Conjunctive use of Fe, Zn and Bio-fertilizers on Growth, Nodulation and Chlorophyll Content of Urdbean (*Vigna mungo* L.)

ABSTRACT

An experiment was conducted on urdbean during *kharif* 2021 at Instructional Farm, College of Agriculture, Ummedganj, Kota (Rajasthan). The experiment comprised 10 treatments viz. (Control, 75 % RDF, 100 % RDF, 75 % RDF + *Rhizobium* @ 600 g ha^{-1} seed inoculation, 75 % RDF + *PSB* @ 600 g ha^{-1} seed inoculation, 75 % RDF + *Rhizobium* @ 600 g ha^{-1} + *PSB* @ 600 g ha^{-1}, 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage, 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *Rhizobium* @ 600 g ha^{-1}, 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *PSB* @ 600 g ha^{-1}, 75% RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *Rhizobium* 600 g ha^{-1}+ PSB @ 600 g ha^{-1}) was carried out in randomized block design with three replications. Ssignificantly higher plant height (25.28 and 34.43 cm) at 60 DAS and at harvest, nodules $plant^{-1}$ (29.22) at 45 DAS, nodule fresh weight (86.31 mg $plant^{-1}$) at 45 DAS, chlorophyll content (3.19 mg g^{-1}) at 45 DAS and dry matter accumulation (2.71, 5.94 and 9.99 g $plant^{-1}$) at 30, 60 DAS and at harvest were recorded with application of 75% RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *Rhizobium* 600 g ha^{-1}+ PSB @ 600 g ha^{-1} in urdbean over rest of treatments. Among these treatments, the application of 75% RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *Rhizobium* 600 g ha^{-1}+ PSB @ 600 g ha^{-1} produced maximum seed yield (1269.30 kg ha^{-1}) of urdbean.

Key words: Bio-fertilizer, chlorophyll, Ferrous, nodules, urdbean and zinc.

INTRODUCTION

Pulses are the integral part of Indian dietary system due to richest source of protein and other nutrients. Indian population is predominantly vegetarian and protein requirement for the growth and development of the human being is mostly met with pulses. The availability of pulses per capita per day has proportionately declined from 71 g to 52 g against the minimum requirement of 70 g day^{-1}/capita [1]. Pulses are improving soil fertility by atmospheric nitrogen fixation through

Rhizobium culture. In India pulses are grown an area of 28.34 million ha with total production of 23.15 million tonnes and productivity of 817 kg ha^{-1} [2]. Among the pulses, urdbean [*Vigna mungo* (L.) Hepper], is an important crop in India. Urdbean is the third most important pulse crop after chickpea and pigeonpea in India. It is highly nutritious containing 24-26% protein, 1.3% fat and 60% carbohydrates on dry weight basis and it is rich source of calcium, iron and vitamins [3].

The major constraints for low yield of urdbean are lack of micronutrient application and non-adoption of proper agronomic practices. Micronutrients are equally important in plant nutrition as major nutrients. The incidence of micronutrient deficiencies in crops has increased markedly in recent years due to intensive cropping, loss of top soil by erosion, losses of micronutrients through leaching, liming of acid soils, decreased proportions of farmyard manure or other organic sources compared to chemical fertilizers and use of marginal lands for crop production [4]. The optimum supply of micronutrients under balanced condition is very important for achieving higher productivity of urdbean. Foliar application of micronutrients has been proved to be an important asset in fertilizer application with a specific aim of increasing nutrient availability at the time of need, especially at the later stage of plant growth. Foliar application of nutrients using water soluble fertilizers is one of the possible ways to enhance the productivity of pulses. The foliar fertilization of Zn and Fe at flowering stage improves growth and yield urdbean [5].

Bio-fertilizer plays a vital role in maintaining long term soil fertility and sustainability [6]. Incorporation of FYM alone or bio-fertilizers improves available nutrient status of the soil with enhanced soil biological activity which in turn provides a congenial physical condition and improved availability of nutrient in the rhizosphere, resulting in an improvement in the crop growth and providing a better source–sink relationship [7]. Among bio-fertilizers, *Rhizobium* inoculation is cheapest, easiest and safest method of supplying nitrogen to legumes through well-known symbiotic nitrogen fixation process. Inoculation of appropriate strain enhances nodule formation resulting better nitrogen fixation. *Rhizobium* species in association with plant roots in urdbean improved soil fertility by fixing atmospheric nitrogen and produces plant growth substances in the soil. *Rhizobium* inoculation can increase the grain yield of pulse crops [8].

