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# Effect of Foliar Application of Liquid Biofertilizer of Buffalo Dung and Gram Bran with Combinations of Biopesticides on the Growth, Flowering and Productivity of Soybean (*Glycine max* L.) Crop

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**Abstract:** Excessive use of chemical fertilizers and pesticides in agricultural field for the huge production of agricultural products causes adverse effect on environment as well as on human health. Animal dungs and agricultural wastes are serious problem for society so it needs to be managed properly. Vermicomposting is the best process for the management of these wastes with the help of earthworm *Eisenia fetida* and the by-products released from these vermicompost are used in enhancing the agricultural productivity. Soybean (*Glycine max* L.) is a short season leguminous crop which has been recognised as pulse-oil seed. It is a highly nutritive crop which contains 43% of protein and 20% of oil approximately but due to various reasons its production rate is decreasing day by day. The vermiwash and neem, garlic and tobacco based biopesticides are being used as an alternative for chemical fertilizers and pesticides. The aim of present study was to observe the growth, flowering and productivity of soybean plant after foliar spray of vermiwash obtained from gram bran (GB) and buffalo dung (BD) with combinations of aqueous extracts of garlic, neem and tobacco leaves. The significant growth (cm) and productivity (g/m$^2$) were observed after the foliar spray of vermiwash (GB+BD) and aqueous extracts of biopesticides (garlic, neem and tobacco leaves). The highest growth of soybean (49.2±2.6 cm) was observed after foliar application of vermiwash (GB+BD) with aqueous extract of neem leaves in ratio of 10:1 whereas, the maximum significant early flowering period (32.3±2.5 days) and productivity (423±14 g/m$^2$) were obtained after treatment of vermiwash (GB+BD) with aqueous extract of neem, garlic and tobacco leaves in ratio of 10:1:1:1. The use of vermiwash with biopesticides in increasing agricultural product is a boon for crops as it is environmentally beneficial and cost-effective alternative to chemical fertilizers and synthetic pesticides.

**Keywords:** Biological waste, Gram bran, Buffalo dung, Vermiwash, Biopesticides, *Glycine max,* Productivity, *Eisenia fetida*

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## Introduction

Chemical fertilizers are inorganic or organic materials which are added to soil to enhance the food productivity but at the same time it is harmful for the society and environment (Tilman *et al.*, 2002). Excessive use of pesticides and chemical fertilizers influence the soil properties

like nutrient content, texture of soil and also affect the environment and human health (Prashar and Shah, 2016). In agricultural systems use of non-chemical fertilizers and pesticides is among the common implementations that have been used nowadays. These non-chemical fertilizers and pesticides consist of bio-fertilizers which are produced through the efficient management of biological wastes. India needs to increase the per unit area productivity of crops to feed the ever growing populations.

The Soybean (*Glycine max* L.) crop is one of the most demanded crops worldwide. Soybean is an important oilseed crop and source of high quality protein for the consumption of human and used as fodder for animals. It is also important in improved crop rotation systems (Manyong *et al.*, 1996; Carsky *et al.*, 1997). Soybean is a short season leguminous crop which grows well in a warm climate with intermediate to heavy rainfall and also known as a food kharif crop (Deosthali *et al.*, 2005). It is a highly nutritive crop which consists approximately 43% of protein and 20% of oil (Trikha, 1986; Crescent, 2003). During the period of cropping, various pest attacks the soybean crop (Singh, 2004; Shukla and Upadhyay, 2007) which can be protected by the implementation of biofertilizers and biopesticides without harming the environment.

Use of plant products which possess biopesticidal activity has been in practice, especially from last 30 years (Lale, 2001). These plant based pesticides are also called as biopesticides. Extracts of some plants seem to be very effective in the control of pests and diseases of agricultural plants without showing any toxic effect (Koul *et al.*, 2008; Koul and Walia, 2009). In the growth of the plant, garlic (*Allium sativum* L.) is known for its stimulating properties on plant and it also protects the plants because of its bactericidal and fungicidal activities (Curtis *et al.*, 2004; Krebs *et al.*, 2006; Latha *et al.*, 2009). The extract of garlic has good properties of antioxidant (Bozin *et al.*, 2008; Rahman *et al.*, 2012; Chen *et al.*, 2013). In organic farming garlic is used as a preventive substance because of its repellent action against arthropod pests. Garlic protects the plants against some species of nematodes when it is directly added to the soil.

