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Full Length Research Paper

Alterations on the plasma concentration of hormonal and non hormonal biomarkers in human beings submitted to whole body vibration exercises

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Vibration is a mechanical stimulus that is characterized by an oscillatory motion. When there is a direct contact of a person, in general standing on the base of this type of platform, the vibration that is produced in these machines is transmitted to the body of the subject producing whole body vibration (WBV) exercises. Biological effects can be associated with the WBV exercises with desirable and undesirable consequences. These effects of the WBV exercises seem to be related to a direct effect in a tissue/organ/system and/or or to indirect effects due to alteration of the plasma concentration of some hormonal and non hormonal biomarkers. The aim of this investigation is to present a revision about hormonal and non hormonal biomarkers in human beings submitted to WBV exercises that have suffered alteration in the plasma concentrations. Searches were performed in the PubMed and Scopus databases with the key words "whole body vibration". Papers were selected following defined criteria. Considering the WBV exercise, hormonal fluctuations of testosterone, growth, insulin-like growth factor1, epinephrine, norepinephrine, cortisol, irisin, parathyroid hormone and sclerotin are observed. Non hormonal biomarkers have suffered alterations in response to WBV, as glucose, free fatty acids, adiponectin, transforming growth factor-beta1, nitric oxide, osteopontin, interleukin-1beta, bone-specific alkaline phosphatase, cartilage oligometric matrix protein and tumor necrosis factor-alpha in plasma concentration. In conclusion, putting together the findings related to the alterations of the concentration of hormonal and non hormonal biomarkers due to the WBV exercises, it is possible that the fluctuations in the plasma concentrations of these biomarkers might help us understand better the biological effects of this kind of exercises, probably due to neuroendrocrine responses.

Key words: Whole body vibration exercise, vibration, hormonal and non hormonal fluctuations

INTRODUCTION

General approaches about whole body vibration exercises

Vibration is a periodic, sinusoidal and deterministic mechanical stimulus that is characterized by an oscillatory motion. Vibrations are also generated in oscillating/vibratory platform. When there is a direct contact of a person, in general standing on the base of this type of platform; the vibration that is produced in these machines is transmitted to the body of the subject producing whole body vibration (WBV) exercises (Cardinale and Wakeling, 2005; Rittweger, 2010).

The possibility of applications of the exercises generated by the WBV in Health Sciences in patients with several clinical disorders has been discussed (Rittweger, 2010). Nevertheless, Rittweger (2010) has pointed out that this modality of exercise is still widely unknown to the scientific community.

Hand et al. (2009) have reported that WBV is a technology that was firstly developed in the second half of the 20th century with the aim to reduce bone density loss and muscle atrophy in astronauts exposed to zero-gravity conditions. Nazarov and Zilinsky (1984), Nazarov and Spivak (1985) and Issurin et al. (1994) firstly, have used the vibration as a type of training in athletes. WBV exercises can produce beneficial effects to the healthy in a subject, including improvements in muscle strength in athletes (Fagnani et al., 2006; Fort et al., 2012; Cheng et al., 2012) and in patients with several clinical conditions, as Parkinson disease (Arias et al., 2009), osteoarthritis (Trans et al., 2009) and multiple sclerosis (Wunderer et al., 2010). Moreover, improvements in (a) the walking function (Ness and Field-Fote, 2009), (b) the bone mineral density (BMD) in elderly (Gusi et al., 2006), (c) the back pain (Del Pozo-Cruz et al., 2011), (d) health-related quality of life, (e) fall risk (Bruyere et al., 2005) and (f) gait (Lam et al., 2012; Unger et al., 2013) have been described.

Despite the positive effects of the WBV, undesirable side effects of these exercises can occur and have been reported. Crewther et al. (2004) observed that untrained participants exposed to acute vibration frequencies, amplitudes and postures (standing, squat) suffered from side-effects, such as hot feet, itching of the lower limbs, vertigo and severe hip discomfort. Cronin et al. (2004) reported that untrained participants suffered from vibration pain of jaw, neck and lower limbs from an acute intermittent WBV. Monteleone et al. (2007) reported a case of significant morbidity following one session of WBV training in a patient with asymptomatic nephrolithiasis. Franchignoni et al. (2013) have reported that a healthy elite athlete (steeplechase runner) suffered two episodes of hematuria after WBV training. It is suggested that platforms providing side-alternating vibration may pose some health risks with high amplitudes (the feet are positioned too far from the axis of rotation in this kind of platform. Cochrane (2011) has pointed out that some of the related side-effects to the use of WBV would be due to lack of familiarization of the participants with the WBV.

Rittweger (2010) has suggested that the exercise would evoke endocrine responses that could be understood as regulatory signals. At least, considering the enhanced bone remodeling, Prisby et al. (2008) revealed that understanding of the physiological responses of the endocrine system during the acute and chronic vibratory protocols would be imperative. Other reports about the hormonal fluctuations due to the WBV have been also published (Bosco et al., 2000; Goto and Takamatsu, 2005; Cardinale et al., 2010; Elmantaser, 2012). Prisby et al. (2008) have also pointed out that the hormonal fluctuations subsequent to the WBV have not been measured in other populations, as among the postmenopausal women. In addition, the concentration of non hormonal biomarkers has been also altered due to the WBV (Goto and Takamatsu, 2005; Prisby et al., 2008; Rittweger, 2010).

Rittweger (2010) has suggested that the fluctuations of the concentration of plasma biomarkers due to the endocrine responses related to the exercises would permit to follow an exercise task, as well as to be mediators to follow the effect of training. In addition, Cochrane (2011) has reported that despite the wide use of WBV in sport, exercise and health, the physiological responses of this kind of exercise remain equivocal due to studies that have used different protocols, various methods of application, several physical parameters of the vibration, training duration and exercises performed with the WBV.

WHOLE BODY VIBRATION PLATFORMS

There are some models of platforms that can generate vibrations capable to produce WBV exercises (Rauch et al., 2010; Marin et al., 2010; Signorile, 2011) in a person. Indeed, it is necessary to point out that biomechanical parameters must be considered (frequency, displacement peak to peak, and amplitude) (Rauch et al., 2010; Cochrane, 2011).

WBV exercise is normally practiced with the subject's feet on the base of the oscillating/vibratory machine. These machines are also known as platforms. They have a base that is the region of contact between the machine

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and the subject. Among the different types, three of them would be capable of transferring energy (vibration) to a person's body (Rauch et al., 2010; Signorile, 2011).

In one of the types, vibration is transferred to both feet synchronously, with an up and down movement of the base. In another type, the base operates with a central pivot in an alternated way, like a teeter-totter; when the right foot is low, the left foot is high, and vice versa. In a third device, a triplanar machine, the movements of the base vary between up and down, forward and back, and side-to-side (Rittweger, 2010, Signorile, 2011).

