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Effect of Foliar Application of Humic Acid, Yeast and Garlic Extracts on Growth, Yield and Quality in Forage Cowpea

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ABSTRACT

A field experiment was conducted at Giza research station, Agriculture research center, Egypt through the two sequential summer seasons 2018 and 2019 to investigate the influence of foliar treatment of humic acid, yeast and garlic extracts on yield and quality of forage, seed yield and its components. Plants were sprayed with foliar application, humic acid, yeast and garlic extract with rate of 8 ml L^{-1}, 1 gm L^{-1} and 10 gm L^{-1} at 21, 45, 75, 100, 120 days after sowing. The results indicated that, the treated plants with humic acid, garlic and yeast extracts achieved the higher values in all studied characters compared with the untreated plants. The values of fresh and dry forage yield were (13.91, 1.98, 13.47, 1.91 and 11.01, 1.53 ton fed^{-1}) when apply of humic acid, garlic and yeast extracts, respectively. While the values of control treatment were 9.89 and 1.35 ton fed^{-1} for fresh and dry forage yield, respectively. The values of seed yield were 528.17, 471.43 and 402.03 kg fed^{-1} for the treated plants with humic acid, garlic and yeast extracts as compared with the control treatment which recorded 324.99 kg fed^{-1}. Furthermore, the increase of N, P and K contents, ash, protein, digestible crude protein and total digestible nutrients. The regulating influence of these substances may be attributed to its nutritional effects or its richness in phytohormones and phenolic components, so it may be applied as plant growth regulators. It could be suggested that increase the forage yield, quality and seed yield by foliar application of humic acid, natural of garlic and yeast extracts with easy preparation, low cost, it has no harmful side effects on humans and animal and environmentally safe.

***Keywords*: :** Foliar application, Humic acid, Yeast Extract, Garlic extract, Forage yield, Quality, Seed yield, Cowpea

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is one of the main valuable leguminous grain cultivated in the equatorial and semi equatorial regions of the world, existence the main source of food proteins, calories, dietary fiber, minerals and vitamins for a great part of the world's population (Phillips *et al.,* 2003). In addition to grain, cowpea can produce good yields of fodder for ruminant feeding systems Ali *et al.,* (2004). Cowpea straw is a balanced nutritious feed for animals and has key roles in animal nutrition throughout the drought period in West Africa (Singh *et al.,* 2003 and Ilknur *et al.,* 2012).

Humic acid is a trade compound composed of 44-58% C, 42-46% O, 6-8% H and 0.5-4% N, in addition to more different substances that enhance soil fecundity and raise the obtainability of nutrients through preserving it on a mineral surface (Larcher, 2003). Humic substances enhance yield and quality of many plant species, specially grains (Ulukan, 2008). Humic acid enhance plant physiological approaches by improving the obtainability of Macro and micro nutrients element in addition to improve the vitamins, aminoacids, and also auxin and cytokinin contents of the plants (Vanitha and Mohandass, 2014). Turan *et al.,* (2011) reported that humic acid had effective effects on dry weight and the N, P, K uptake of plants. Sebastiano *et al.,* (2005) showed that humic acid had an effective influence on plant growth, seed yield and quality, and photosynthetic assimilation of plant. The foliar treatment of humic acid leads to a transitional production of plant dry matter. El-Galad *et al.,* (2013) detected that humic acid treatment considerably enhanced the soil available elements such as potassium, iron, manganese and Zink. Abd El-Gawad (2013) stated that the 8 kg fed^{-1} dose of humic acid improving the protein proportion of seeds.

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Despite the progress of cultivation and production of cowpea crop in Egypt, its cultivation still suffer from many problems that led to a decrease in seed yield per unit area, so it has become necessary to find means various modern methods to increase production, and one of the most important of these is the development of plant nutrition and fertilization with organic materials that are safe in the environment cheap and economically viable. Therefore, this study came to the use one of biofertilizer, which is bread yeast extract which has proven its effectiveness on many crops by improving and increasing growth and yield characteristics, and yeast is one of the eukaryotic single-cell microorganisms and reality of nuclear, they reproduce by simple division or budding (Al-Esawy, 2015) yeast cell diameter ranges from 5-10 micrometer and is devoid of chlorophyll.

