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Research article

Effect of *Jeevamrutha* on physicochemical property of Garlic (*Allium sativum* L.)

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Abstract: *Jeevamurtha*, a biodynamic preparation widely used in organic farming, has shown promise in enhancing plant growth and improving soil health. In this study, we investigate the effect of *Jeevamrutha* application on the physicochemical properties of garlic (*Allium sativum*). A controlled experiment was conducted where garlic plants were treated with varying concentrations of *Jeevamrutha i.e.*, 0 ml (control), 10 ml, 20 ml, 30 ml and 40 ml per pot, while a control group received no treatment. Physiological parameters, including plant height, leaf area, chlorophyll content, and nitrogen, were measured at regular intervals over the growth period. Our results indicate that *Jeevamrutha* application positively influenced the physicochemical properties of garlic. Treated garlic plants exhibited significantly higher growth rates, evident through increased plant height and larger bulb size compared to the control group. Moreover, *Jeevamrutha*-treated garlic plants displayed higher chlorophyll content, suggesting improved photosynthetic efficiency. The findings from this study provide valuable insights into the beneficial effects of *Jeevamrutha* on garlic physiology. The enhanced growth and improved physiological properties observed in garlic plants treated with *Jeevamrutha* suggest its potential as an eco-friendly and sustainable option to promote crop productivity.

Keywords: Organic farming, Jeevamrutha, Chlorophyll content, Nitrogen, Garlic.

INTRODUCTION

Garlic (*Allium sativum* L.) is a tuberous vegetable crop widely cultivated in various countries around the world (Fig. 1). It has a long history and is considered one of the oldest crops originating from the Asian continent. Classified under the Amaryllidaceae family, it shares a close relationship with plants like blood lily (Cape tulip), Cornish lily (Nerine), belladonna lily, onions (*Allium cepa*), and French shallots (*Allium oschaninii*).

Figure 1. Garlic (*Allium sativum* L.)

Garlic holds great significance as a commodity due to its diverse functional uses, particularly as a cooking ingredient and medicinal herb. It is consumed both fresh and dried, adding a distinctive flavour to various vegetable and non-vegetable dishe (Memane *et al*., 2008). From a biochemical perspective, garlic bulbs contain antibiotic substances such as allicin and scordinine, which contribute to boosting the human body's immune system. Additionally, in the culinary world, garlic is renowned for its spicy and aromatic properties, enhancing the taste of dishes, thanks to the presence of methyl allyl disulfide compounds.

On the other hand, *Jeevamrutha*, a significant liquid manure, is prepared from cow urine and dung. Its application promotes higher growth, increased yield, and improved quality of chili crops (Kurubetta *et al*., 2017).

Nutritional Value of Garlic

Garlic has very high nutritional value and contains protein, fat, carbohydrate, vitamin B1, vitamin B2, vitamin C, niacin, and so forth. Besides, it also contains magnesium, sodium and other trace elements necessary for the human

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body, and these trace elements are indispensable nutritional ingredients to the human body. The content of each nutritional ingredient in various varieties of garlic is different (Block, 1985).

Functional Ingredients of Garlic

Garlic has unique edible and medicinal value because of its chemical composition. Sulfur compounds are the main component of garlic as medicine, and more than 30 kinds of sulfur compounds have been found at present. In addition, garlic contains amino acid, protein, vitamin, enzymes, glycoside, fatty acid and rare metal elements such as selenium and germanium (Ahmad, 1996) and shows obvious pharmacological characteristics.

i. Alliin

The chemical name of alliin is S-allyl-L-cysteine sulfoxide, and its molecular formula is C6H10NO3S. It is colourless and odourless acicular crystal. Alliin in the cytoplasm of garlic is stable an odourless. After garlic is broken, its cell membrane ruptures, and Alliin generates thiosulfonates (allicin) under the effect of allinase and pyridoxine phosphate coenzyme. Allicin is unstable and can be degraded into volatile sulfur compounds, such as diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), etc. (Han *et al*., 2012). These sulfur compounds form the special flavour of Garlic and make garlic have many kinds of biological activity.

ii. Amino acids

Garlic is rich in amino acids, and amino acids are divided into amino acids constituting proteins and non-protein amino Acids. The former mainly includes cysteine, histidine, arginine, Aspartic acid, etc. The latter mainly includes S-allyl-L-cysteine Sulfoxide, S-allyl-L-cysteine, etc. (Ginting *et al*., 2017).