WBV exercise can be considered a forced oscillation (Rittweger, 2010), and the energy of the vibration is transferred from the machine to the subject that is in contact with it. In general, these vibrations have sinusoidal shape, and can be described by amplitude, a displacement peak to peak, and a frequency (Cardinale and Wakeling, 2005; Rittweger, 2010; Rauch et al., 2010). Furthermore, in the protocol the time of work, the time of rest and the number of sessions must also be considered, as in the recommendations suggested by Rauch et al. (2010).

During a WBV exercise, the body is accelerated due to the stimulus of the vibration. In consequence it causes a reactive force by and within the body of the subject (Cardinale and Wakeling, 2005; Rittweger, 2010; Rauch et al., 2010; Signorile, 2011). It is important to consider that, under determined conditions (high frequency and amplitude) and the work time, these forces can be potentially undesirable and harmful to the body (Monteleone et al., 2007; Rauch, 2010; Cochrane, 2011; Franchignoni, 2013).

GENERAL APPROACHES ABOUT THE ACTION MECHANISM OF THE WBV: NEUROMUSCULAR AND NEUROENDOCRINE RESPONSES

Pribsy et al. (2008) have discussed the mechanisms associated with the effectiveness of WBV in enhancing skeletal mass in the elderly, individuals with low-bone mineral density, and adolescents. They suggested that the mechanisms by which this effect in the skeletal mass may be related to indirect effects due to tissue perfusion, fluctuations in systemic hormones, and/or via a direct effect related to mechanical stimulation.

Considering the indirect effects of the WBV exercises, besides the changes on the hormone concentration in the plasma (Bosco et al., 2000; Di Loreto et al., 2004; Goto and Takamatsu, 2005; Martin et al., 2009; Cardinale et al., 2010; Çidem et al., 2014; Huh et al., 2014), other biomarkers (Goto and Takamatsu, 2005; Humphries et al., 2009) can have their concentration altered in the plasma.

Concerning the direct effect in a subject under a WBV exercise, the muscles and tendons act as spring-like elements that store and release mechanical energy (Cardinale and Wakeling, 2005; Rittweger, 2010). In addition to the spindle afferents, Ib-afferents from Golgi tendon organs are likewise responsive to muscle vibration (Burke et al., 1976; Hayward et al., 1986; Cardinale and Wakeling, 2005; Rittweger, 2010). Spindle discharge will induce excitatory effects upon the a-motoneurone (monosynaptic or polysynaptic) pathways and contractions of the homonymous muscle (Granit et al., 1956; Rittweger, 2010). In consequence, the passive muscle vibration generates a reflex contraction, the tonic vibration reflex (TVR) (Matthews, 1966; Rittweger, 2010).

Thinking about the indirect effect of the WBV, it is important to review the study presented by Cardinale and Bosco, 2003 that have pointed out that it is also relevant to consider the influence of vibratory stimulation on central motor command. This statement is based on the investigation performed by Naito et al., 2000, which showed that the primary and secondary somatosensory cortex, together with the supplementary motor area, constitutes the central processing unit of afferent signals. They have demonstrated, using positron emission tomography, that vibration is capable of producing kinesthetic illusion that will activate the supplementary motor area, the caudal cingulate motor area, and area 4a of the brain. Moreover, the supplementary motor area of the brain that is activated by vibration (Naito et al., 2000) would be activated early during self-initiated movements (Cunnington et al., 2002).

The mechanism involved in the WBV exercises are still being discussed, nevertheless, it is thought that WBV causes a rapid reflex and stretch-shortening (Ritzmann et al., 2010) where a temporal association exists between electromyographic activity and muscle contractile movement (Cochrane et al., 2009) that is likely to involve the TVR (Matthews, 1966), thereby activating the muscle spindles and enhancing the excitatory drive reflex of the alpha motoneurons (Rittweger, 2010). Moreover, WBV may have a positive influence on motor cortex excitability and voluntary drive. Authors have demonstrated that when acute WBV was applied to the lower-body during or between resistance training sets, it significantly increased upper body performance (Marin et al., 2010). In addition, acute effects of WBV on neuromuscular responses have been reported (Marin et al., 2011; Pollock et al., 2012; Marin et al., 2012; Giunta et al., 2013). In consequence, different effects can be observed (Abercromby et al., 2007; Mileva et al., 2009; Rittweger, 2010; Cochrane, 2011; Menicucci et al., 2013). Mileva et al. (2009) reported increased corticospinal excitability and alteration of intracortical processes through WBV. It was suggested that vibration stimulus would influence the excitatory state of the peripheral and central structures, which could facilitate subsequent voluntary movements.

In addition, Cardinale and Bosco (2003) have suggested that during strength training exercise, rapid endocrine activation is triggered by collaterals of the central motor command and transmitted to the

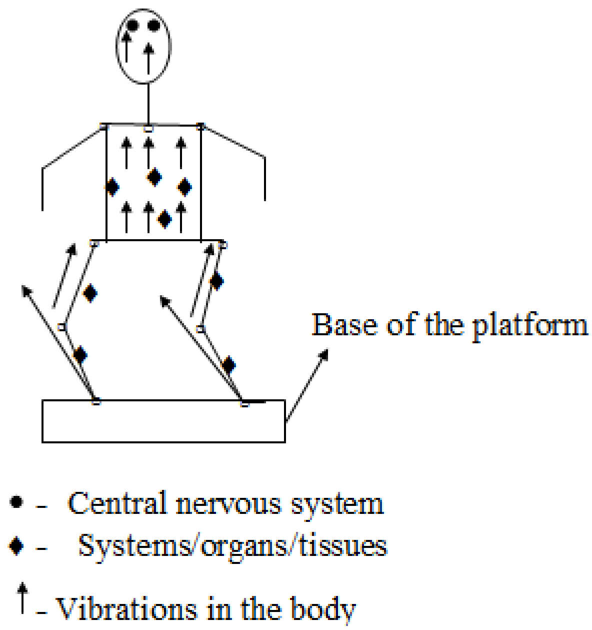

Figure 1. Interaction of vibration in the body.

hypothalamic neurosecretory and autonomic centres. The responses would be supported by the feedback influences from proprioceptors and metaboreceptors in the muscle. The mechanical characteristics of vibration could provide an adequate stimulus for specific hormonal secretion in association with external load (Giunta et al., 2013) or alone (Viru, 1992). It is stimulating to suggest that other biomarkers, besides hormones, can have their plasma concentration altered due to different exercises, as the WBV exercises (Goto and Takamatsu, 2005).

Putting together the findings reported in the literature, Rittweger (2010) has suggested that the exercise would evoke endocrine responses that could be understood as regulatory signals. At least, considering the enhanced bone remodeling by Prisby et al. (2008) the understanding of the physiological responses of the endocrine system during the acute and chronic vibratory protocols would be imperative. Prisby et al. (2008) have also pointed out that the hormonal fluctuations subsequent to the WBV have not been measured in other populations, as among the postmenopausal women. Rittweger (2010) has suggested that the fluctuations of the concentration of plasma biomarkers due to the endocrine responses related to the exercises would permit to follow an exercise task, as well as to be mediators to follow the effect of training. In addition, Cochrane (2011) has reported that despite the wide use of WBV in sport, exercise and health, the physiological responses of this kind of exercise remain equivocal due to studies that have used different protocols, various methods of application, several vibration parameters (frequency, peak to peak displacement), training duration and exercises performed with the WBV. The aim of this Investigation is to present a suitable revision about hormonal and non hormonal biomarkers in human beings submitted to WBV exercises that have suffered alteration in the plasma concentrations. It is expected that the findings described in this work can contribute to stimulating further research in this field to try to understand better about the biological effects associated with the vibrations generated in the oscillating/vibratory platforms.

