^d	4.12^d	84.60	6.00^b	9.40^a	LS	2.470^b	4.28^b	1.40^a	0.50^a	0.90^a	0.210^b	4.96^a	3.15	0.35^a	0.220^a	10.80^a	5.95^b	85.92^b
T5	6.27^e	4.22^a	86.60^a	6.00^b	7.40^b	LS	3.21^a	5.560^a	1.30^b	1.00^a	0.30^a	0.280^a	4.25^b	3.23^a	0.360^a	0.02^a	9.340^{ab}	5.98^b	86.08^a

Table: *Effect of treatment on soil properties after harvest.*

Means in the same column with the same letter are not significantly different ($P < 0.05$).

Discussion

The obtained results showed that soybean inoculated with Nodumax (irrespective of adhesive agents) could increase the root length, nodule numbers, root dry weight and number of roots than untreated control; due to increase in the activity of *rhizobium* and other indigenous soil microorganism thereby enhances Nitrogen fixation and promote root growth, so as to increase the yield of legume plants. Inoculated soybeans gave significantly higher number of nodules and roots with subsequent increase in root weights. This agrees with the work of Abdul Jabbar and Saud (2012), Aminu., *et al* [21]. However inoculation effect on root growth characteristics and yield depends on the efficiency of adhesive agent used during inoculation of seed.

In this study, the high yield obtained in the use of gum arabic as adhesive agent showed that gum Arabic bound more inoculant to seeds than other adhesive or stickers at the time of coating, which was sufficient for effective Nitrogen fixation. This showed that gum arabic maintain the survival of *rhizobium* in the face of inadequate nutrients in acidic soil of cultivated area. It has been reported that effective adhesive agent is one that can produce more than 50 nodules per plant at anthesis [22]. Using sticking agent with proper type and amount of inoculants greatly increased the number of rhizobia adhering to the seed (Hoben., *et al*. 1991). In this study gum Arabic was found to produce more 50 nodules at 8 and 10 WAP compare to other treatments and control. This is in line with work of Chang., *et al*. (2005) and Singh [23] respectively who recorded the higher nodulation with soybean inoculated with *Bradyrhizobium japonicum* compared to uninoculated control.

Wang and Heggo (2003) conducted an experiment on soybean and found a higher number of nodulation in *Bradyrhizobium* inoculated plant than uninoculated plant

The result obtained in physicochemical parameters of soil showed that Nodumax inoculation irrespective of adhesive agent used generally improved the soil physical and chemical properties of soil after harvest. Chinelo., *et al.* [24] found similar result that there was increase in physico-chemical characteristics of the soil by *rhizobium* inoculation of soybean.

Inoculation of soybean with Nodumax has led to increase in soil organic carbon, organic matter, nitrogen, phosphorous potassium and other Macro elements content compare to control. This is in conformity with the work of Bambara and Ndakidemi [25], Nyoki and Ndakidemi [26] who reported the same result on soybean inoculated with *rhizobium*.

Lower concentration of organic matter, N, P and other cations under soybean grown without Nodumax could depend on the reduced microbial activity consequently reduced soil fertility. According to Schimmel and Bennet [27], N mineralization by soil microbes is the key event in the N cycle making mineral N available, whereas plants uptake mineral N changes in Nitrogen dynamics in soils are closely connected with altering the microbial activities involved in N cycle by abiotic and biotic factors [28]. pH changes observed in this study was slightly acidic after the experiment which may lead to reduction in the availability of nutrients, this is conformity with work of Mobasser., *et al*. [29] who reported that Biological nitrogen fixation induces changes in the soil pH resulting in limited availability of some plant nutrients in the soil, however it is in disagreement with that of Bambara and Ndakidem [25] who reported a significant an increase in soil pH following *Rhizobium* inoculation on *Phaseolus vulgaris.*

Conclusion

Nodumax inoculation altered most of the chemical properties of soybean cultivated soil in this study. The soil chemical properties such as pH, OC, OM and macro- and micronutrients (N, P, Ca, Mg and Na and Fe and Zn, resp.) were significantly increased in the Nodumax inoculated soybean plots over the control. These results strongly support the use of Nodumax to improve soil chemical properties for improved Soybean growth, development and production. Also for higher growth indices, nodule numbers and root biomass and increase yield, adhesive agents like gum Arabic honey, powdered milk and sugar should be used. Considering the fact that gum Arabic is not always available and expensive, local farmers should be encouraged to use honey, powered milk and sugar or any other local sticky agent that will not be harmful to the rhizobia for inoculation before planting.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper

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Volume 5 Issue 10 October 2019