MATERIALS AND METHODS

A field experiment was conducted on urdbean during *kharif* 2021 at Instructional Farm, College of Agriculture, Ummedganj, Kota (Rajasthan). Geographically, is situated at 25.11^0 North latitude and 75.50^0 East longitude at an altitude of 258 meters above mean sea level (MSL). In Rajasthan, this region falls under the Agro-Climatic Zone-V (Humid South Eastern Plain Zone). This zone possesses typical sub-tropical conditions with maximum temperature range in summer is 42.2- 43.0 ^{0}C and minimum 12 - 27^{0}C. In this zone annual average rainfall is received 840 mm. The soil of experimental site was clay loam in texture, slightly saline in reaction, medium in available nitrogen (264 kg ha^{-1}) and phosphorus (21.7 kg ha-1) while high in potassium (388 kg ha-1) and sufficient in DTPA extractable micronutrients with pH neutral (7.61) and EC (0.52 dS m^{-1}). Source of nutrients were applied urea for nitrogen, DAP for phosphorus and mutate of potash for potassium. The full dose of fertilizer 100 % RDF (20:40:30 NPK kg ha^{-1}) was applied as basal dose. Before sowing, seed of urdbean was inoculated with liquid *rhizobium* culture @ 10 ml kg^{-1} seed and liquid *PSB* culture @ 10 ml kg^{-1} seed as per treatment. Foliar fertilization of zinc sulphate ($ZnSO_4$) and ferrous sulphate ($FeSO_4$) were applied at pre flowering and pod formation stage. Spraying was done with knapsack sprayer and the leaves were wetted thoroughly with fine mist. The absorption of solution by urdbean leaves, a sticker was added in the spray solution.

The experiment laid out in randomized block design with three replications. The experiment comprised 10 treatments viz. (Control, 75 % RDF, 100 % RDF, 75 % RDF + *Rhizobium* @ 600 g ha^{-1} seed inoculation, 75 % RDF + *PSB* @ 600 g ha^{-1} seed inoculation, 75 % RDF + *Rhizobium* @ 600 g ha^{-1} + *PSB* @ 600 g ha^{-1}, 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage, 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *Rhizobium* @ 600 g ha^{-1}, 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *PSB* @ 600 g ha^{-1}, 75% RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *Rhizobium* 600 g ha^{-1}+ PSB @ 600 g ha^{-1}) was carried out in randomized block design with three replications. For recording pre and post-harvest observations, five plants were randomly selected for each plot and tagged with labels for various observations on growth parameters and yield. Data were recorded plot wise as per standard procedures and statistically analysed by adopting appropriate method of standard analysis of variance [9].

Chlorophyll content (mg g^{-1})

Total chlorophyll content of leaves at 45 DAS was determined by the method advocated by [10]. Take 20 mg of leaf sample and crush and suspended in test tube containing 5ml of Dimethyl Sulphoxide (DMSO). Test tube were incubated at $60^{0}C$ for one hour in oven after that take reading on spectrophotometer at 645 and 663 nm.

$$\text{Total chlorophyll content (mg g}^{-1}) = \frac{(20.2 \times A_{645}) + (8.03 \times A_{663})}{1000 \times \text{weight of leaf sample taken (g)}}$$