Among various biopesticides, neem (*Azadirachta indica*) has been found as a highly reliable source of biopesticides (Raizada *et al.*, 2001). In the list of 2400 botanicals, neem is on the top list which is used as biopesticide world wide. Due to its amazing properties neem has been known as the "Tree of the 21st century" by the United Nations (UNEP, 2012). All the parts of neem plant like seeds, leaves, barks, roots and flowers are having fungicidal, insecticidal as well as nematicidal properties (Bajwa and Ahmad, 2012). Due to the deleterious effects of leaves and seed extract of neem on insect pests they have been widely used (Nathan *et al.*, 2008). It has been reported that azadirachtin is an active element of neem which is used as effective biopesticide (Lowery and Isman, 1995). Various biochemicals like Nimbolide, Margolene, Mahoodin etc. have been purified from neem plant (Krishnaiah *et al.*, 2007; Olabinri *et al.*, 2013). The extracts and the by-products of the neem plant can be used alone or mixed with other compounds to produce the final product (Sara *et al.*, 2004).

Tobacco (*Nicotiana tabacum*) is an herbaceous plant of the family Solanaceae which has been farmed for its leaf. Tobacco leaves has been exploited as an insecticide and pesticide from a long time (Abdul-Ghany *et al.*, 2011). Tobacco is derived from organic sources and is beneficial to environment, so it is referred as an ecological or green insecticide. Tobacoo is recognized as a good pesticide and is also used as an insect or tick repellent to prevent vector-borne infections in traditional pharmacopoeia (Jufri *et al.*, 2016; Pavela *et al.*, 2016). Tobacco quality is mostly determined by the key chemical components of tobacco leaves, such as nicotine, protein and carbohydrates.

Millions of tonnes of animal and agro wastes are produced in India every year (Gupta, 2005; Garg *et al.*, 2006). If proper management of these

wastes will not be done it will create hazard to the society as well as the environment (Bhartiya and Singh, 2012; Chauhan and Singh, 2012; Chauhan and Singh, 2013; Singh and Chauhan, 2015). Vermiwash is a liquid extract of vermicompost which consists of micro- and macro- nutrients as well as several plant growth hormones, vitamins and enzymes. These elements altogether enhance the growth and productivity of plant and also provide protection against various diseases (Anand *et al.*, 1995). The formulation of liquid biofertilizer is one of the promising and new technology which helps in increasing the productivity in agricultural system. It must be diluted before applying to the plants. It is one of the most cost-effective and ecologically friendly trash disposal solutions available (Jayashree, 2016). By providing the suitable medium which is sufficient for the entire crop cycle, these biofertilizers facilitate the long survival of the organisms (Mahdi *et al.*, 2010). Foliar application of vermiwash made by the vermicomposts of buffalo dung+gram bran has enough capacity to increase crop growth, flowering, production and reduce insect infestation (Nath and Singh, 2011).

The aim of the present study was to observe the effect of combination of vermiwash of buffalo dung and gram bran along with the biopesticides for the proper growth, flowering and productivity of the Soybean (*Glycine max* L.).

## Materials and Methods

*Collection of Wastes and Experimental Set up for Vermicomposting*:

Buffalo dung was collected from farm houses of the Gorakhpur city, India. Gram bran was collected from rural and urban parts of Gorakhpur district. Vermibeds of buffalo dung and binary combination of buffalo dung and gram bran (1:1 ratio) were prepared and for pretreatment exposed to the sunlight for 15 to 20 days to remove the various harmful organisms and noxious gases. After pretreatment, adult earthworms *Eisenia fetida* were inoculated in each vermibed for vermicomposting. The culture of the earthworm *Eisenia fetida* used during the experiment was procured from the Vermibiotechnology Laboratory, Department of Zoology, D.D.U Gorakhpur University, Gorakhpur, India. The vermireactors were covered with a fine mesh screen in order to prevent the worms in bed and allow gas exchange and also moisten daily up to 40-50 days for maintaining the moisture (40% to 60% RH). Vermibeds were turned manually after the interval of one week. After 90 days the brown colour tea like granules appeared on the upper surface of each vermibed. Fresh vermicompost was collected for the extraction of vermiwash (Nath *et al.*, 2009; Singh *et al.*, 2021).

*Extraction of vermiwash*:

Vermiwash were extracted from prepared vermicompost along with earthworms by the help of vermiwash collecting device. The apparatus is made of plastic drum having capacity of 2 L and a tap at the bottom. The drum is filled with broken bricks, about 3 cm thickened which is followed by sand layer of 2-3 cm thickness, lastly filled with 5 kg vermicompost with earthworms *Eisenia fetida* population. Simultaneously 2.5 L fresh water was added into the drum. After 24 h opened the tap and allowed the tea like liquid extract of vermiwash by drop by drop in the collecting beaker (Nath *et al.*, 2009).

