The Bioscan 11(1): 449-453, 2016 (Supplement on Agronomy)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EVALUATION OF DIFFERENT SOIL EXTRACTANTS FOR ASSESSING BORON SUPPLY AND ESTIMATION OF CRITICAL LIMITS FOR SUNFLOWER IN SOILS OF *KUMAON* REGION OF UTTARAKHAND

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KEYWORDS
Boron
Bray's per cent yield
Calcium chloride
Calcium chloride + mannitol
Critical limit
Dilute hydrochloric acid method

Received on :
20.08.2015

Accepted on :
13.02.2016

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ABSTRACT

Twenty surface (0-15 cm) soil samples collected from different sites of *Kumoun* region of Uttarakhand (India) were analyzed for extractable soil B using five different soil extractants and soil test values were evaluated for predicting the response of B application in hybrid sunflower(Sankar Sunflower 6460).The soil extractants used in the study could be arranged in the following order of their B extraction capacity: Mehlich 3 (pH 2.0) > 0.01M $CaCl_2$ + 0.05 M mannitol (pH 8.5) > 0.01 M $CaCl_2$ > 0.05 M HCl > Hot water.Among soil extractants used in the study, B extractable in hot water, 0.05 M HCl and Mehlich-3 extractants correlated significantly with Bray's per cent yield. The critical limits of B in soil were 0.27, 0.37 and 9.2 mg kg^{-1}B extractable in hot-water, 0.05 M HCl and Mehlich-3 extractants. The critical limit of boron in hybrid sunflower plants was 27.4 mg B kg^{-1} dry plant tissue at 40 days after emergence.

INTRODUCTION

Amongst the micronutrients, boron is one of the most important micronutrient required for normal growth and development of plant. Although B requirement plants is relatively small, yet it requires special attention as the concentrations range between deficiency and excess is very narrow. The suitability of extractants for determining available B can be assessed by correlating the amount of extractable B in untreated soil with Bray's per cent yield, uptake and tissue B concentration of plants (Datta *et al.,* 1998.

Nable *et al*. (1997) suggested the several soil extractants for measuring the plant available fraction of B in soils *viz*. ammonium oxalate, saturation extract, hydrochloric acid, hot water extraction with variation and improvement, calcium chloride, mannitol exchangeable, Mehlich-3 and ammonium bicarbonate diethylenetriamine pentaaceticacid (AB-DTPA).The hot water soluble procedure, measures B capacity, extracts B from organic, adsorbed and soluble pools of the soil (Offiah and Axley, 1993). However, The hot water soluble procedure faces various challenges including B contamination from glassware, limited numbers of samples run at one time, inconsistency in refluxing time and lack of repeatable results. Shuman *et al*. (1992)noted that the Mehlich-3 procedure extracted available soil B level comparable with the hot water extraction method, making it a more suitable alternative method in soils of low pH. In a given agroclimatic condition, suitability of different soilextractantneed to be checked in predicting the response of particular nutrient application(Watham et al. 2014). Keeping above facts in view, the present investigationwas undertaken to evaluate some soil test methods for available boron and determination of critical limits of boron in hybrid sunflower in soils of *Kumaon* region of Uttarakhand.

MATERIALS AND METHODS

Twenty soil samples (0-15 cm) having no previous history of boron application were collected from different sites of *Kumoun* region of Uttarakhand. The soil texture were analyzed by Bouyoucos Hydrometer method (Black, 1965); soil pH (Bower and Wilcox, 1965); electrical conductivity (Jackson, 1973), organic carbon was determined by wet oxidation method of Walkley and Black (1934) and hot-water soluble boron (Berger and Troug, 1944) (Table 1).

These soil samples were estimated for extractable B using five extractants viz., hot-water soluble, calcium chloride (0.01 M $CaCl_2$), calcium chloride and mannitol (0.01 M $CaCl_2$ + 0.05 M mannitol, pH = 8.5), dilute hydrochloric acid (0.05 M HCl)

and Mehlich-3 (0.2M CH_3COOH + 0.25 M NH_4NO3 + 0.103 M HNO_3 + 0.001 M EDTA, pH = 2.0). The boron content is extracted aliquat was determined by Azomethine-H method (Bingham, 1982).