PLASMA BIOMARKERS RESPONSES TO WBV

Figure 1 shows the penetration of the vibration in the body and the possible interactions in the systems/organs/tissues through its displacement and the transference of energy of this physical agent. Part of the energy of the vibrations is absorbed; however, part of it can reach the central nervous system. In consequence, authors have reported that WBV exercise is capable of interfering in the concentration of various plasma substances, hormonal and non hormonal biomarkers (Bosco et al., 2000; Di Loreto et al., 2004; Goto and Takamatsu, 2005; Cardinale et al., 2010; Çidem et al., 2014; Huh et al., 2014).

Hormonal responses to WBV

Viru (1992) has suggested that exercise can be responsible in evoking endocrine effect that can be related both to regulatory signals necessary to the exercise, and also as mediators for the training effect. In addition, Menicucci et al., 2013 suggest that sustained strenuous exercise produced a stereotyped cardiovascular early recovery whose speed could be conditioned by the immune and stress-related hormonal milieu.

The hormone responses to exercise have been documented for several hormones, as testosterone (54), growth hormone/IGF axis (Kindermann et al., 1982; Kraemer et al., 1990; Giunta et al., 2013), catecholamines (Kindermann et al., 1982), cortisol (Kindermann et al., 1982; Kvorning et al., 2006), triiodothyronine (Neto et al., 2013) and others (Behboudi et al., 2011; Reichkendler et al., 2013; Bemben et al., 2015).

Considering the WBV exercise, hormonal fluctuations could inform us about the specific physiological processes related to this type of exercise. In Table 1, some hormones that have their concentration altered due to the WBV exercises are shown.

Testosterone, which is a steroid hormone, has a complex variety of roles in the male physiology. Moreover, it is essential for the development and maintenance of various organs and their physiological function in men. It exerts biological effects throughout a man's entire life (Ohl and Quallich, 2006). Concerning the

Table 1. Hormones altered due to the WBV exercises.

Hormone	WBV exercise effect	References
Testosterone	Increase	Bosco et al., 2000; Santos-Filho et al., 2011; Nameni, 2012; Di Giminiani et al., 2014
Adiponectin	Increase	Humphries et al., 2009
Growth hormone	Increase	Bosco et al., 2000; Kvorning et al., 2006; Di Giaminiani et al., 2014
IGF-1	Increase	Cardinale et al., 2010
Epinephrine	Increase	Di Loreto et al., 2004; Cardinale et al., 2010
Irisin	Increase	Huh et al., 2014
Norepinephrine	Increase	Di Loreto et al, 2004; Goto and Takamatsu, 2005
Cortisol	Decrease	Bosco et al., 2000; Kvorning et al., 2006
Parathyroid hormone	Increase	Martin et al., 2009
Sclerotin	Increase	Çidem et al., 2014

IGF-1 - Insulin-like growth *factor* 1.

effect of the WBV exercise in the plasma level of testosterone, Bosco et al., 2000; Santos-Filho et al., 2011; Nanemi, 2012 and Di Giminiani et al., 2014 have reported significant increase in testosterone levels in response to these exercises. However, other authors have not found increase in the concentration of testosterone due to WBV exposure (Cardinale et al., 2006; Erskine et al., 2007). Ullah et al., 2014 have reported that, in addition to the development of secondary sexual characteristics in males, testosterone has other physiological actions, such as maintaining lean muscle and bone mass, and glucose metabolism. Considering that the WBV exercises could interfere with these last functions, it is possible to suggest that this interference would be associated with the increase of the concentration of testosterone.

The secretion of the growth hormone (GH) is pulsatile. It stimulates the linear growth of the children directly and indirectly (via the synthesis of Insulin-like growth factor 1-IGF-1). It is also associated with other metabolic effects, such as increase of the lipolysis and lipid oxidation that leads to the mobilization of stored triglyceride. GH is also related to the stimulation of protein synthesis and the antagonism of the insulin action and phosphate, water, and sodium retention. The peak GH secretory activity occurs within an hour after the onset of deep sleep. Exercise, physical activity, trauma, and sepsis are associated with increased GH secretion (Salvatori, 2004; Goldenberg and Barkan, 2007; Salvatori, 2009). Doessing et al., 2010 have discussed the importance of the IGF-1 and of the GH. The predominant action of GH is to stimulate hepatic synthesis and secretion of IGF-1. As a differentiating and growth factor, IGF-1 is a critical protein induced by GH, and is likely responsible for most of the growth-promoting activities of GH (Salvatori, 2004; Goldenberg and Barkan, 2007; Doessing et al., 2010). Ueland, 2005 has reported that endogenous GH is critical for the maintenance of bone mass in adults; and Sherlock and Toogood, 2007 have described that elderly subjects have a reduction in the levels of plasma GH.

Insulin-like growth factor-I (IGF-I) is a hormone with a potent growth and differentiation factor. It is the major mediator of the GH-stimulated somatic growth, as well as a mediator of GH-independent anabolic responses in many cells and tissues. *In vitro*, GH and IGF receptors have been demonstrated on osteoclasts and both GH and IGF-I may directly modify osteoclast function and activity. IGF-I is synthesized by multiple mesenchymal cell types, and two major mechanisms to the regulation of the IGF-I have been reported (Sherlock and Toogood, 2007). Regarding the IGF-I that is synthesized in the liver and secreted into the blood, it is described that this is under the control of GH. Considering the autocrine and paracrine IGF-I synthesis in the peripheral tissues, such as bone, this would be controlled by the GH and by other factors that are secreted locally by the surrounding cell types (Juul, 2003; Salvatori, 2004; Ueland, 2005; Goldenberg and Barkan, 2007; Salvatori, 2009).

Concerning the studies of the influence of WBV exercises in IGF-1 levels, Cardinale et al., 2010 have found an increase in the concentration of the IGF-1 in elderly people, suggesting a vibration-specific effect on the GH-IGF axis. Putting together these results, they could justify the effect of the WBV exercise in the muscle and in the skeletal (Cardinale and Wakeling, 2005; Prisby et al., 2008; Rittweger, 2010).

Schoorlemmer et al. (2009) have presented that high cortisol level is associated with several undesirable clinical conditions, as osteoporosis, hypertension, diabetes mellitus and susceptibility to infections. Moreover, they demonstrated that high salivary cortisol levels are associated with increased mortality risk in a general older population. Fluctuations within the plasma concentration of the cortisol have been reported by several authors due to the WBV exercises (Bosco et al., 2000; Kvorning et al., 2006; Di Giminiani et al., 2014). Bosco et al., 2000 showed a decrease in the plasma cortisol levels, whereas Kvorning et al., 2006 showed a decrease in a certain groups and Di Giminiani et al., 2014 have shown an increase in the GH levels after vibration exercise. However, Cardinale et al., 2006 found a non significant increase in plasma cortisol levels, while Di Loreto et al., 2004 and Goto and Takamatsu, 2005 have not found alterations in the concentration of this hormone after WBV exercise.