iii. Superoxide dismutase

Garlic is one of natural plants rich in superoxide dismutase (SOD). SOD is a metal enzyme that exists widely in an organism and can catalyse superoxide anion to take part in the disproportionation reaction. The enzyme can clear away superoxide radicals from in an organism effectively, thereby preventing the toxic action of reactive oxygen on the organism. It can resist radiation and tumour and delay the aging of an organism, so it has important application value in health care products, medicine and cosmetics.

iv. Garlic polysaccharide

Garlic polysaccharide, also one of the active ingredients rich in garlic, is relatively stable. Most studies on garlic polysaccharides are about its primary structure, while there is no study on secondary structures Kim & Kim (2011). Garlic polysaccharide is a water-soluble polysaccharide and has very good hygroscopicity and moisture retention and a variety of biological activities (including antioxidant activity and good property of protecting liver), so it has wide development and utilization prospects. Clinical research shows that garlic polysaccharides can clear away superoxide radicals and hydroxyl radicals, so it can decrease blood pressure, protect liver, avoid atherosclerosis and prevent ageing.

Medicinal uses

In the early 1970s many epidemiological and experimental studies provided evidence that garlic influences risk factors associated with heart disease; however, the more recent and rigorous meta-analyses have found only a modest or insignificant effect on cholesterol and triglyceride levels, and no significant effect on HDL cholesterol (Bordia *et al*., 1998; Banerjee & Maulik, 2002; Sheneni & Enesi 2024). It has antibacterial, antifungal (Hughes & Lawson, 1991) and antiviral (Meng *et al*., 1993). A study showed garlic can lower serum cholesterol by as much as 9 percent by stimulating the release of bile by the gall bladder and by decreasing the production of cholesterol in the liver (Yeh *et al*., 1999). Garlic may also aid in the lowering of blood pressure by slowing the production of the body's pressure-raising hormones (Holladay, 1995). Another benefit of garlic is its ability to relax vascular smooth muscle, which prevents the acute hypoxic increase in pulmonary pressure (Fallon *et al*., 1998).

Garlic is also able to stimulate the immune system's macrophages, white blood cells that destroy foreign organisms. It also increases the activity of T-helper cells, and can be used to treat upper respiratory viral infections because of its ability to clear mucous from lungs (Holladay, 1995), and help patients with asthma (Grieve, 1995). During W.W.I. garlic was used as an antiseptic for wounds (Grieve, 1995) and to treat typhus and dysentery. Researchers have found that garlic blocks the action of certain enzymes that help infectious microbes survive in host tissue (Yeh *et.al* 1999).

Cultivation of Garlic

Garlic is normally cultivated vegetatively. Garlic produces best in a rich, moist, sandy soil, but can also be grown in a loam or clay soil. A little lime should be added to the soil. The bulb should be divided into individual cloves, and they are planted separately about 6 inches apart and 2 inches deep. A sunny spot is best and weeding is recommended while occasionally gathering the soil up around the roots (Grieve, 1995).

MATERIALS AND METHODS

The pot experiment was conducted in the Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, Uttar Pradesh, India from February 2023 to May 2023. Kharif season (April to September) and Rabi season (October to March) were considered. The cloves of garlic of uniform size were selected for plantings. The average weight and diameter of the cloves were 1.6 - 2.1 g and 0.9 - 1.0 cm, respectively. Soil was collected from the Department of Botany, D.D.U. Gorakhpur University. These cloves are placed into plastic pots. Each pot contained a total amount of 2 kg of soil.

Preparation of Jeevamrutha

Jivamrita/Jeevamrutha is a fermented microbial culture. It provides nutrients also helps to prevent fungal and bacterial plant diseases. *Jeevamrutha* is prepared by mixing 10 kg fresh local cow dung with 10 liters aged cow urine, add 2 kg local jiggery, 2 kg pulse flour and handful of garden soil (rhizosphere soil) or from the bund of the farm and the volume made up to 200 liters (Fig. 2). Stir the solution well and let it ferment for 48 hours. Keep the drum in shade covered with wet gunny bag. Now *Jeevamrutha* is ready for the application. 200 liters of *Jeevamrutha* is sufficient for one acre of land to boost the plant growth and give a good yield (Aminot & Rey, 2000). It also gives resistance against pests and diseases with increased beneficial organism activity and promotes organic carbon in the soil (Lichtenthaler & Buschmann, 2001).