Two kg of processed soil was filled in polyethylene lined plastic pots (20 × 5 × 2 cm) and boron was applied @ 0.0, 0.25, 0.5, 1.0 and 2.5 mg B Kg^{-1} soil through stock solution of borax. The treatments were replicated twice in a completely randomized design. A basal dose of nitrogen, phosphorus and potassium @ 50: 60: 40 N: P_20_5:K_20 ha^{-1} in the form of urea and potassium dihydrogenphosphate (KH_2PO_4) stock solution were uniformly applied to each pot and the soil of the respective pots was thoroughly mixed after the addition of desired treatments and pots were irrigated and allowed to equilibrate for about 3 days. Hybrid sunflower (*Helianthus annuus L.*)variety Sankar Sunflower 6460 seeds were sown and thinned to 4 plants in each pot after seven days of sowing. The moisture level was maintained at field capacity in all the pots by irrigating with distilled water as and when required. The crop was harvested at head initiation stage (40 days after emergence). The plant samples were decontaminated by sequential washing with tap water, 0.1 N HCl and finally with distilled water to remove dirt and then oven-dried at 60°C for 48 hours till constant weight is achieved and dry matter yield from each plot is recorded and relative yield was calculated as follows:

$$\text{Relative yield (\%)} \quad \frac{\text{Dry matter at 0 mg kg}^{-1}\text{ soil}}{\text{Dry matter at optimum level of B}} \quad 100$$

The samples were ground and the powdered samples were analyzed for plant boron by taking one gram of ground plant sample in silica crucibles and kept in muffle furnace for dryashing at 550°C for about 2 hours until ashingwas completed leaving a white residue. After cooling, 5 mL of 6 NHCl was added to dissolve residue in silica crucibles and then it was filtered using Whatman no. 42 filter paper by repeated washing upto 50 mL in plastic vials and 2mL of aliquot was taken in plastic beaker to determine boron by Azomethine-H method (Bingham, 1982).

The dry matter yield, boron concentration and boron uptake by hybrid sunflower data were statistically analyzed by following standard procedures as outlined by Snedecor and Cochran (1967) and the test of significance was conducted at 5 per cent level of significance and were also subjected to simple correlation analysis. The critical limits of boron in soil and hybrid sunflower were estimated following the statistical method proposed by Cate and Nelson, 1971.

Table 1: The range and mean values of soil properties of investigated soils

Soil Characteristics	Range	Mean
Sand(%)	8.1 - 92.2	44.9
Silt(%)	1.0 - 58.0	32.8
Clay(%)	6.5 - 43.8	22.2
pH	5.6 - 8.6	7.53
E.C (dSm^{-1})	0.021 - 0.101	0.06
Organic carbon (%)	1.6 - 12.0	4.49
Hot-water soluble B (mg kg^{-1} soil)	0.26 - 1.52	0.86

RESULTS AND DISCUSSION

Extraction of available boron

The ranges and mean values for extractable B by different extractants for hybrid sunflower crop are presented in Table 2. The ranges noted for extractable soil boron were 0.18 to 0.53 mg B kg^{-1} soil (mean 0.35 mg B kg^{-1}) for hot water soluble boron, 0.34 to 0.76 mg B kg^{-1} soil (mean 0.57 mg B kg^{-1}) for 0.01 M $CaCl_2$, 0.31 to 0.78 mg B kg^{-1} soil (mean 0.59 mg B kg^{-1}) for 0.01M $CaCl_2$ + 0.05 M mannitol (pH 8.5), 0.20 to 0.90 mg B kg^{-1} soil (mean 0.42 mg B kg^{-1}) for 0.05 M HCl and 5.24 to 16.69 mg B kg^{-1} soil (mean 9.15 mg B kg^{-1}) for Mehlich 3 extractant (pH 2.0).

The range reported here for $CaCl_2$ extractable B was well within the concentration limits (0.24 to 1.79 mg $kg^{-1}$with a mean value of 0.77 mg kg^{-1}) reported for arid soils of western Rajasthan by Chaudhary and Shukla (2004). On the basis of mean extractable B content in soils, the extractants employed in the present study could be arranged in the following order of their B extraction capacity: Mehlich 3 (pH 2.0) > 0.01M $CaCl_2$ + 0.05 M mannitol (pH 8.5) > 0.01 M $CaCl_2$ > 0.05 M HCl > Hot water.However, Aitken *et al*. (1987) reported low levels of B with mannitol-$CaCl_2$ in comparison to those displaced from soil by the refluxing procedures in acidic soils of Queensland. The inability of mannitol to extract B from these soils was discussed in relation to the chemistry of mannitol B complexes and it was suggested that mannitol would not be an effective extractant for B in acid soils. Sarkar *et al*. (2008) also reported the extraction of boron from the acidic soil was in the order: calcium chloride > hot-water > phosphorus dihydrogen phosphate > tartaric acid > mannitol + calcium chloride. Higher extractability of B in Mehlich 3 could be related to extremely acidic pH.