Catecholamines are hormones produced in the adrenal glands and the main ones are dopamine, norepinephrine, and epinephrine. They play a major role as neurotransmitters in the central and peripheral nervous systems, and have a close relationship with human health (Kjaer, 1998; Hu et al., 2013). Catecholamines are released into the blood generally when a person is under physical or emotional stress (Zouhal et al., 2008). Moreover, the metabolism of carboydrates and lipids are influenced by these hormones. Stallknecht et al., 2001 have reported that lipolysis during endurance exercise is stimulated primarily by catecholamine release and is suppressed by insulin. Di Loreto et al., 2004 and Goto and Takamatsu, 2005 have reported that the plasma concentration of epinephrine and norepinephrine are increased after WBV exercises. Ishitake et al., 1999 have suggested that short-term exposure to WBV can suppress the gastric myoelectric activity as a responses that would be mediated by neurohumoral effects due to the mechanical effect of WBV. It is possible to consider that this neuroendocrine response has been proposed to be the reason for reduced gastric motility during WBV exercise, although direct mechanical influences could also play a role.

Mirza et al. (2010) have pointed out that sclerostin is known to act in a local paracrine fashion in the microenvironment of bone and they have demonstrated that sclerostin also enters in the circulation and suggest the possibility that, in addition to its local actions, it may regulate bone mass by acting as an endocrine hormone. Çidem et al., 2014 have shown that the plasma sclerostin level measured at 10 min after the WBV treatment increased 91% on the first day and decreased 31.5% on the 5th day in the whole-body vibration group.

Parathyroid hormones (PTH) produced in the parathyroid gland, as well as the estrogens and other hormones produced in different sites, are involved in the bone remodeling in a continuous process (Chrstensen et al., 2014). PTH contributes to several physiological processes, such as the maintenance of the optimal physiological calcium concentration in extracellular fluids. This fact indicates the relevance of this hormone in the control of osseous construction. Martin et al., 2009 have hypothesized that a short term WBV training would provoke an endocrine response in elderly subjects with results favorable to the osseous construction processes. They found that the PTH blood concentration increased due to the low-intensity WBV training lead to a positive hormonal profile on PTH, which can benefit the osseous construction processes on elderly people.

Humphries et al. (2009) have reported an increase in the concentration of adiponectin, that modulates various metabolic process and other molecules in healthy active women.

Irisin, a hormonal molecule, is an identified myokine, suggested to mediate the beneficial effects of exercise by inducing browning of white adipocytes and thus increasing energy expenditure (Daskalopoulou et al., 2014). Huh et al. (2014) have pointed out that the regulation of irisin by exercise is not completely understood in human beings. Moreover, they have demonstrated that WBV acutely increase the concentration of this biomarker in the plasma.

General biomarkers responses to WBV

The concentration of some non hormonal biomarkers has suffered alterations in response to vibration exercise. In Table 2, some of these substances that have their concentration altered due to the WBV exercises are shown.

Di Loreto et al. (2004) have demonstrated that WBV exercise decreases the glucose levels (non significantly) in the plasma. These authors suggest that WBV leads to an enhancement of the glucose influx into the muscle. Moreover, after training intervention with WBV, a slight, but not significant, reduction in the fasting glucose concentration was found in type-2 diabetic patients. Nevertheless, Behboudi et al. (2011) have shown a significant decrease of fasting glucose in the exercise groups, one including WBV exercise.

Goto and Takamatsu (2005) have reported an increase in serum free fatty acids after the WBV exercise; however, they did not find changes in the concentration of the glycerol. These authors have suggested that a WBV session would stimulate secretions of catecholamine and GH and subsequently enhance lipolysis, resulting in increased FFA and glycerol concentrations of blood. They discussed that their results are partially consistent with their hypothesis and suggest that the WBV session stimulates lipolysis during the recovery period. The lipolysis enhancement might be caused by epinephrine and norepinephrine secretions seen immediately after the WBV session.

Humphries et al. (2009) have reported an increase in

Table 2. Concentration of non hormonal biomarkers altered due to the WBV exercises.

Biomarkers	WBV effect	References
Glucose	Decrease	Di Loreto et al., 2004*, Behboudi et al., 2011
Free fatty acids	Increase	Goto and Takamatsu, 2005
TGF-β1	Increase	Humphries et al., 2009
nitric oxide	Increase	Humphries et al., 2009
Osteopontin	Decrease	Humphries et al., 2009
interleukin-1beta	Decrease	Humphries et al., 2009
Bone ALP	Increase	Bemben et al., 2015
COMP	Decrease	Liphardt et al., 2009
TNF-α	Decrease	Humphries et al., 2009

Bone ALP - Bone-specific alkaline phosphatase, TGF-β1 -Transforming growth factor-beta1, TNF-α - Tumor necrosis factor-alpha, COMP - Cartilage oligometric matrix protein. *- non significant.

the concentration of adiponectin (hormonal molecule), transforming growth factor-beta1, and nitric oxide with an accompanying decrease in osteopontin, in interleukin-1beta, and in tumor necrosis factor-alpha plasma concentration in healthy active women. These authors discussed that these results indicate that whole-body vibration exposure may be effective in improving the bone mineral density by increasing bone deposition while also decreasing bone resorption. Moreover, WBV exercise may also provide an efficient stratagem for young women to achieve peak bone mass and help stave off osteoporosis later in life and provide a new form of physical training.

Bemben et al. (2015) have reported that WBV exercise is also capable in increasing significantly the concentration of bone-specific alkaline phosphatase (Bone ALP).

Posey et al. (2008) have described that the cartilage oligomeric matrix protein (COMP) is a non-collagenous extracellular matrix protein expressed primarily in cartilage, ligament, and tendon. This protein has been studied due to mutations in the gene cause two skeletal dysplasias, pseudoachondroplasia and multiple epiphyseal dysplasia. COMP is also a biomarker for joint destruction associated osteoarthritis, rheumatoid arthritis, joint trauma, and intense activity. Serum cartilage oligomeric matrix protein levels are higher in aggressive cases of arthritis and levels are used to predict future disease progression. Liphardt et al. (2009) have found that interventions with WBV exercises decrease the serum COMP concentrations.

DISCUSSION

In this investigation, it is shown that the plasma concentration of several biomarkers (hormonal and non hormonal) can be altered in a subject submitted to WBV exercise. It is expected that the findings described in this work can contribute to increasing the knowledge related with biological effects due to the vibrations generated in oscillating/vibratory platforms. The understanding of the consequence of fluctuations in the concentration of these biomarkers can help prevent side-effects as well to improve the use of these vibrations.