RESULTS AND DISCUSSION

Effect of Fe, Zn and bio-fertilizers on growth parameters

The growth of urdbean crop was measured in terms of plant population, plant height, nodules $plant^{-1}$, nodule fresh weight, chlorophyll content and dry matter production presented in Table 1. A perusal of data indicated that the plant populations at 30, 60 DAS and at harvest and plant height at 30 DAS did not significantly influence, among all the treatments. It is evident from the data, plant height increased with the advancement in the age of the plant and reached the maximum at harvest. Significantly higher plant height at 60 DAS (25.28 cm) and at harvest (34.43 cm) was recorded with application of 75% RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ foliar spray at pre flowering and pod formation stage + *Rhizobium* 600 g ha^{-1}+ PSB @ 600 g ha^{-1} culture inoculation in urdbean as compared to control (12.33 and 16.18 cm), 75 % RDF (14.67 and 19.41 cm), 100 % RDF (16.42 and 21.96 cm), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} seed inoculation (17.48 and 23.32 cm), 75 % RDF + *PSB* @ 600 g ha^{-1} seed inoculation (17.26 and 22.73 cm), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} + *PSB* @ 600 g ha^{-1} (18.23 and 24.67 cm), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage (17.46 and 23.42 cm), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *Rhizobium @* 600 g ha^{-1} (20.59 and 26.06 cm), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *PSB* @ 600 g ha^{-1} (19.39 and 26.35 cm) in urdbean. The number of nodules $plant^{-1}$ at 45 DAS (29.22) was registered significantly higher under 75% RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ foliar spray at pre flowering and pod formation stage + *Rhizobium* 600 g ha^{-1}+ PSB @ 600 g) ha^{-1} culture inoculation over control (14.06), 75 % RDF (18.42), 100 % RDF (20.12), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} seed inoculation (22.30), 75 % RDF + *PSB* @ 600 g ha^{-1} seed inoculation (21.0), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} + *PSB* @ 600 g ha^{-1} (22.58), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage (22.68), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage +

Rhizobium @ 600 g ha^{-1} (24.99), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *PSB* @ 600 g ha^{-1} (24.03) in urdbean. Significantly higher fresh nodule weight of urdbean (86.31 mg $plant^{-1}$) at 45 DAS was observed with application of 75% RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ foliar spray at pre flowering and pod formation stage + *Rhizobium* 600 g ha^{-1}+ PSB @ 600 g ha^{-1}culture inoculation control (41.17 mg $plant^{-1}$), 75 % RDF (49.71 mg $plant^{-1}$), 100 % RDF (55.28 mg $plant^{-1}$), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} seed inoculation (59.10 mg $plant^{-1}$), 75 % RDF + *PSB* @ 600 g ha^{-1} seed inoculation (58.20 mg $plant^{-1}$), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} + *PSB* @ 600 g ha^{-1} (61.40 mg $plant^{-1}$), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage (60.03 mg $plant^{-1}$), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *Rhizobium* @ 600 g ha^{-1} (68.66 mg $plant^{-1}$), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *PSB* @ 600 g ha^{-1} (67.96 mg $plant^{-1}$).

The chlorophyll content at 45 DAS (3.19 mg g^{-1}) was recorded significantly higher under 75% RDF + Fe 0.1 % $FeSO_4$ + Zn 0.5 % $ZnSO_4$ foliar spray at pre flowering and pod formation stage + *Rhizobium* 600 g ha^{-1}+ PSB @ 600 g ha^{-1} culture inoculation over control (1.71 mg g^{-1}), 75 % RDF (1.93 mg g^{-1}), 100 % RDF (2.04 mg g^{-1}), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} seed inoculation (2.17 mg g^{-1}), 75 % RDF + *PSB* @ 600 g ha^{-1} seed inoculation (2.13 mg g^{-1}), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} + *PSB* @ 600 g ha^{-1} (2.25 mg g^{-1}), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage (2.25 mg g^{-1}), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *Rhizobium* @ 600 g ha^{-1} (2.61 mg g^{-1}), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *PSB* @ 600 g ha^{-1} (2.38 mg g^{-1}). Among the treatments, application of 75% RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ foliar spray at pre flowering and pod formation stage + *Rhizobium* 600 g ha^{-1} + PSB @ 600 g ha^{-1} culture inoculation exerted significantly higher dry matter accumulation (2.71, 5.94 and 9.99 g $plant^{-1}$) at 30, 60 DAS and at harvest over control (1.14, 2.18 and 4.25 g $plant^{-1}$), 75 % RDF (1.38, 2.53 and 5.15 g $plant^{-1}$), 100 % RDF (1.68, 3.63 and 6.29 g $plant^{-1}$), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} seed inoculation (1.71,3.57 and 6.43 g $plant^{-1}$), 75 % RDF + *PSB* @ 600 g ha^{-1} seed inoculation (1.71, 3.67 and 6.38 g $plant^{-1}$), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} + *PSB* @ 600 g ha^{-1} (1.75, 3.76 and 6.55 g $plant^{-1}$), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage (1.74, 3.70 and 6.43 g $plant^{-1}$), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *Rhizobium* @ 600 g ha^{-1} (2.17, 4.59 and 7.88 g $plant^{-1}$), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *PSB* @ 600 g ha^{-1} (2.09, 4.48 and 7.79 g $plant^{-1}$) at 30, 60 DAS and at harvest in urdbean.