Relationships among boron extractants

The simple correlation coefficients among different soil extractants were highly significant for 0.05 M HCl&Mehlich 3

Table 2: Amount of boron in soils extracted by different extractants for hybrid sunflower crop

Extractant	Range(mg B Kg^{-1})	Mean	Reference
Hot-water	0.18 – 0.53	0.35	Berger &Troug(1944)
Calcium chloride (0.01 M $CaCl_2$)	0.34 – 0.76	0.57	Aitken *et al*. (1987)
Calcium chloride and mannitol (0.01 M $CaCl_2$ + 0.05 M mannitol, pH = 8.5)	0.31 – 0.78	0.59	Cartwright *et al*.(1983)
Dilute hydrochloric acid (0.05 M HCl)	0.20 – 0.90	0.59	Ponnamperuma*et al*.(1981)
Mehlich-3 (0.2M CH_3COOH + 0.25M NH_4NO_3 + 0.103M HNO_3 + 0.001 M EDTA, pH = 2.0)	5.24-9.15	16.69	Mehlich(1984)

Table 3: Simple correlation coefficients (r) among different extractants for hybrid sunflower crop

Extractant	0.01 M $CaCl_2$	0.01M $CaCl_2$ + 0.05M mannitol, (pH = 8.5)	0.05 M HCl	Mehlich-3 (pH = 2.0)
Hot-water	0.379	0.277	0.497*	0.551*
0.01 M $CaCl_2$	1.000	0.168	0.454*	0.351
0.01M $CaCl_2$ + 0.05M mannitol, (pH = 8.5)		1.000	0.327	0.260
0.05 M HCl			1.000	0.699**
Mehlich-3 (pH = 2.0)				1.000

** Significant at p = 0.01; * Significant at p = 0.05

Table 4: Simple correlation coefficients (r) between extractable soil B content, B concentration and B uptake by hybrid sunflower

Soil extractable B(mg B kg^{-1} soil)	Simple correlation coefficient (r) B content(μg B/g tissue)	B uptake (μg/pot)
HWS-B	0.510*	0.428
0.01M $CaCl_2$	0.472*	0.183
0.01M$CaCl_2$ + 0.05M mannitol(pH 8.5)	0.571**	0.459*
0.05 M HCl	0.767**	0.582**
Mehlich 3(pH 2.0)	0.616**	0.601**