Undoubtedly, they used it in the manufacture of bread without knowing what it was. The scientific name Meyen was first given in 1870 AD (Lodder, 1970). And the uses of yeast developed and entered into many industrial fields, including the production of alcohol (ethanol) as a biofuel (El-Helw and Abd El-Wahed, 2010). Bread yeast contains 16 amino acids, 13 vitamins and carbohydrates (Barnett *et al.,* 1990). Most of them are nutrients rich in what the plant needs. It was found that yeast extract activates cell division and nuclear acid formation and proteins (Fathy and Farid, 1996). It was also found that yeast produces auxin, gibberellin and cytokinin. As is known, these plant hormones are important in the germination, growth and differentiation of plant tissues and determine the extent of its response to the surrounding environmental conditions (El-Tohamy *et al.,* 2008).

Garlic bulb (*Allium sativum* L.) which belongs to the family *Liliaceae*, garlic extract is the sap of garlic bulb. It is characterized by holding a large amount of sulfur containing amino acids, like cysteine and methionine (Synge 1971). Furthermore, Garlic contains essential compounds such as volatile oil, sugar, proteins, fibers, minerals (potassium, phosphorus, magnesium, sodium, calcium, iron) and vitamins (Al Mayahi and Fayadh 2015 and Haciseferogullari *et al.,* 2005). The garlic extract has played several roles as a result of its hormonal (auxin-like) nature, which has a key function in lateral expansion and prolongation of cells. Regarding garlic acid influence, Helmy (1992) recorded that treatment of fresh garlic clove extract solution either in ethanol or tap water to summer squash cv. Eskandarani plants at 250 mg DW/plant lead to the preferable effects on the increment of the flowers number. Ahmed *et al.,* (2005) cleared that a higher increment in the number of pods of pea was recorded with post-inoculation application with garlic extract at 10 g/l. It has been supposed that the effective influence of garlic extract in improving crop growth and yield may be attributed to the existence of nutritional substances like minerals, enzymes, phenols, essential oils, vitamins, sulfur substances (Pandya *et al.,* 2011), however, little is known about its content of plant hormones. The present work was carried to evaluate the influence of humic acid, yeast and garlic extracts in forage yield, quality, seed yield and yield components of forage cowpea.

Materials and Methods

A field trial was achieved at Giza Agricultural Research station, ARC, Egypt, during the two sequential summer seasons 2018 and 2019 to investigate the influence foliar treatment of humic acid, yeast and garlic (*Allium Sativum*) extracts on forage yield and quality, seed yield and their components on forage cowpea.

The experiments were laid-out in randomized complete block design with four replications and each experimental unit was 2m x 3m occupying an area of 6 m^2.

Mechanical and chemical properties were analyzed of the experimental soil at Giza Agric. Res. Station, according to Piper (1950) and Page *et al.,* (1982); soil texture class was clay loam, pH 7.8 and 7.6, organic matter 0.85 and 0.82%, and EC 1.83 and 1.79 dsm^{-1} in the first and second seasons, respectively.

Entire recommended farming procedures have been used; 150 kg fed^{-1} super phosphate (15.5% P_2O_5) was applied before sowing; 45kg fed^{-1} of urea (46% N) and 100 kg fed^{-1} potassium sulphate (48% k_2O) were applied before live irrigation.

Humic acid analysis was performed for the purpose of estimating carbon, nitrogen, phosphorous, potassium, organic carbon and organic nutrients according to Jackson (1967). Sulfur was estimated by using the barium chloranilate procedure (Beaton, 1968). Fe, Mn and Zn were estimated by using atomic absorption (GBC 932 model), as described by Cottenie *et al.,* (1961). Macro-micronutrients content in humic acid is recorded in Table (1).

Table 1: Available macro-micronutrients content in humic acid.