*Extraction of Neem, Garlic and Tobacco Leaves*:

Neem leaves were collected from the local area of Gorakhpur city, India. Garlic leaves were collected from the local garden near the Department of Zoolgy, DDU Gorakhpur University, Gorakhpur and tobacco leaves were collected from the local market of Gorakhpur city. These leaves were dried in direct sunlight for two weeks, with periodic turning. The 100 g of neem, garlic and tobacco leaves were crushed separately with 100 ml water, dried and then aqueous extract (w/v) was prepared for each. The aqueous extraction of neem, garlic and tobacco leaves were mixed with vermiwash (v/v) in 1:10 ratio and further diluted ten times with water for foliar application on experimental plants. Prepared vermiwash with biopesticides spread in soybean crop at the

Table 1: Effect of vermiwash of vermicompost of buffalo dung and gram bran with the combinations of aqueous extract of neem, garlic and tobacco leaves (biopesticides) on the growth, flowering and productivity of Soybean (*Glycine max* L.)

| Treatment | Combination Ratio | Growth of the plant (cm) Days after sowing | | | Flowering Period (days) | Productivity ($g/m^2$) |
|---|---|---|---|---|---|---|
| | | 20 | 30 | 40 | | |
| Control | | 10.8±2.1 | 15.7±2.2 | 20.9±2.1 | 50.8±2.4 | 103±19 |
| Vermiwash | | 17.2±2.8* | 24.5±3.4* | 33.3±3.6* | 43.9±3.4* | 288±33* |
| VW+GL | 10:1 | 18.2±1.9* | 26.8±2.4* | 37.2±2.3* | 38.2±2.6* | 320±18* |
| VW+NL | 10:1 | 25.4±4.3* | 31.4±2.9* | 49.2±2.6* | 43.3±2.3* | 343±16* |
| VW+TL | 10:1 | 27.3±2.2* | 34.5±2.6* | 46.6±2.9* | 39.3±2.7* | 393±17* |
| VW+GL+NL+TL | 10:1:1:1 | 32.3±2.1* | 37.6±2.4* | 47.4±2.2* | 32.2±2.5* | 423±14* |

VW= Vermiwash, GL=Garlic leaf, NL=Neem leaf, TL=Tobacco leaf
Each value represents mean ± SE of 6 replicates; *Significant ($P<0.05$) t test between treated and control group

interval of 15 days and first spray done 10 days after sowing.

*Measure the growth, flowering and productivity of Soybean (Glycine max L.)*:

The seeds of Soybean (*Glycine max L.)* were sown in the agricultural field. Six different areas of $1m^2$ of Soybean crop were selected for treatment of each combination. The growth (cm) of plant was measured by Auxanometer at the interval of 10 days after 20 days of sowing. The productivity was measured as $g/m^2$ of Soybean crops. The different combinations of vermiwash and biopesticides of neem, garlic and tobacco leaves were sprayed over the crop after each 10 days interval for the measurement of growth, whereas, at the time of starting of flowering after each 7 days interval. The different combinations of vermiwash with biopesticides were sprayed over the crop and control has no treatment.

*Statistical Analysis*:

All the data are presented as mean ±SE of six replicates. Student 't' test was applied to determine the significant ($P< 0.05$) difference between treated and control group (Sokal and Rohlf, 1973).

## Results

The foliar spray of vermiwash obtained from gram bran and buffalo dung with combination of the aqueous extract of garlic, neem and tobacco leaves significantly increased the growth of soybean plant (Table 1). There was time dependent significant growth which was observed after foliar spray of vermiwash with different aqueous extract of biopesticide (garlic, neem and tobacco leaves). The maximum growth of soybean i.e. 49.2±2.6 cm was observed in VW+NL combination after 40 days (Table 1).

All the combinations of vermiwash with aqueous extract of garlic, neem, tobacco leaves showed significant early flowering in soybean plant. The earliest flowering in soybean plant i.e. 32.2±2.5 days was observed after foliar spray of combination of VW+GL+NL+TL (Table 1).

Foliar spray of vermiwash obtained from gram bran and buffalo dung with combination of the aqueous extract of garlic, neem and tobacco leaves significantly increased the production of soybean. The maximum productivity i.e. 423±14 $g/m^2$ was observed in soybean plant which was treated with VW+GL+NL+TL combination (Table 1).