The number of publications in the PubMed database using the keyword “whole body vibration” in the last six years corresponds to 52.83% of the total of publications in this subject. Considering this finding, it is stimulating to discuss the importance of this kind of exercise (WBV exercise) to the human beings.

Three types of machines that produce vibration and can generate WBV are available (Cardinale and Wakeling, 2005; Rittweger, 2010; Signorile, 2011). It is suggested that a device with a side-alternating vibration would evoke rotational movements around the hip and lumbo-sacral joints (Rittweger, 2010). Abercromby et al. (2007) has suggested that this movement would be responsible by an additional degree of freedom and, accordingly, whole-body mechanical impedance is smaller in side-alternating than in synchronous WBV. Cronin et al. (2004) reported several undesirable conditions in untrained participants with WBV. Franchignoni et al. (2013) have reported hematuria due to WBV in healthy elite athlete and they suggested that platforms providing side-alternating vibration may pose some health risks with high amplitudes. The action mechanism of the vibration in the WBV exercise is complex and is not fully understood. Nevertheless, it is possible to speculate that fluctuations in hormonal and non hormonal biomarkers could be associated with some of the effects (desirable and undesirable).

In addition, several factors contribute to try to increase this complexity of the action mechanism of the WBV. Some of them are; the various protocols, different methods of application, variable biochemical parameters (frequency, peak to peak displacement), results obtained with trained and untrained groups, and health and unhealthy persons used in the investigations (Cochrane, 2011). Nevertheless, direct and/or indirect effects might be associated with the biological responses. An effect in the muscle with a local consequence and/or with interference in the central nervous system can be suggested as direct effect. Moreover, the action in the central nervous system can induce fluctuations in the plasma concentration of hormonal and non hormonal biomarkers (Prisby et al., 2008; Rittweger, 2010; Cochrane, 2011). Importance of the neuroendocrine response to the vibrations seems to be clear due to the hormone fluctuations demonstrated for several authors (Di Loreto et al., 2004; Goto and Takamatsu, 2005; Cardinale et al., 2010; Santos-Filho et al., 2011; Di Giminiani et al., 2014) in subjects submitted to this physical agent. In addition, the alterations of the plasma concentration of non hormonal biomarkers (Goto and Takamatsu, 2005; Humphries et al., 2009; Behboudi et al., 2011) could be also associated with effects of vibration in the neuroendocrine system. In addition, it is possible to speculate that the negative effects of the WBV could be also associated with the fluctuations of hormonal and non hormonal biomarkers.

Considering the hormonal biomarkers, alterations in concentrations in the plasma are found by some authors, but there is not a consensus, since other authors do not find alterations. As an example, Bosco et al., 2000, Santos-Filho et al., 2011 and Nameni, 2012 have observed an increase in the level of testosterone, however, (Di Loreto et al., 2004, Kvorning et al., 2006, Erskine et al., 2007 and Cardinale et al., 2010) have not found alteration in the concentration of this hormone. This fact has also been observed to other hormones. Probably, the biomechanical parameters, as frequency and amplitude can have influence in this effect. In addition, Kraemer and Ratamess (2005) have pointed out that the increase in the concentrations of some hormones, such as testosterone and GH, are dependent on the recruited muscle volume related to the exercise intensity. As discussed by Di Giminiani et al. (2014) another question concerned with the GH to be considered is the pulsatile characteristics of this hormone that could bring limitation to some investigations.

Regarding the non hormonal biomarkers, there are only a limited number of studies. Nevertheless, the findings are very important, but with non significant (Di loreto et al., 2004) and significant (Behboudi et al., 2011) reduction of glucose and the increase in the plasma concentration of free fatty acids reported by Goto and and Takamatsu, 2005. Effect of the WBV in other biomarkers, the irisin, can also be relevant. Humphries et al. (2009) have reported an increase in the concentration of adiponectin, transforming growth factor-beta1, and nitric oxide with an accompanying decrease in osteopontin, in interleukin-1beta, and in tumor necrosis factor-alpha plasma concentration. These authors discussed their results that indicate that whole-body vibration exposure may be effective in improving bone mineral density by increasing bone deposition while also decreasing bone resorption. Concerning effect of the WBV on the bone, Bemben et al. (2015) have reported that this kind is also capable of increasing significantly the concentration of Bone ALP. Considering the importance of the COMP as a biomarker and its being high in aggressive cases of arthritis, Liphardt et al. (2009) have found a relevant result. The WBV exercises decrease the serum COMP concentrations. In addition, considering other functions related to the bone, Tossige-Gomes et al. (2012) have demonstrated that the proliferative response of $TCD4^+$ cells showed a significant decrease (23%) in the WBV group compared to the control group, while there was no difference between groups regarding the proliferative response of $TCD8^+$ cells. These authors suggest that the data indicate the addition of WBV to squat exercise training might modulate T-cell-mediated immunity, minimizing or slowing disease progression in elderly patients with osteoarthritis of the knee.

Considering possibility of the use of WBV in trained and untrained subjects and in patients with some clinical disorders (Issurin et al., 1994; Bosco et al., 1998; Bosco et al., 1999; Bosco et al., 1999; Kerschan-Schindl et al., 2001; Rittweger et al., 2000; Rittweger, 2002; Issurin, 2005), it is relevant to try to understand better the action mechanisms associated with this kind of exercise. It is noteworthy that the side-effects related to the utilization of the WBV also stimulate these investigations (Crewther et al., 2004; Cronin et al., 2004; Monteleone et al., 2007; Franchignoni et al., 2013). Moreover, thinking about the different findings on effect of the WBV reported by the authors and the plasma concentration o a same biomarkers, one reason for inconsistent results could be associated with the personal patient characteristics.

Conclusion

Putting together the findings concerning the effect of WBV exercise in the concentration of hormonal and non hormonal biomarkers, it is possible to verify the importance of studies in this field. Although, the comprehension of these effects is complex, these fluctuations in the plasma concentrations of these biomarkers might aid a better understanding of the effect of the WBV exercises. In addition, it is suggested that this kind of exercise would neuroendrocrine responses.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Scientific Research and Essays

Full Length Research Paper

Enhancing germination and seedling vigour in cluster bean by organic priming

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The experiment was conducted at Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai during 2014-2015, to find out the effect of organic seed priming with cow urine at different concentrations. The cluster bean seeds were soaked for 3 h with different concentrations *viz.,* 2, 4, 6, 8 and 10% along with water and control (no treatment). The seed quality parameters *viz.*, speed of germination, germination, root length, shoot length, vigour index I, vigour index II and dry matter production were evaluated. The best performance was observed in cow urine (2%) by recording highest seed quality parameters. The percentage increase over control was 10.52, 8.16, 9.8, 8.0, 16.34, 15.36 and 7.8 for speed of germination, germination percentage, root length, shoot length, vigour index I, vigour index II and dry matter production, respectively.

Key words: Bovine urines, cluster bean, pre sowing seed treatment, vigour index-germination.