N %	P %	K %	S %	Fe mg/kg^{-1}	Mn mg.kg^{-1}	Zn mg.kg^{-1}	C %	C/N ratio	O.M %	EC (dSm^{-1})
1.79	0.45	3.71	3.88	378	221	23.25	43.85	24.50	61.74	1.62

Yeast extract has been prepared by dissolving the specified weight of dry yeast in a liter of warm water, adding to it the same weight of sugar 1:1 and leaving it for 12 hours for the yeast to grow and multiply then the process of spraying until wet in the early morning.

Garlic bulbs were weighed and ground separately at the rates of 10 g/l and then steeped into two teaspoonfuls vegetable oil. Each garlic and vegetable oil infusion was mixed with detergent soap and latter dissolved in a liter of water. Mixture was first stirred and latter filtered then diluted in 10 liters of water and treated as foliar spray on growing cowpea.

The following treatments were applied:

1- Untreated (Control, Mineral fertilizers in recommended doses).
2- Foliar treatment of humic acid at rate of 8 m L^{-1}.
3- Foliar treatment of yeast extract at rate of 1 g L^{-1}.
4- Foliar treatment of garlic extract at rate of 10 g L^{-1}.

Foliar application of humic acid, yeast and garlic extracts after 21, 45, 75, 100 and 120 days from sowing, respectively.

One cutting was taken after 60 days from sowing then plants were left for flowering and seed production. To measure the plant growth characters, a sample of 10 plants from each plot were randomly taken.

The following characters were studied

Forage yield and its components

Plant height (cm), number of branches, number of leaves plant^{-1}, fresh forage yield (ton fed^{-1}), dry forage yield (ton fed^{-1}) and dry matter %.

Seed yield and its components: pod length (cm), number of seeds pod^{-1}, number of pods plant^{-1}, seed weight plant^{-1} (g), 100 seed weight (g), seed yield (kg fed^{-1}),and strew yield (kg fed^{-1}).

Relative yield: - Was calculated according to Barakat *et al.,* (2002) as follows:-

$$\text{Relative yield} = \frac{\text{Fresh or dry forage or seed yield at treatment}}{\text{Fresh or dry forage or seed yield at control}} \times 100$$

Forage quality

Plant samples for the first cut were used to determine crude fiber (CF %) and ash percentage (Ash %) according to A.O.A.C. (2000). Nitrogen was estimared by micro keldahl as described by Jackson (1967). Phosphorus was estimated spectrophotometrically using ammonium molybdate/stannous chloride procedure as described by Chapman and Pratt (1961). Potassium was estimated by flame photometer as described by Page *et al.,* (1982). Raw protein content was determined using conversion of nitrogen percentage to protein (Kang *et al.,* 2012). Protein % calculated by the equation= N% X Conversion factor (6.25).

Organic matter (OM %) were estimated by using the following equation:

OM% =100 – (Ash %). Digestible crude protein (DCP) was calculated according to Bredon *et al.,* (1963).

-Total digestible nutrients (TDN); was determined as described by Adams *et al.,* (1964), using the following equation where:

TDN= 74.43 + 0.35 CP – 0.73 CF

-Digestible energy (DE) = k cal / g dry matter; was calculated according to Heaney and Pigden (1963). Where: DE = 0.546 + 0.055 TDN.

Statistical analysis

The collected results were statistically analyzed following the methods summarized by Steel *et al.,* (1980) using the computer program PLABSTAT (Utz, H. F., 2004). Differences among means were tested by L.S.D at 0.05 level of significance. Bartlett's test was achieved the test of similarity of error variance. The test show insignificant differences for entire assessed features, so, the two season's data were combined.

Results and Discussion

1. Growth characters and forage yield

Mean values of forage yield and related characters for forage cowpea as affected by humic acid, yeast and garlic extracts from the combined analysis across seasons are presented in Table (2). Analysis of variance showed significant differences among all treatments as compared with control treatment in all traits, but it was not significant differences between humic acids and garlic extract in plant height, number of leaves plant^{-1}. The results in Table (2) showed that using humic acid was superior in plant height, number of branches and number of leaves plant^{-1}, recording the values 118.07 cm, 6.67 and 37.73, respectively. Followed by garlic extract which recorded 114.50 cm, 6.00 and 37.47 for the same traits, respectively.