Figure 2. Preparation of *Jeevamrutha*.

Experimental Design and Treatment

The experiment was laid out with five treatments with three replicates. The treatment levels of the recommended dose of *Jeevamrutha* were: 0 ml (control), 10 ml, 20 ml, 30 ml, 40 ml. per pot, respectively (Fig. 3). Each plastic pot contained a total of 2 kg of soil. Different concentrations of *Jeevamrutha* first split were applied to soil 1st day of the planting of cloves and the second split was applied at 30 days. Around three cloves were planted in each pot and three replicates were prepared. Morphological parameters like shoot length, root length, fresh and dry weight of plant and biochemical parameters like chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll, carotenoid, nitrogen content and percentage of protein were calculated by following methods. Plant height, shoot length and root length was calculated from root to shoot tip *via* a measuring scale.

Figure 3. Image showing 5 pot having different concentrations of *Jeevamrutha via* – containing 40 ml in 1st pot, 30 ml in 2nd pot, 20 ml in 3rd pot, 10 ml in 4th pot and 0 ml (control) *Jeevamrutha* in 5th pot.

Estimation of Chlorophyll Content

The amount of Chlorophyll "a" and Chlorophyll "b" and Total Chlorophyll in Allium sativum was measured in the primary leaves by the method of Arnon (1949). The optical density (OD) of the solution was recorded at 663 nm and 645 nm (for chlorophyll a and b, respectively) and 440 nm (for carotenoids) using a spectrophotometer (the values of photosynthetic pigments were expressed in milligrams per gram fresh weight (Sikandar *et al*., 2018). Calculated in terms of mg per gram fresh weight of leaf by the following formulae:

$$\text{Chlorophyll "a"} = [12.7\ (D663) - 2.69\ (D645)] \times V/1000 \times W$$

$$\text{Chlorophyll "b"} = [22.9\ (D645) - 4.68\ (D663)] \times V/1000 \times W$$

$$\text{Total chlorophyll} = [20.2\ (D645) + 8.02\ (D663)] \times V/1000 \times W$$

$$\text{Carotenoid} = [\text{OD at } 440 \times V/196 \times W]$$

Where, D = Optical density observed for chlorophyll extract at the particular indicated wavelength, V = Final Volume of the Chlorophyll extract in 80% acetone, W = Fresh weight of leaves in mg.

Estimation of Nitrogen Content

Nitrogen content was estimated in the dried leaves obtained from salt-stressed and control plants. For this 50 mg of dried leaves were homogenised in 5 ml of 80% ethanol. The volume of homogenate was adjusted to 20 ml. After centrifugation at 3000 rpm for 20 minutes, both soluble and insoluble fractions were separated and digested by Doneen's (1932) micro-kjeldahl method (Lanzotti, 2006). The absorbance of the pale-yellow developed was measured at 440 nm in a UV/VIS systronic spectrophotometer No 118. The amount of soluble nitrogen was calculated in terms of mg N2 g^{-1} dry weight of sample using a standard curve prepared from ammonium sulphate.

Estimation of Total Protein Content

For measurement of Protein content in dried leaves the amount of insoluble nitrogen fraction, as obtained by micro – Kjeldahl digestion method was multiplied by a factor 6.25.

RESULT AND DISCUSSION

Effect of Jeevamrutha on Morphological Parameters of garlic plants

The application of *Jeevamrutha*, influences the growth and yield of plants, including various morphological parameters. The results of the study indicate that the vegetative growth characters, shoot length, root length, fresh and dry weight of the garlic plant significantly increased at all the different concentrations of *Jeevamrutha* after 30 days from sowing the plant (Figs. 4 & 5). Shoot length with 40 ml of *Jeevamrutha* is 22.23 cm as compared to control 10.33 cm. Root length of the garlic plant with 40 of *Jeevamrutha* is 5.40 cm and in control 2.33 cm. Fresh and dry weight of the garlic plant with 40 ml of *Jeevamrutha* is 2.14 gm and 1.67 gm as comparison to control with 0 ml of *Jeevamrutha* is 1.14 gm and 0.56 gm, respectively. The effect of *Jeevamrutha* on plant growth parameters can vary depending on the concentration. Data reveal that garlic plants with different concentrations of *Jeevamrutha* caused a significant increase in the contents of chlorophyll a, chlorophyll b, carotenoids in garlic leaves, and total protein content in different concentrations (Adaki *et al*., 2014). The remarkable significant increase was measured at 40 ml of *Jeevamrutha* (Table 1).