INTRODUCTION

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub] (2n=14) is an under exploited leguminous vegetable belonging to the family Fabaceae. It is commonly known as *Guar, Chavli kayi, Khurtti.* Guar is grown in *kharif* season in arid and semi arid regions of India. It is a drought hardy, deep rooted, summer annual legume. Guar is the most important and potential vegetable cum industrial crop grown for its tender pods for vegetable purpose and for endospermic gum (30 to 35%).

In India, cluster bean occupies an area of 2.20 million ha with a production of 0.60 million tonnes (2005). In North Indian states like Rajasthan, Haryana, Gujarat and Punjab it is mainly cultivated for guar gum production and for forage, whereas in South India it is being cultivated for vegetable purpose. From India, cluster bean is mainly exported to USA, Germany, Netherlands, UK, Japan, and France value at Rs. 200 million rupees annually (Sing et al., 2009). Due to environmental concerns, there is an urgent need to reduce the use of chemical fertilizers and pesticides in agriculture and horticulture and alternative to chemicals are being sought to improve crop establishment and health. One option is the use of organics nutrients or growth regulators to seed or roots, which may promote plant growth or provide diseases control through a variety of mechanisms, including supply of organic nutrients production of plant hormones,

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antibiotic or enzyme; induced systemic resistance; direct parasitism of plant pathogen or deleterious micro-organisms; or competition with pathogen for or nutrients. Further, organic seed is a crucial link in the chin from research to organic seed production and ultimate supply of high quality seed at reasonable price to the commercial seed producing farmers for promotion of organic seed production. Hence, the safe and feasible approach is the priming of seeds with organics which are safe, eco friendly, economical and easily available. Organic seed priming provides hardiness to high temperature, low moisture especially in semi arid tropics. It promotes faster germination, higher seedling vigour leading to higher crop productivity. The main benefits of organic seed treatments include increased phosphate levels, nitrogen fixation and root development.

Cow urine contains about 1.0% nitrogen, traces of P_2O_5 and 1.0% of K_2O. Approximately 2400 to 2500 L of urine are produced per year per animal (Yawalker et al., 1996). If this urine were not conserved, nitrogen in the urine, which is mainly in the form of urea, would be quickly lost as ammonia. It is also considered as a natural disinfectant and pest repellent and forms the main component of Panchagavya (an organic crop booster prepared and sprayed by Indian farmers) (Tharmaraj et al., 2011). Organic seeds priming is more affordable so even small scale farmers can practice. Keeping into view its importance as a vegetable and its adaptability to arid drought conditions, there is need for its improvement for yield. This can be achieved by maintaining plant population by organic seed priming.

MATERIALS AND METHODS

Genetically pure seeds of cluster bean (Pusa Navbahar) used for the study. The experiment was conducted at Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai during 2014. The seeds were treated with cow urine at the concentration of 2, 4, 6, 8 and 10% along with water and dry seed as control. Seeds were soaked for 3 h and shade dried. The seeds were tested for the standard germination test adopting between paper (BP) method as per the ISTA rules (Anon., 1996). The germination room maintained at 25 ± 2^{o}c temperature and 90 ± 3 % RH. The seeds showing radical protrusion were counted daily from third day after sowing until fourteenth day. The speed of germination was calculated using the formula by Maguire (1962). Hundred seeds were placed in between paper using four replications and per cent germination was recorded. At the time of germination count, ten normal seedlings were selected at random from each replication and used for measuring the root length of seedlings. Root length was measured from the point of attachment of seed to the tip of primary root. The mean values were recorded and expressed in centimeter. The seedlings used for measuring root length were also used for measuring shoot length. The shoot length was measured from the point of attachment of cotyledon to the tip of the leaf and the mean values were recorded and expressed in centimeter. Vigour index values were computed using the following formula and the mean values were expressed in whole number (Abdul-Baki and Anderson, 1973). Vigour index I = Germination (%) × Total seedling length (cm) and vigour index II =. Germination (%) × dry matter production (g/10 seedlings). The data from various experiments were analyzed statistically adopting the procedure described by Panse and Sukhatme (1985). Wherever necessary, the percentage values were transformed to arc sine values before carrying out the statistical analysis.

RESULTS AND DISCUSSION

All the concentrations of cow urine increased the seed quality parameters. Among the concentrations, seeds primed with cow urine at 2% recorded increased speed of germination (9.5), germination percentage (98%), root length (14.47 cm), shoot length (16.05 cm), vigour index I (2991), vigour index II (49.98) and dry matter production (0.51 g/10 seedlings) compared to control (8.5, 90, 13.05, 14.75, 2502, 42.30 and 0.47) for speed of germination, germination percentage, root length, shoot length, vigour index I, vigour index II and dry matter production respectively).

Milch animal urine (cow / buffalo) contains about 1.0% nitrogen, traces of P_2O_5 and 1.0% of K_2O and approximately 2400 to 2500 L of urine are produced per year per animal (Yawalker et al., 1996). The reason for increased seed physiological parameters observed in the study may be due to the fact that bovine urine contains physiologically active substances *viz*., growth regulators, nutrients (Kamalam and Rajappan, 1989) and trace elements (Munoz, 1988). Illango et al. (1999) reported increased total chlorophyll content (1.80 mg/g fresh weight) and soluble protein (2.78 mg/g) upon soaking *Albizia lebbeck* seeds in cow urine in comparison to check (1.66 and 2.5 mg/g). Significantly higher plant height (74.21 cm), leaf dry weight, more number of tillers (137.4) were recorded 60 days after sowing, higher leaf area duration (2.47), higher straw yield (3388 kg / ha) was recorded for wheat seeds soaked in 10% cow urine (Shivamurthy, 2005).The cow's urine treatment with 1: 10 concentration was found very suitable to treat seeds of finger millet for good germination and seedling vigour. Shankaranarayanan et al. (1994) also reported that soaking of tamarind seeds in 10% cow urine or cow dung solution for 24 h increased the germination and vigour index as compared to that of untreated seeds. Our results were in close conformity with the findings in *Albizia lebbeck* (Ilango et al., 1999), jamun (Swamy et al., 1999), asparagus (Misra et al., 2002), Shivamurthy (2005) in wheat and Sivasubramaniyam et al. (2012) in pulses (Tables 1 and 2; Figure 1).

Conclusion

It could be concluded that cow urine (2%) can be recommended as organic seed priming for increasing the vigour in cluster bean under rain fed ecosystem.

Conflict of Interest

The authors have not declared any conflict of interest.

Table 1. Effect of organic seed priming with cow urine on speed of germination, germination percentage and root length (cm) in cluster bean.

Treatments	Speed of germination	Germination (%)	Root length (cm)
Cow urine (2%)	9.5	98 (82.17)	14.47
(4%)	9.4	98 (82.17)	14.22
(6%)	9.2	97 (80.15)	13.75
(8%)	9.0	96 (79.06)	13.50
(10%)	8.7	93 (74.79)	13.20
Hydro priming	9.1	97 (80.15)	13.68
Control	8.5	90 (71.56)	13.05
Mean	9.1	96 (79.06)	13.70
SEd	**0.188**	**1.990**	**0.285**
(P=0.05)	**0.392****	**4.139****	**0.593****

(Figures in the parentheses are arc sine transformed values), **- Significant at 5% level.