Table 2: Mean values of fodder yield and its characters for forage cowpea (combination of two seasons 2018 and 2019) as affected by humic acid, yeast and garlic extracts.

Treatments	Plant height (cm)	Number of branches	Number of leaves plant^{-1}	Fresh forage yield (ton fed^{-1})	*Relative yield (%)	Dry forage yield (ton fed^{-1})	*Relative yield (%)
Control	93.39	4.33	25.83	9.89	100.00	1.35	100.00
Humic acid	118.07	6.67	37.73	13.91	140.65	1.98	146.67
Yeast extract	106.77	5.33	33.60	11.01	111.32	1.53	113.33
Garlic extract	114.50	6.00	37.47	13.47	136.20	1.91	141.48
Mean	108.18	5.58	33.66	12.07		1.69	
LSD at 0.05	6.63	0.62	3.18	1.10		0.15	

*The relative fresh and dry fodder yields were calculated for treatments as a percentage from the control.

While the lowest values 106.77 cm, 5.33, 33.60 for the same characters, respectively, were recorded with yeast extract treatment.

Means of forage cowpea as affected by humic acid, yeast and garlic extracts for fresh and dry fodder yields (ton fed^{-1}), and their relative yield in comparison to control during the both successive seasons are shown in Table (2).

Data presented in Table (2) cleared significant variations between all treatments as compared with control treatment in fresh and dry fodder yields. While, it was not significant differences between humic acids and garlic extract. Foliar application of humic acid produced the highest values of fresh and dry forage yields (13.91 and 1.98 ton fed^{-1}), respectively and transcended the control treatment by (40.65 and 46.67%), respectively. Followed by foliar application of garlic extract which recorded (13.47 and 1.91 ton fed^{-1}) for fresh and dry forage yields, respectively. and exceeded the control treatment by (36.20 and 41.48%), Meanwhile, Foliar application of yeast extract appeared the lowest fresh and dry fodder yields (11.01 and 1.53 ton fed^{-1}), respectively and it was transcended than the control treatment by (11.32 and 13.33%), respectively. Sebastiano *et al.,* (2005) showed that humic acid had an effective influence on plant growth, seed yield and quality, and photosynthetic assimilation of plant. The foliar treatment of humic acid leads to a transitional production of plant dry matter. On the other hand garlic extract regulation in vegetative growth involving leaf, stem and branch and elongation in plants stimulated by garlic extract treatment may be related with the necessary components exist in garlic. Nevertheless, this improvement in the plant growth may also as a result of auxins and other plant hormones existence in the garlic extract. It is well known that auxins regulate cell extension, stem growth, plant height, leaves number and root initiation in addition t0 induce plant development (Colla *et al.,* 2014 and Elzaawely *et al.,* 2018). Gibberellins also stimulate cell and stem prolongation, cell division and leaf extension (Hedden and Sponsel, 2015). This is also due to the role of yeast extract in

increasing the efficiency of the vital processes that occur inside the plant, as the yeast extract contains a group of amino acids that act as building units in the reactions producing amino acids inside the plant, as well as containing nitrogen and some mineral elements that work on activate growth and actively contribute to building chlorophyll in leaves. In addition, yeast extract is a natural source of cytokinins that play a major role in cell division and proliferation and increase their growth rates, which affects by increasing branching and root formation, and this means that the plant can express itself at the best levels with the nutritional richness that this extract contains (Branett *et al.,* 1990 and Day, 1990).

2. Forage quality

Mean values of forage quality characters as affected by humic acid, yeast and garlic extracts from the combined analysis across seasons are presented in Tables (3 and 4). Analysis of variance showed significant differences among all treatments as compared with control treatment in all traits, but it was not significant differences between humic acid and garlic extract. The results in Tables (3 and 4) showed that using humic acid was superior in crude protein (CP%), digestible crude protein (DCP%), Ash%, dry matter %, total digestible nutrients (TDN%), digestible energy (DE%), N%, P% and K% recording the values 21.01%, 16.61%, 14.19%, 14.23%, 65.58%, 4.15%, 3.36%, 0.38% and 1.48% respectively. Followed by garlic extract which recorded 20.86%, 16.47%, 14.07%, 14.17%, 65.32%, 4.14%, 3.34%, 0.35% and 1.44% for the same traits, respectively. The current data is a coincidence with Shaban *et al.,* (2012) found that foliar treatment of humic acid lead to a significant enhance in protein content. While the lowest values 19.93%, 15.57%, 12.60%, 13.87%, 64.31%, 4.08%, 3.19%, 0.33% and 1.31% for the same characters, respectively, were recorded with yeast extract treatment.