In the present study application of ferrous and zinc and bio-fertilizers affected the overall growth of urdbean plants measured in terms of plant height, fresh weight of nodule, chlorophyll content and dry matter accumulation. The significant increase in plant height was found due to application of ferrous, zinc and bio-fertilizers. *Rhizobium* inoculation, which may maintain favourable balance between the applied nutrients in the plant for its optimum growth while elongation and chlorophyll biosynthesis. Tripathi et al. [11] found similar result related to plant height with [12]. The foliar application of nutrient helps in spreading of root system and gives more site for rhizobia infection, and increase their proliferation in rhizospere, helps in forming more effective root nodules. These results were in accordance with the findings of [13]. The increase in these growth attributes due to foliar application of Zn and Fe were also reported by [14]. The increase in chlorophyll synthesis due to application of Fe, Zn and bio-fertilizers enhances the activity of participation of Fe in the formation of chlorophyll and Zn enzymatic role in starch formation and in protein synthesis. The higher nitrogen, phosphorus and zinc content was increased the availability throughout the growth period and reproductive period of black gram leading to increase the number of cell and size resultant profound vegetative growth in terms of plant height, dry matter accumulation, nodule count and their fresh weight was observed [15]. The significant increase of dry matter accumulation was due to the fact that nitrogen helps in maintaining higher auxin level which might have resulted in better plant height, leaf area and presumably chlorophyll content of the leaves [16]. Increase in chlorophyll content due to the stimulation effect of Fe, Zn and Bio-fertilizers on plant processes helped in cell division and root elongation. These results were in accordance with the findings of [17]. Zinc helps in chlorophyll formation and plays an important role in nitrogen metabolism. The application of zinc in a deficient soil improved the overall growth and development of plant was observed by [18].

Effect of Fe, Zn and bio-fertilizers on seed yield

Seed yield is a function of various growth and yield attributing parameters. The data pertaining to seed yield (kg ha^{-1}) as influenced by application of Fe, Zn and Bio-fertilizers was presented in Table 1. Significantly higher seed yield (1269.30 kg ha^{-1}) was recorded with application of 75% RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ foliar spray at pre flowering and pod formation stage + *Rhizobium* 600 g ha^{-1}+ PSB @ 600 g ha^{-1} culture inoculation as compared to control (605 kg ha^{-1}), 75 % RDF (733 kg ha^{-1}), 100 % RDF (817 kg ha^{-1}), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} seed inoculation (867 kg ha^{-1}), 75 % RDF + *PSB* @ 600 g ha^{-1} seed inoculation (854 kg ha^{-1}), 75 % RDF + *Rhizobium* @ 600 g ha^{-1} + *PSB* @ 600 g ha^{-1} (881 kg ha^{-1}), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage (902 kg ha^{-1}), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *Rhizobium* @ 600 g ha^{-1} (1004 kg ha^{-1}), 75 % RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ at pre flowering and pod formation stage + *PSB* @ 600 g ha^{-1} (993 kg ha^{-1}) seed yield of urdbean,