**Significant at p = 0.01;* Significant at p = 0.05

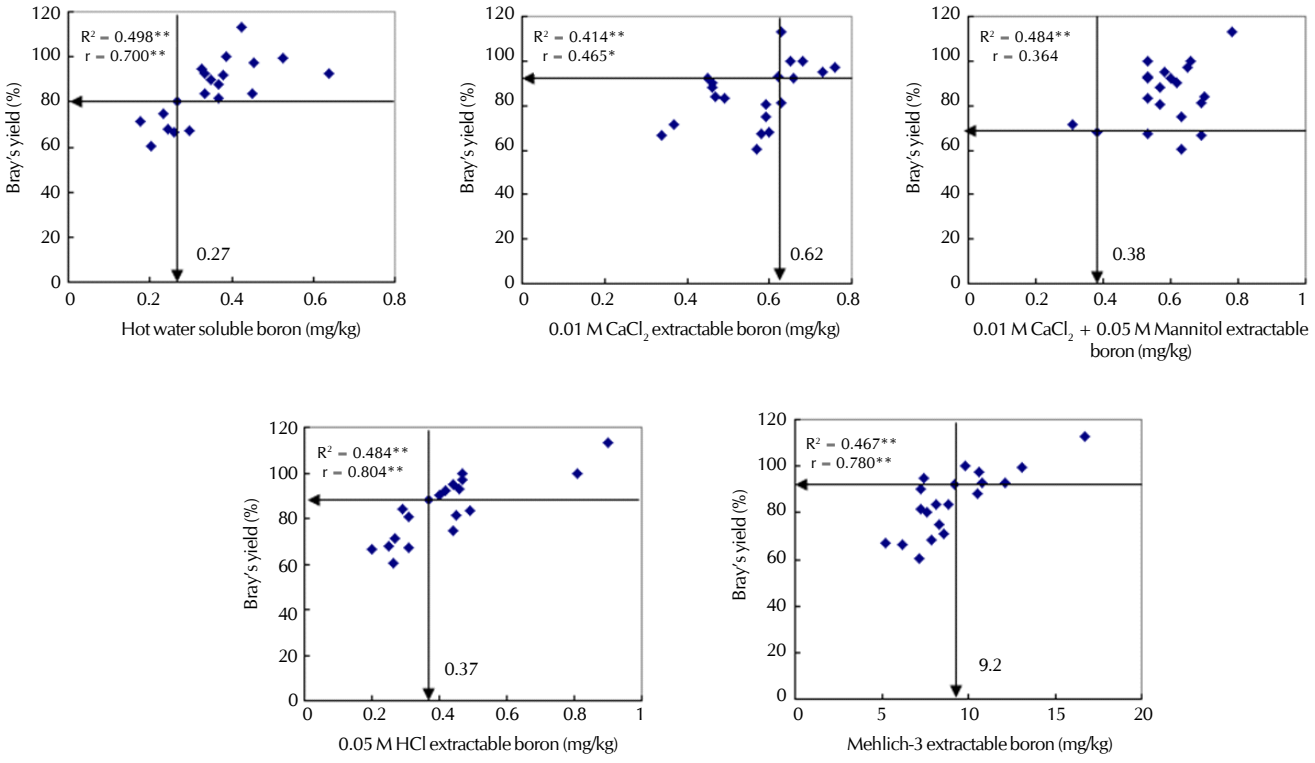

Figure 1: Scatter diagram ploted between B concentration versus Bray's percent yield of hybrid sunflowers. ** Significant at p = 0.01 and *Sinificant at p = 0.05

(p < 0.01; Table 3). Significant correlation coefficient was also observed among HWS-B and 0.05 M HCl , HWS-B andMehlich 3 and 0.01 M $CaCl_2$ and 0.05 M HCl (p < 0.05). These significant correlation coefficient values among different soil extractants signified that all the examined extractants extracted B from a similar pools of B in these soils. Zbíral and Nemec (2009) also reported a statistically significant correlation between hot water and Mehlich 3 extractable B in soils of Czech Republic. Highly significant correlation between hot-water soluble B and 0.05 M HCl extractable B was also observed by Tsadilas *et al*. (1997) in the soil samples collected around the vines of kiwi fruits. Both 0.05 MHCl extractable B and Mehlich 3 extractable B were found to be highly correlated to hot-water soluble-B which indicated that both 0.05 M HCl and Mehlich-3 method could be used as an alternative method to hot water soluble-B. However, Tsadilas and Kazai

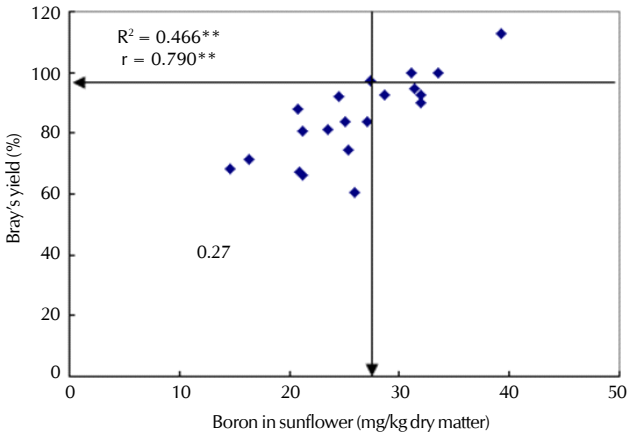

Figure 2: Scatter diagram ploted between B concentration versus Bray's percent yield of hybrid sunflowers. ** Significant at p = 0.01 and *Sinificant at p = 0.05

(2005)while evaluating the efficiency of hot water extraction, saturation paste, resin, mannitol and hydrochloric acid methods for determination of soil B available to apple tree varieties (*Malus domestica*, var. golden and black) found that hot water extractable boron was strongly correlated with B extracted by mannitol and HCl and these two extractants could be used as alternatives to hot water extraction method.