## Discussion

The foliar application of vermiwash with aqueous extracts of biopesticides i.e. garlic, neem and tobacco leaves increased the growth, flowering and productivity of soybean plant. The vermiwash contains high concentration of some important accessible elements so it increased the growth of the plant. Vermiwash which are obtained by different wastes are rich source of enzymes, vitamins, plant growth hormones (such as IAA, gibberellins, cytokines) and provides nutrients like phosphorus, potassium, calcium etc. to the plants (Nath and Singh, 2012). The significant increase in the growth of soybean plant was observed due to foliar spray of vermiwash with the combination of aqueous extract of neem leaves. The treatment of vermiwash with aqueous extract of biopesticide showed significant growth in soybean plant because the presence of plant growth hormones, vitamins, enzymes along with the presence of micro- and macro-nutrient in vermiwash (Anand *et al.*, 1995; Buckerfield *et al.*, 1999; Karuna *et al.*, 1999; Yadav *et al.*, 2005). Biopesticide obtained from the aqueous extract of neem leaves were important components which reduced the pest infestation and boosted the growth of the crop (Lowery and Isman, 1995; Isman, 1999; Hemavathy and Balaji, 2007). It has been reported that azadirachtin can reduce feeding behaviour of various larval insect pest and contains an antifeedent activity (Schmutterer and Singh, 1995).

The aqueous extract of garlic showed dual nature. It acts both as a fertilizer and a pesticide of organic origin. It also helps in the enrichment of soil and controls the growth of soil pest and bacteria. It provides macro-nutrients which are important for the growth of the plants. It is a bio-degradable, eco-friendly and an excellent soil conditioner (Lokanadhan *et al.*, 2012).

Tobacco leaves, powder, extracts or fumigants have been used to get rid of agricultural pests or parasites (McIndoo, 1943). Tobacoo has been used as an insect or tick repellent to prevent vector-borne infections so it is recognized as an excellent biopesticide (Jufri *et al.*, 2016; Pavela *et al.*, 2016). Tobacco leaves are made of many different components, and crude leaf methanolic extracts are extremely complex combinations (Eich, 2008; Leffingwell, 1999; Rodgman and Perfetti, 2013). Tobacco extracts has been used for ages to control insect pests (Bradbury, 2008). The quality of tobacco is determined from the chemical components of the leaves of tobacco, which are nicotine, protein and carbohydrates. Vermiwash with various biopesticides such as neem, garlic and tobacco resulted in a significant reduction in the economically harmful insect pests resulting in improved crop growth, early flowering and productivity (Rajput *et al.*, 2003; Nath *et al.*, 2011; Nath and Singh, 2011).

The significant reduction in the flowering of soybean was observed in vermiwash obtained from buffalo dung and gram bran with the aqueous extract of garlic, tobacco and neem leaves. The extracts of vermicompost improve early flowering period (Atiyeh *et al.*, 2001; Atiyeh *et al.*, 2002). High concentration of total kjeldahl and total phosphorus stimulate the crops to start its early flowering period (Muscolo *et al.*, 1999). During vermicomposting large amounts of humic acids were produced which have positive response on the growth and flowering of plants (Manivannan, 2004; Ramamoorthy, 2004). The increased productivity in soybean was observed in vermiwash obtained from buffalo dung and gram bran with the aqueous extract of garlic, tobacco and neem leaves. It is due to the combination of vermiwash with biopesticides which increased the number and flowers and reduced the infestation of pests which resulted more number of healthy pods and ultimately increased the productivity of the crop. The bioavailability of phosphorus is increased by Gibberellins and auxins (Subler, 1995; Lalitha *et al.*, 2000; Erich *et al.*, 2002). Vermiwash contains the enzymes like protease, amylase, urease and phophatase enzyme cocktail. It also contains nitrogen-fixing bacteria like *Azotobacter* species, *Agrobacterium* and

*Rhizobium* species as well as some phosphate-solubilising bacteria (Zambare *et al.*, 2008). It has been reported that the physiology of plant was promoted by the foliar applications of vermiwash which ultimately caused higher yield and quality of crops (Gamaley *et al.*, 2001).

## Conclusion

The combinations of vermiwash prepared from buffalo dung and gram bran with the biopestides made of the aqueous extract of neem, garlic and tobacco leaves is a good option for the growth and productivity of Soybean crop. Combination of vermiwash obtained from buffalo dung + gram bran with the aqueous extract of neem leaves is very suitable combination for the growth of Soybean crop as well as the combination of vermiwash obtained from buffalo dung + gram bran with the aqueous extract of GL+NL+TL is very effective for the flowering and productivity of Soybean. It can also be stated that vermiwash obtained from buffalo dung and gram bran have sufficient efficiency to enhance the growth, flowering and productivity of the crop. By the foliar spray of vermiwash the subjected plant gets all the necessary nutrients for the growth and productivity. The farmers of our country can easily make these combinations of vermiwash and biopesticides for various types of agricultural as well as horticultural plants for better growth and productivity of the plant. These by-products can be prepared easily. So, it is the best alternative for chemical fertilizers and this process is less expensive as well as eco-friendly.

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