The significant improvement in seed yield under the influence of application of Fe, Zn and bio-fertilizers was largely a function of improved growth and the consequent increase in different yield attributes [19]. It might also be due to availability of nutrients to the urdbean crop which increased number of pods $plant^{-1}$. This might have significantly increased the number of pods $plant^{-1}$. Similar results were also reported by [20] for number of pods of chickpea when nutrients applied at the initial stages, might have been effectively absorbed and translocated to the pods resulting in more number of pods $plant^{-1}$. Similar result was also found by [21]. The result showed that test weight was not significantly influenced by the different treatments due to adequate supply of zinc and iron contributed to accelerate the enzymatic activity and auxin metabolism in plants, as auxins are involved in cell division and root formation resulted in more number of pods $plant^{-1}$ and number of seeds pod^{-1}. These results are in close conformity with those of [18], who reported significant increase in the number of pods $plant^{-1}$ and number of seeds pod^{-1} in urdbean due to application of zinc and iron. The profound influence of nutrient application on biological yield seems to be on account of its influence on vegetative and reproductive growth [22]. The increase in growth attributes due to application of 75% RDF might have improved the yield parameters of black gram [23]. Application of *Rhizobium* and PSB significantly increased pods $plant^{-1}$, seeds pod^{-1} as well as seed, straw and biological yield over absolute control due to the fact that *Rhizobium* inoculation increased the root nodulation through better root development and more nutrient availability ultimately there was beneficial effect on seed yield. The test weight and harvest index remain non-significant due to application of different biofertilizers. The findings of this investigation confirm the observations of earlier workers, [24] and [25]. The significant increase in yield due to zinc and iron fertilization could be attributed to the increased plant growth and biomass production, possibly as a result of the uptake of nutrients. These findings are supported by [26] and [18] in various crops.

CONCLUSION

Amongst all the treatments, application of 75% RDF + 0.1 % $FeSO_4$ + 0.5 % $ZnSO_4$ foliar spray at pre flowering and pod formation stage + *Rhizobium* 600 g ha^{-1}+ PSB @ 600 g ha^{-1} culture inoculation gave maximum productivity. Therefore, it is suggested that most suitable dose for urdbean to obtain higher yield.

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Table 1: Effect of Fe, Zn and Bio-fertilizers application on growth parameters and seed yield of urdbean.

Treatments	Plant height (cm)			Nodules plant^{-1}	Nodule fresh weight (mg plant^{-1})	Chlorophyll content (mg g^{-1})	Dry matter accumulation (g plant^{-1})			Seed yield (kg ha^{-1})
	30 DAS	60 DAS	At harvest				30 DAS	60 DAS	At harvest	
T_1: Control	8.97	12.33	16.80	14.06	41.17	1.71	1.14	2.18	4.25	604.78
T_2: 75 % RDF	9.33	14.67	19.41	18.42	49.71	1.93	1.38	2.53	5.15	733.36
T_3: 100 % RDF	9.96	16.42	21.96	20.12	55.28	2.04	1.68	3.63	6.29	817.20
T_4: T_2 + *Rhizobium* @ 600 g ha^{-1} seed inoculation	10.12	17.48	23.32	22.30	59.10	2.17	1.71	3.57	6.43	867.24
T_5: T_2 + PSB @ 600 g ha^{-1} seed inoculation	9.99	17.26	22.73	21.00	58.20	2.13	1.71	3.67	6.38	853.63
T_6: T_2 + *Rhizobium* @ 600 g ha^{-1} + *PSB* @ 600 g ha^{-1}	10.18	18.23	24.67	22.58	61.40	2.25	1.75	3.76	6.55	881.23
T_7: T_2 + 0.1 % FeSO4 + 0.5 % ZnSO4 at pre flowering and pod formation stage	10.84	17.46	23.42	22.68	60.03	2.25	1.74	3.70	6.43	901.78
T_8: T_2 + T_7 + *Rhizobium* @ 600 g ha^{-1} seed inoculation	11.16	20.59	26.06	24.99	68.66	2.61	2.17	4.59	7.88	1003.66
T_9: T_2 + T_7 + *PSB* @ 600 g ha^{-1} seed inoculation	10.93	19.39	26.35	24.03	67.96	2.38	2.09	4.48	7.79	993.05
T_{10}: T_2 + T_7 0 + *Rhizobium* 600 g ha^{-1} + PSB @ 600 g ha^{-1} seed inoculation	11.86	25.28	34.43	29.22	86.31	3.19	2.71	5.94	9.99	1269.30
SEm±	0.59	1.00	1.37	0.97	4.02	0.11	0.10	0.20	0.39	43.86
CD (p=0.05)	NS	2.98	4.06	2.89	11.95	0.33	0.30	0.60	1.17	130.30