Means of crude fiber (CF %) and organic matter (OM %) gave the highest values by using yeast extract, it was recorded 23.42% and 87.40%, respectively. While the lowest values were recorded by using humic acid, it was recorded 22.19% and 85.81%, respectively.

Similar data were gained by Gad *et al.,* (2012), they demonstrating that the foliar treatment of humic acid caused the greatest rates of N, P and K (%) concentration in plant in comparison to untreated plants. Hussien and Hassan (2011) showed that the foliar treatment of humic acid improved the uptake of phosphorus and potassium. Moreover, inorganic nutrient elements such as N, P and K were excessed in snap bean leaves and pods after garlic extract treatment. This may be due to the great amounts of minerals and important nutrients in the garlic extract (Shakir and Al-Rawi 2017)

This may be due to promoting the growth and enhancement of forage cowpea plants and keeping for the high yield in terms of quantity and quality. Furthermore the bioactive substances exist in humic acid, garlic, and yeast extracts, their plant hormones act as a plant growth stimulator.

Table 3: Forage quality of forage cowpea as affected by humic acid, yeast and garlic extracts (combined across 2018 and 2019 seasons).

Treatments	Dry matter %	Ash %	CF %	OM%	TDN%	DE%
Control	13.61	10.77	24.61	89.23	63.05	4.01
Humic acid	14.23	14.19	22.19	85.81	65.58	4.15
Yeast extract	13.87	12.60	23.42	87.40	64.31	4.08
Garlic extract	14.17	14.07	22.48	85.93	65.32	4.14
Mean	13.97	12.91	23.18	87.09	64.57	4.10
LSD at 0.05	0.15	1.15	0.91	1.36	0.93	0.05

CF: Crude Fiber**, OM:** Organic matter, **TDN:** Total digestible nutrients, **DE:** Digestible energy

Table 4: Forage quality of forage cowpea as affected by humic acid, yeast and garlic extracts (combined across 2018 and 2019 seasons).

Treatments	N%	P%	K%	CP %	DCP %
Control	3.01	0.30	1.22	18.82	14.51
Humic acid	3.36	0.38	1.48	21.01	16.61
Yeast extract	3.19	0.33	1.31	19.93	15.57
Garlic extract	3.34	0.35	1.44	20.86	16.47
Mean	3.23	0.34	1.36	20.15	15.79
LSD at 0.05	0.11	0.03	0.07	0.89	0.76

3. Seed yield and its components

Data in Table (5) clear that pod length (cm), number of pods plant^{-1}, seed weight plant^{-1} (g), number of seeds pod^{-1} and 100- seed weight (g) were significant differences among treatments and control. But it was not significant differences between humic acid and garlic extract in all above mention traits. The highest values were found by using humic acid, it was recorded 15.57 cm, 21.73, 15.25g, 15.73 and 13.19 for pod length, number of pods plant^{-1} seed weight plant^{-1} (g), number of seeds pod $^{-1}$ and 100- seed weight (g). It was exceed than control by 43.4%, 33.9%, 10.2%, 38.8% and 23.6%, respectively. Followed by garlic extract which recorded 14.43 cm, 21.13, 14.99 g, 14.03 and 13.08, respectively for the same characters by exceeding values than control 32.9%, 30.2%, 8.6%, 23.8% and 22.6%. Meanwhile, the lowest values were recorded by using yeast extract it was gave 13.93 cm, 16.47, 14.31 g, 12.03 and 11.78 for pod length, number of pods plant^{-1} seed weight plant^{-1}, number of seeds pod $^{-1}$ and 100- seed weight, respectively.