Figure 4. Vegetative growth of garlic plant at different concentration of *Jeevamrutha* after 30 days from sowing the plant

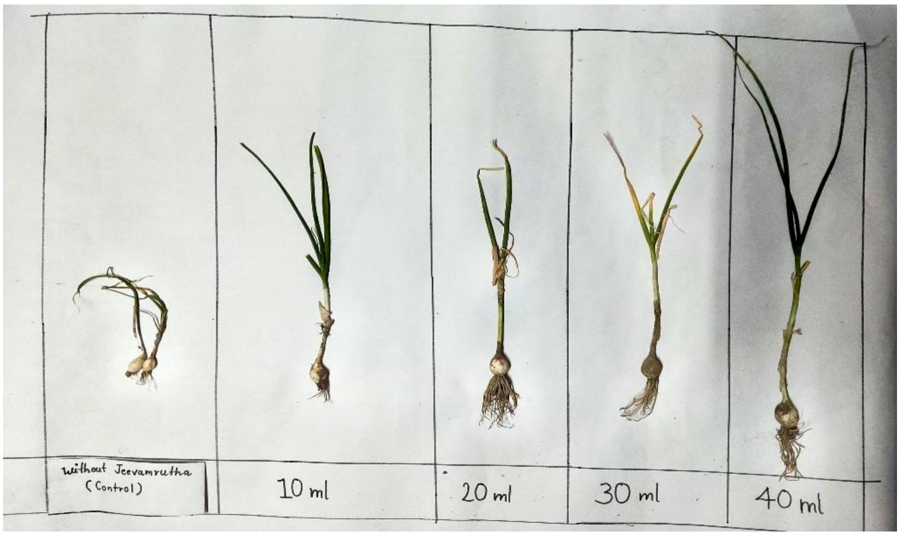

Figure 5. Vegetative growth of garlic plant at different concentrations of *Jeevamrutha* after 60 days from sowing the plant.

Table 1. Effect of different concentrations of *Jeevamrutha* on the morphological characters of garlic plant after 30 days from sowing.

Days after Sowing (DAS)	Treatments	Concentrations (ml)	Shoot length (cm)	Root length (cm)	Fresh wt. (g)	Dry wt. (g)
After 30 DAS	Control	0 ml	10.33	2.33	1.14	0.56
	Jeevamrutha 10	10 ml	14.00	3.33	1.30	0.75
	Jeevamrutha 20	20 ml	16.33	4.00	1.52	0.97
	Jeevamrutha 30	30 ml	19.33	4.83	1.90	1.09
	Jeevamrutha 40	40 ml	22.33	5.40	2.14	1.67
After 60 DAS	Control	0 ml	13.00	2.00	1.05	0.75
	Jeevamrutha 10	10 ml	20.00	3.00	2.49	1.72
	Jeevamrutha 20	20 ml	22.33	5.00	3.01	1.83
	Jeevamrutha 30	30 ml	27.33	5.89	3.34	1.97
	Jeevamrutha 40	40 ml	34.00	6.33	5.19	2.20

Effect on Chlorophyll a, Chlorophyll b, Total Chlorophyll and Carotenoid

Jeevamrutha, an organic liquid formulation, has been shown to have a positive effect on the chlorophyll and carotenoid content of plants. As the results indicate in table 2 chlorophyll a, chlorophyll b, total chlorophyll and carotenoid in 0 ml of *Jeevamrutha* (control) is 1.68, 3.657, 4.410, 0.154 and in 40 ml of *Jeevamrutha* it is 3.293, 5.110, 5.754 and 0.377 after 30 DAS respectively and it increases grad after 60 DAS, where chlorophyll a, chlorophyll b, total chlorophyll and carotenoid in control (0 ml) 1.312 , 2.733, 2.684, and 0.150 and in 40 ml of *Jeevamrutha*, it is 4.428, 7.471,7.704 and 0.397 respectively. Singh & Lal (2019) study on sweet basil subjected to NaCl-induced salt stress found that *Jeevamrutha* improved the photosynthetic pigments, including chlorophyll 'a', 'b', and carotenoid content. Gopal & Gurusiddappa (2014) also showed that the application of *Jeevamrutha* can lead to an increase in chlorophyll content, resulting in better foliage with dark green leaves.