Table 2. Effect of organic seed priming with cow urine on shoot length (cm), vigour index I and vigour index II in cluster bean.

Treatments	Shoot length (cm)	Vigour index I	Vigour index II
Cow urine (2%)	16.05	2991	49.98
(4%)	15.25	2888	49.00
(6%)	15.20	2808	47.53
(8%)	15.12	2748	47.04
(10%)	14.86	2610	44.64
Hydro priming	15.03	2785	47.53
Control	14.75	2502	42.30
Mean	15.18	2762	46.86
SEd	**0.316**	**57.576**	**0.976**
CD (P=0.05)	**0.657****	**119.737****	**2.031****

**- Significant at 5% level.

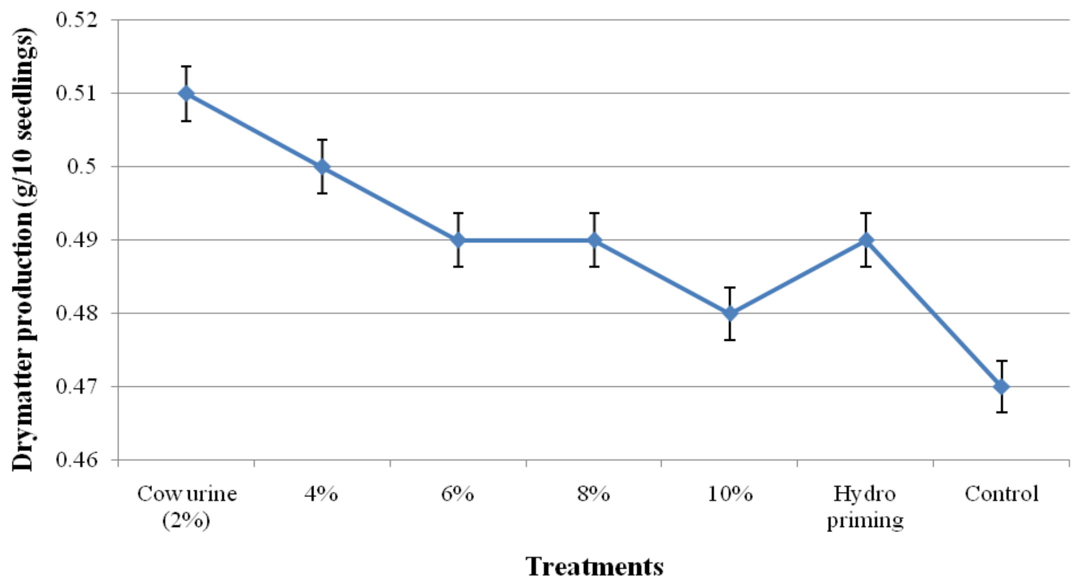

Figure 1. Effect of organic seed priming with cow urine on dry matter production (g/10 seedlings) in cluster bean.

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Scientific Research and Essays

Full Length Research Paper

Group balanced block design for comparisons among oilseed *Brassicae*

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The *brassica* genus contains the most genetically diverse collection of agriculturally important plant species, including oilseed, mustard and cruciferous species. Comparisons for seed yield and its components were drawn among brown (*Brassica rapa var. brown sarson*), gobhi (*Brassica napus*) and yellow sarson (*B. rapa var. trilocularis*). Brown sarson was found high yielder by virtue of its high siliquae number per plant and recorded maturity duration between the two. *B. napus* entries were late and showed intermediate seed yield. Significant mean squares were noted for all the traits among and within groups.

Key words: *Brassica rapa, Brassica napus,* sarson, maturity, yield.

INTRODUCTION

Brassica is a genus of the *Brassica*ceae (*Cruciferae*), commonly known as the *Cruciferae* family and are among the oldest cultivated plants known to humans with written records dating back to ca. 1500 BC (Prakash, 1980) and archaeological evidence of its importance dating back to 5000 BC (Yan, 1990) .

The *Brassica*ceae, which *currently* includes 3709 species and 338 genera (Warwick et al., 2006), is one of the ten most economically important plant families (Rich, 1991). The genus *brassica* has mainly Mediterranean distribution, but it extends to Asia and Africa, including India. Oilseed rape has been cultivated for thousands of years in Asia and the Indian subcontinent and then later in Europe.

The research carried by Morinaga (1934) revealed that *brassica*s consist of six species, three of them are monogenomic diploids viz *Brassica nigra* (n=8), *Brassica oleraceae* (n=9) and *Brassica Campestris* (n=10) while as, three are chromosome digenomic tetraploids, *Brassica carinata* (n=17), *Brassica juncea* (n=18) and *Brassica napus* (n=19), which evolved in nature through convergent allopolyploid evolution between any of the

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two diploid species. Brassica species are widely used in human diet mainly as an important source of vegetables, condiments, and edible oils (Branca and Cartea, 2011). Rapeseed-mustard seed is rich in oil and protein and contains 40 to 46% oil and 18 to 22% protein. In addition the oil content of *Brassica* seed meal contains about 40% protein with well-balanced amino acid (Miller et al., 1962) but lower than would be desired. The use of the related crops is cited in some ancient civilized regions such as in the Mediterranean and in Asia (Shaukat et al., 2014).

Rapeseed was the third largest source of vegetable oil in the world (after soybean and palm) and the second world source of protein, although it reached only a fifth of the soybean production. To assess and predict the possibilities and consequences of inter-specific hybridization, besides other factors it is important to know the flowering chronology and other important agro-morphological traits related to seed yield across different *Brassica* species (Anonymous, 1999). Usually among the three ecotypes brown sarson is more adaptable to environment in Kashmir, while gobhi sarson tends to be late.

Available germplasm of yellow sarson has shown little tolerance to cold. The study was undertaken to establish the differences with respect to yield and its component attributes within and among the genotypes in three *Brassica* species viz., *Brassica rapa var. brown sarson* (or brown sarson), *B. rapa var. trilocularis* (or yellow sarson) and *B. napus* (gobhi sarson).

MATERIALS AND METHODS

The experimental material comprised of 10 genotypes of brown (*B. rapa var. brown sarson*), gobhi (*B. napus*) and yellow sarson (*B. rapa var. trilocularis*) each were laid in a group balanced block design with 3 replications during *rabi* 2008-09 at MRCFC,Khudwani, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, India. The said experimental material was procured from Directorate of Rapeseed-mustard Bharartpur.

Each genotype was grown in a 5 m row length with three rows/plot with a crop geometry of 30 x 10 cm. The analysis of variance was performed following Gomez and Gomez (1983). The observations were recorded for 12 agro-morphological characters such as, plant height (PH), days to 50% flowering (DF), days to maturity (DM), primary branches per plant (PB), secondary branches per plant (SB), main raceme length (RL), siliquae on main raceme (SM), total siliquae per plant (TS), siliqua length (SL), 1000-seed weight (SW), seeds per siliqua (SS) and seed yield per plant (SY).