Table 5: Mean values of seed yield related characters for forage cowpea as affected by humic acid, yeast and garlic extracts (combined across 2018 and 2019 seasons).

Treatments	Pod length (cm)	Number of pods plant^{-1}	Seed weight plant^{-1} (g)	Number of seeds pod^{-1}	100-seed weight (g)
Control	10.86	16.23	13.80	11.33	10.67
Humic acid	15.57	21.73	15.25	15.73	13.19
Yeast extract	13.93	16.47	14.31	12.03	11.78
Garlic extract	14.43	21.13	14.99	14.03	13.08
Mean	13.70	18.89	14.59	13.28	12.18
LSD at 0.05	1.16	4.26	0.49	1.87	1.04

Means of forage cowpea as affected by humic acid, yeast and garlic extract for seed yield (kg fed^{-1}), Strew yield (kg fed^{-1}), Biological yield (kg fed^{-1}), Harvest Index (%) and relative yield in comparison with control through the two seasons are shown in Table (6).

Data in Table (6) cleared significant variations between all treatments as compared with control treatment in seed yield, strew yield, biological yield, harvest index and relative yield. While, it was not significant differences between humic acid and garlic extract. Foliar application of humic acid produced the highest values of seed yield (528.17 kg fed^{-1}) and exceeded the control treatment by (62.52%), highest strew yield (289.57 kg fed^{-1}), biological yield (817.74 kg fed^{-1}) and harvest index (64.59%), respectively. Followed by foliar application of garlic extract which recorded (471.43 kg fed^{-1}) and exceeded the control treatment by (45.06%), strew yield (277.77 kg fed^{-1}), biological yield (749.20 kg fed^{-1}) and harvest index (62.92%), respectively Meanwhile, Foliar application of yeast extract produced the lowest seed yield (402.03 kg fed^{-1}) and it was exceeded significantly than the control treatment by (23.71%), strew yield (226.27 kg fed^{-1}), biological yield (628.30 kg fed^{-1}) and harvest index (63.99%), respectively. These results are in agreement with Branett *et al.,* (1990) and Day, (1990).

Table 6: Mean values of seed yield and its characters for forage cowpea as affected by humic acid, yeast and garlic extracts (combined across 2018 and 2019 seasons).

Treatments	Seed yield (kg fed^{-1})	*Relative yield (%)	Strew yield (kg fed^{-1})	Biological yield (kg fed^{-1})	Harvest Index (%)
Control	324.99	100.00	184.72	509.71	63.76
Humic acid	528.17	162.52	289.57	817.74	64.59
Yeast extract	402.03	123.71	226.27	628.30	63.99
Garlic extract	471.43	145.06	277.77	749.20	62.92
Mean	431.66		244.58	676.24	
LSD at 0.05	63.60		39.06	78.15	

*The relative seed yield was calculated for treatments as a percentage from the control.

In the current trial in two growing seasons, garlic extract improved the yield components of forage cowpea involving pod length and number, which concomitant with the noticed enhance in pod yield. The improvement in pod yield may be attribute to the raise in chlorophyll content in the forage cowpea leaves lead to the possible improve in the rate of photosynthesis and accumulation of dry materials , hence pod yield. Moreover, gibberellins and cytokinins have been recorded to enhance pod

set and afterwards pod number that was related with an improve in seed yield in several plants such as soybean (Dyer *et al.,* 1987) and snap bean (Emongor 2015; Elzaawely *et al.,* 2017 and Elzaawely *et al.,* 2018). Therefore, it can be suggested that increasing of seed yield fed^{-1} as a result of foliar applications is mainly caused by the raises in number of branches, number of pods $plant^{-1}$ and 100 seed weight (gm). The improvement in yield and its components may be attributed to the influence of all foliar applications on improving criteria for forage cowpea.

Conclusion

In this study, humic acid, garlic and yeast extracts foliar treatment cleared a regulate influence on forage cowpea plants causing by enhanced growth and physiological functions involving photosynthetic capacity and endogenous plant hormones that can ensure raised production of forage cowpea with high quality. Humic acid, garlic and yeast extracts could be desirable in agricultural production cussed by their different properties involving easy preparation, low cost and environmentally secure.

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