Correlation of soil boron with yield and uptake

The values of simple correlation coefficients (r) between extractable B content in soils estimated by different soil extractants, B concentration and B uptake by hybrid sunflower under control treatment (0 mg B kg^{-1} soil) are presented in Table 4.

Boron content in hybrid sunflower plants correlated significantly to soil extractable B estimated by 0.01 M $CaCl_2$ + 0.05 M mannitol(pH 8.5)($p < 0.05$) , 0.05 M HCl and Mehlich 3 (significant at $p < 0.01$) and with hot water and 0.01 M $CaCl_2$ (both significant at $p < 0.05$). Boron uptake by hybrid sunflower plants correlated significantly to extractable soil boron estimated by only 0.01 M $CaCl_2$ + 0.05 M mannitol(pH 8.5) (at $p < 0.05$) , 0.05 M HCl and Mehlich 3 extractants (both significant at ($p < 0.01$). However, B extracted by hot water and 0.01 M $CaCl_2$ could not relate significantly to B uptake by hybrid sunflower. The close correlation between concentration in sunflower tissue and 0.01M $CaCl_2$ + 0.05 M mannitol extractable, 0.05M HCl extractable and Mehlich 3 extractable B indicated that both these B procedures provided a good estimate of readily available B in the soils.

Critical level of Boron in soil

It was observed that the critical limit of boron in the soils varied with methods of boron extraction. The critical levels of boron in soils determined by statistical method of Cate and Nelson (1971)ranged from 0.27 to 9.2 mg B kg^{-1}soil(Fig. 1)These plots clearly showed that the critical limits of B in soil were 0.27 mg hot water soluble B, 0.62 mg 0.01M $CaCl_2$ extractable B , 0.38 mg 0.01M $CaCl_2$+ 0.05 M mannitol extractable B, 0.37 mg 0.05 M HCl extractable B and 9.2 mg Mehlich extractable B kg^{-1} soil.The critical limits for soil B determined by hot water and $CaCl_2$ + mannitol extractions were well within the limits reported earlier by Tsadilas and Chartzoulakis (1995) for olive trees in Greece who reported these values as 0.33 and 0.41 mg kg^{-1} soil, respectively. The critical levels of soil B reported by Arora (2001) using hot water and 0.01 M $CaCl_2$at 60 days of berseem growth were 0.47 and 0.42 mg kg^{-1} soil, respectively. The critical limit of B in soils of apple orchards of Himachal Pradesh was worked out to be 0.46 mg kg^{-1} soil by Singh (2000). The R^2 values were significant ($p = 0.01$) for all the extractants tested. Considering the magnitude of R^2-values, different soil extractants can be arranged in the following order of reliability: Hot-water > 0.01 M $CaCl_2$ + 0.05 M mannitol = 0.05 M HCl > Mehlich 3 > 0.01 M $CaCl_2$. Boron extracted by 0.01 M $CaCl_2$extractant showed significant correlation ($p < 0.05$) with Bray's percent yield of Sunflower while B extracted by hot water, 0.05 M HCl and Mehlich 3 showed highly significant correlation (at $p < 0.01$). Thus, these critical limits determined in different soil extractant could be used to distinguish boron responsive soil from unresponsive soil ones. Datta *et al.* (1998) conducted a green house pot experiment with four levels of B (0, 0.5, 1.0, and 2.0 mg kg^{-1}) in 25 acid soils from lateritic and alluvial tracts of Southern West Bengal and estimated the critical values of extractable B in soils for soybean plants as 0.51 mg for hot water, 0.61 mg for $CaCl_2$, 0.27 mg for ammonium acetate and 0.45 mg kg^{-1} soil for salicylic acid.

Critical level of boron in hybrid sunflower

The results revealed that the critical level of boron in hybrid sunflower was 27.4 mg B kg^{-1} dry plant tissue at 40 d after emergence (Fig. 2). The R^2-value was statistically significant at $p = 0.01$.

Bray's per cent yield of hybrid sunflower also correlated significantly to B concentration of hybrid sunflower tissue (r = 0.790**, significant at $p = 0.01$).This result was also corroborating with the findings of Datta *et al*. (1998) where critical B concentration in soybean plants was 18 mg kg^{-1} on dry weight basis.

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