Barring DF and DM, observations for all the traits were recorded on 10 randomly selected competitive plants per entry per replication. All the recommended package and practices were adapted to raise a good crop.

RESULTS AND DISCUSSION

The perusal of the results revealed that among the three *Brassica* groups viz., brown sarson, gobhi sarson and yellow sarson, significantly high average mean values over the constituent genotypes were exhibited by brown sarson group with respect to traits PH, RL, SM and TS followed through gobhi sarson to yellow sarson (Table 1). Highest average mean values for DF and DM were recorded for gobhi sarson which established that this *Brassica* groups tend to be late in maturity (231 DAS) under Kashmir conditions while, yellow sarson matures on an average 14 days earlier than brown sarson (205 DAS).

Since gobhi sarson usually shows delayed maturity than brown sarson, at least here by 26 days, this remains perhaps the biggest deterrent to fit elite gobhi sarson germplasm in rice-rapeseed rotation. The trait SW weight exhibits highest average mean for gobhi sarson followed by yellow sarson and brown sarson sequentially. Statistically non-significant differences were recorded for SS between gobhi and brown sarson group, however, the trait recorded highest mean (27 seeds per siliqua) over the genotypes in yellow sarson group (Sinhamahapatra et al., 2003).

This was because of the tetra-locular siliquae of the genotypes in yellow sarson compared to bi-chambered nature of most of the gobhi and all the brown sarson entries. More importantly, highest average SY was exhibited by brown sarson (1167 kg/ha) against other two groups those showed yield at *par* between them. Brown sarson out-yielded two other sarson types by virtue of significantly higher SM and TS than either of the two. Similar comparisons were made between three *Brassica* groups by Varshney et al. (1986) and Shikari and Sinhamahapatra (2004).

Analysis of variance revealed that highly significant variation existed among three *Brassica* groups for all the twelve characters (Table 2). The *Brassica* species recorded significant to highly significant variability within the groups for all the characters under study except SB and SL for yellow and brown sarson respectively. Also, non-significant mean squares for DF were noted within brown sarson group.

High significant mean squares were shown by three primary yield attributes that is, SS, TS and SW among and within the groups except within yellow sarson group for TS.

Since brown sarson is the only *Brassica* oilseed grown in Kashmir valley, the comparisons made above highlights the importance of breeding early maturing gobhi sarson types with high TS, SW and SS. Yellow sarson usually remains low yielder out of the three, though could be used in hybridization for improving SW, maturity traits and oil content.

Conflict of Interest

The authors have not declared any conflict of interest.

Table 1. Comparison using Group Balanced Block Design among and within groups representing three *Brassica* species for yield and its components.

Group/ statistic	Plant height (cm)	Days to 50% flowering	Days to maturity	Number of primary branches	Number of secondary branches	Main raceme length (cm)
Range (Gobhi sarson)	68.87-97.60	164.67-175.00	226.67-235.33	3.87-5.87	5.27-8.70	34.47-40.87
Range (Yellow sarson)	34.64-63.32	136.33-158.40	164.33-203.87	3.10-5.21	2.31-3.67	17.97-35.07
Range (Brown sarson)	81.13-109.47	161.33-165.67	191.67-214.33	3.60-6.17	1.40-5.90	44.53-62.40
Mean (Gobhi sarson)	84.94	170.67	231.30	4.66	6.85	37.91
Mean (Yellow sarson)	51.97	151.00	191.30	4.07	3.02	27.37
Mean (Brown sarson)	100.64	163.37	205.43	4.62	3.57	50.96
Grand mean over groups	79.18	161.68	209.34	4.45	4.48	38.74
CD (at 5%) among groups	5.87	1.20	5.30	0.36	0.62	7.13
CD(at 5%) within groups	4.98	1.38	1.88	0.36	0.53	1.84
CV (%) among groups	7.38	0.74	2.52	7.98	13.67	18.30
CV (%) within groups	7.99	1.08	1.14	10.36	14.94	6.04

Group/ statistic	Siliqua on main raceme	Siliquae/plant	Siliqua length (cm)	1000- seed weight (g)	Seeds/ siliqua	Seed yield / hectare (kg)
Range (Gobhi sarson)	27.87-41.80	48.88-136.82	4.18-4.99	3.07-4.58	16.44-22.62	464.76-1246.00
Range (Yellow sarson)	16.67-31.67	40.43-67.58	3.11-4.06	3.27-4.20	21.30-30.93	494.56-868.05
Range (Brown sarson)	42.73-60.67	99.80-225.50	4.78-5.49	1.97-2.93	13.43-20.68	838.89-1475.00
Mean (Gobhi sarson)	34.02	97.90	4.60	3.79	18.75	753.02
Mean (Yellow sarson)	24.40	55.63	3.58	3.59	27.02	644.73
Mean (Brown sarson)	51.12	127.49	5.22	2.32	17.24	1167.81
Grand mean over groups	36.51	93.67	4.46	3.23	21.00	855.18
CD (at 5%) among groups	2.08	5.44	0.76	0.05	2.33	125.32
CD(at 5%) within groups	2.36	8.22	0.21	0.07	0.92	59.90
CV (%) among groups	5.67	5.78	16.97	1.50	11.04	14.58
CV (%) within groups	8.21	11.14	5.85	2.80	5.55	8.89

Table 2. Mean squares among and within different *Brassica* species grown in group balanced block design.

Source of variation	df	Mean sum of squares					
		Plant height (cm)	Days to 50% flowering	Days to maturity	Number of primary branches	Number of secondary branches	Main raceme length (cm)
Replications	2	46.40	0.18	20.35	0.35	0.39	62.37
Groups	2	18515.72**	2963.94**	12344.18**	3.20**	128.57**	4188.52**
Error (a)	4	34.10	1.42	27.77	0.13	0.37	50.29
Gobhi sarson	9	194.03**	30.74**	20.26**	1.03**	5.13**	20.73**
Yellow sarson	9	267.38**	194.58**	484.73**	1.34**	0.59	77.86**
Brown sarson	9	232.60**	5.96	215.93**	1.74**	5.12**	90.41**
Error (b)	54	40.06	3.06	5.67	0.21	0.45	5.48

Source of variation	df	Siliqua on main raceme	Siliquae/plant	Siliqua length (cm)	1000- seed weight (g)	Seeds/ siliqua	Seed yield / hectare (kg)
		7.99	35.87	0.58	0.01	4.34	6374.13
Groups	2	5495.88**	39122.89**	20.62**	18.92**	831.94**	2286927.22**
Error (a)	4	4.29	29.34	0.57	0.01	5.38	15553.82
Gobhi sarson	9	66.15**	1881.00**	0.17*	0.91**	13.40**	182184.68**
Yellow sarson	9	66.95**	213.54	0.24**	0.20**	25.95**	52209.25**
Brown sarson	9	89.49**	3955.99**	0.14	0.34**	22.20**	145937.57**
Error (b)	54	8.99	108.82	0.07	0.01	1.36	5784.54

** and * indicates P (<0.01) and P (<0.05) respectively.

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