Table 2. Effect of different concentrations of *Jeevamrutha* on chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll & carotenoid of garlic plant after 30 days from sowing the plant.

DAS (Days after Sowing)	Treatments	Concentrations (ml)	Chl "a" (mg g^{-1})	Chl "b" (mg g^{-1})	Total chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})
30 DAS	Control	0 ml	1.682	3.657	4.140	0.154
	Jeevamrutha 10	10 ml	1.913	4.427	4.955	0.221
	Jeevamrutha 20	20 ml	2.113	4.769	5.452	0.295
	Jeevamrutha 30	30 ml	2.229	5.025	5.654	0.350
	Jeevamrutha 40	40 ml	3.293	5.110	5.754	0.377
60 DAS	Control	0 ml	1.312	2.733	2.684	0.150
	Jeevamrutha 10	10 ml	2.296	5.886	5.784	0.352
	Jeevamrutha 20	20 ml	3.064	6.066	6.128	0.353
	Jeevamrutha 30	30 ml	3.408	6.423	6.528	0.365
	Jeevamrutha 40	40 ml	4.428	7.471	7.704	0.397

Effect on Nitrogen and Protein content of the plant

The use of *Jeevamrutha*, a cow-excreta-based bio-formulation, has been found to have a positive impact on the protein content of plants. Research has shown that *Jeevamrutha* can lead to increased growth and nutritional characteristics of plants, including higher protein content (Bhattacharjee & Uppaluri, 2023; Krishnamoorthy *et al*., 2019). This effect is attributed to the presence of beneficial microflora, macronutrients, essential micronutrients, vitamins, essential amino acids, and growth-promoting factors in *Jeevamrutha*, which contribute to the overall improvement in the nutritional status of the plants (Gopal & Gurusiddappa, 2014; Krishnamoorthy *et al*., 2019). Therefore, the application of *Jeevamrutha* can be beneficial in enhancing the protein content of plants, thereby contributing to improved plant growth and productivity.

Table 3. Effect of different concentrations of *Jeevamrutha* on nitrogen and total protein content of garlic plant.

DAS (Days after Sowing)	Treatments	Concentrations (ml)	Nitrogen (mg N2 g^{-1})	Protein in percentage (%)
30 DAS	Control	0 ml	1.550	10.13
	Jeevamrutha 10	10 ml	1.727	11.23
	Jeevamrutha 20	20 ml	1.829	11.89
	Jeevamrutha 30	30 ml	1.915	12.45
	Jeevamrutha 40	40 ml	2.300	14.98
60 DAS	Control	0 ml	1.560	10.14
	Jeevamrutha 10	10 ml	1.880	12.23
	Jeevamrutha 20	20 ml	2.127	13.83
	Jeevamrutha 30	30 ml	2.223	14.45
	Jeevamrutha 40	40 ml	2.550	16.58

After 60 days from sowing, the plant is considered following. parameters such as plant height, root height, fresh and dry weight of garlic plant significantly increased at all the different concentrations of *Jeevamrutha*. Also, the chlorophyll content and total protein increase were estimated.

CONCLUSION

Application of the recommended dose of *Jeevamrutha* applications at each stage of planting and vegetative stage will enhance the growth parameters of the garlic (Table 1). In addition, the increases in metabolic activity of the garlic plant might have resulted from a corresponding increase in photosynthetic pigments (Table 2), which in turn increased in protein due to the increase in nitrogen content (Table 3). This ultimately results in a higher yield level in garlic. *Jeevamrutha*, an organic liquid manure, has several beneficial effects on agriculture. Research has shown that the application of *Jeevamrutha* promotes immense biological activity in the soil, enhances nutrient availability to crops, and increases the activity of microbes by solubilization and uptake of nutrients. This, in turn, leads to higher growth and yield of crops, including incremental yield, microbial load, and growth hormones, ultimately resulting in higher crop yield. These liquid formulations of *Jeevamrutha* are efficient organic substitutes, and they can be applied along with organic manures in an integrated approach for obtaining higher crop yield, besides improving the nutrient status of the soil. Hence, this experiment has shown the advantages of organic liquid formulations and thus, they can be widely utilized in crop production.

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